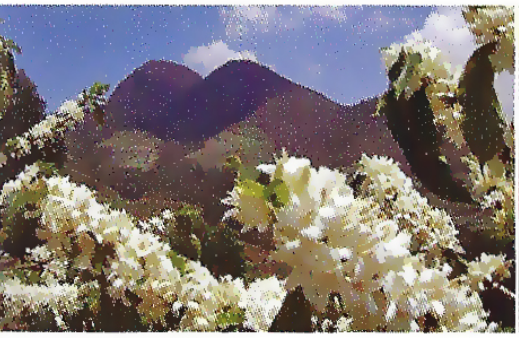
ASIC 2008

22nd International Conference on Coffee Science

Campinas, SP - Brazil

September 14-19, 2008



ASIC

ASSOCIATION FOR SCIENCE AND INFORMATION ON COFFEE
ASSOCIATION POUR LA SCIENCE ET L'INFORMATION SUR LE CAFÉ

VOLUME 2

22nd International Conference on Coffee Science
Campinas, 14th – 19th September 2008

22ème Colloque Scientifique International sur le Café
Campinas, 14 – 19 septembre 2008

Table of Contents

VOLUME 2

Coffee Genomics & Biochemistry

Communications

- Differential Gene Expression Response of *Coffee arabica* and *C. liberica* to Coffee Berry Borer Attack
  Gongora C., Idarraga S., Sanchez A., Gaitan A., Rivera L., Cristancho M., Yepes M., Buell R. (Colombia)

- High Density Genetic Mapping of Resistance Gene to Race II of Hemileia vastatrix in Hibrido de Timor Genotype
  Diola V., Brito G.G.G., Gallina A.P., Caixeta E.T., Sakyama N.S., Loureiro M.E. (Brazil)

- Study of Drought-Tolerance Mechanisms in Coffee Plants by an Integrated Analysis
  Marraccini P., Ramos H.J.O., Vieira L.G.E., Ferrao M.A.G., da Silva F.R., Taquita J.A., Bloch Jr. C., Andrade A.C. (Brazil)

- Construction of a Genetic Map Based on an Interspecific F2 Population between *Coffea arabica* and *Coffea canephora* and its Usefulness for Quality Related Traits
  Priolli R.G.H., Ramos L.C.S. G., Pot D., Moller M., Gallo P.B., Pastina M.M., Garcia A.A.F., Yamamoto P.A., Lannes S.D., Ferreira L.P., Scholz M.B.S., Mazzafera P., Pereira L.F.P. Colombo C.A. (Brazil)

- Chloroplast Membrane Lipids from *Coffea* sp. under Low Positive Temperatures
  Partelli F.L., Vieira H.D., Pais I.P., Quartin V.L., Campos P.S., Fortunato A., Eichler P., Viana A.P., Ribeiro A., Ramalho J.C. (Brazil)

Posters

- Variability in the Caffeine and Trigonelline Levels in Clones of Robusta Coffee
  Braghini C.A., Salva T.G., Braghini M.T., Silvarolla M.B., Fazuoli L.C. (Brazil)

- Genetic Variability of Agronomic Characteristics in 'Tupi Iac 1669-33' Progenies
  Petek M.R., Mistro J.C., Oliveira A.C.B., Fazuoli L.C., Gallo P.B., Braghini M.T. (Brazil)

- Initial Characterization of Durandé Hybrid F_3 Progenies
  Padilha L., Matiello J.B., Almeida S.R., Carvalho C.H.S., Ferreira R.A., Mendonça S.M., Filho S.L., Japiassu L.B., Santos A.C.R., Paiva R.N. (Brazil)

- Changes in Physiological Quality of *Coffea arabica* and *Coffea canephora* Seeds Submitted to Different Drying Rates
  Fraiz, A.C.R., Tonetti O.A.O, Anderson Cleiton J., Amaral da Silva E.A, Souza Eira M.T. (Brazil)

- Factors Demanding the Conservation of the Coffee Gene-Pool in Montane Rainforests of Ethiopia
  Hindorf H. (Germany)

- Genetic Divergence in Clones of *Coffea canephora* Variety Conilon by Different Methods of Multivariate Analysis
  Ferrão R.G., Ferrão M.A.G., da Fonseca A.F.A., Cruz C.D., Cecon P.R., Crescencio P.C.S. (Brazil)

- Microsatellite Markers from the Brazilian Coffee Genome Project
  Caixeta E.T., Pena G.F., Maciel-Zambolim E.,Missio R.F., Zambolim L., Sakiyama N.S. (Brazil)

- Híbrido de Timor: a Valuable Source of Genetic Variability for Arabica Coffee
  Sakiyama N.S., Caixeta E.T., Setotaw T.A., Zambolim E.M., Maciel-Zambolim L., Pereira A.A. (Brazil)

- Identification of Coffee Genotypes More Adapted to Areas with Water Deficit During Fall and Winter Time
  Carvalho C.H.S., Bento M.A., Matiello J.B., Almeida S.R., Ferreira R.A., Padilha L., Vilela A.F. (Brazil)

- Contribution of Changes in ABA Levels in Root and Leaves and Its Contribution to Drought Tolerance in *Coffea canephora*
  Silva V.A., Antunes W.C., Guimaraes B.L.S., Dimascio P., Damatta F., Almeida A.M., Loureiro M.E. (Brazil)

- Contribution of Root System to Adjustment of Photosynthetic Responses to Drought in *Coffea canephora*
  Silva V.A., Antunes W.C., Guimarães B.L.S., Cordeiro A.T., Damatta F., Almeida A.M., Loureiro M.E. (Brazil)

- PEG Osmotic Stress on Coffee Seedlings
  Rosa V.E., Almeida J.A.S., Gallo P.B., Colombo C., Gonçalves W., Ramos L.C.S. (Brazil)

- Analysis of Phenotypic Plasticity in Response to Water Constraints in Coffee Plants Growing Under Field Conditions
  Marraccini P., Rodrigues G.C., Rocha O.C., Guerra F.A., Andrade A.C., Leroy T., Pot D., Jourdan C., Gion J.M. (Brazil)

- Prospection of Tissue Specific Promoters in Coffee
  de Almeida J.D., Barros L.M.G., Santos D.B.M., Cotta M.G., Barbosa E.A., Cação S.B., Eira M.T.S., Alves G.S.C.. Vinecky F., Pereira L.F.P., da Silva F.R., Andrade A.C., Marraccini P., Carneiro M. (Brazil)

- Potential Utilization of Functional Molecular Markers as Predictors for Partial Resistance to Leaf Rust (*Hemileia vastatrix* Berk. & Br.) in Coffee (*Coffea arabica* L.)
  Herrera J.C., Romero G., de la Torre G., Alvarado G. (Colombia)

- Transcript Profiles in Compatible and Incompatible Host-Coffee Leaf Rust Interactions
  Cadenas F., Galeano N., O'Brien K., Yepes M., Geoffroy M., Buell R., Gaitan A. (Colombia)

- Differential Proteomic Analysis Indicates That Modulation in the Expression of Photosystem II Proteins May Be Involved in Differential Drought Response in *Coffea canephora* Genotypes
  Almeida A.M., Guimarães B.L.S., Soares C.Q.G., Silva V.A., Vieira M.A., Valente R.H., Baracat M.C., Fontes E.P.B., Loureiro M.E. (Brazil)

- Differential Root Proteomic Analysis Indicates Several Proteins Involved in Drought Stress Response in *Coffea canephora* Genotypes
  Almeida A.M., Soares C.Q.G., Guimarães B.L.S., Valente R.H., Vieira M.A., Soares E.A., Silva V.A., Araujo S.H.C., Silva V.A., Fontes E.P.B., Loureiro M.E. (Brazil)

- Photorespiration as a Drought Tolerance Mechanism in *Coffea canephora*
  Guimarães B.L.S., Soares C.Q.G., Silva V.A., Vieira M.A., Valente R.H., Baracat M.C., Fontes E.P.B., Loureiro M.E., Almeida A.M. (Brazil)

- *Galactinol synthase* Expresssion By Heat Stress in *Coffea arabica* L.
  Santos T.B., Marur C.J., Petkowicz C.L.O., Reicher F., Pereira L.F.P., Vieira L.G.E. (Brazil)

- In Silico Characterization and Transcription Analysis of Two Alpha-Expansins Isoforms in *Coffea arabica* L.
  Budzinski I.G.F., Pereira L.F.P., Sera T., Vieira L.G.E. (Brazil)

- Identification and Characterization of Gene Expression of Different Members of Snrk Kinase Family in *Coffea canephora* and *Coffea arabica*
  Paiva R.M.C., Freitas R.B., Fagundes F.J.F., Loureiro M.E. (Brazil)

- Identification and Characterization of Gene Expression of Three Coffee NCED Members under Drought
  Paiva R.M.C, Freitas R.B., Silva V.A., Lopes F.J.F., Loureiro M.E. (Brazil)

- Transcription Analysis of a Pectin Methylesterase Isoform in *Coffea arabica* L.
  Leite T.F., Budzinski I.G.F., Cação S.M.B., Pereira L.F.P., Vieira L.G.E. (Brazil)

- CoffEST is the Complete Resource for *Coffea* spp. EST Analysis
  Barbosa A.V., Sales R.M.O.B., da Silva F.R. (Brazil)

- Optimization of Protein Coffee Leaf Quantification by Fluorescent Immunodetection Method
  Soares E.A., Araujo S.H.C., Soares C.Q.G., Almeida A.M., Loureiro M.E. (Brazil)

- Coffee Beans Contents of Soluble Solids and Chlorogenic Acids in Stock Plants of Robusta
  Fazuoli L.C., Braghini C.A., Salva T.J.G., Braghini M.T., Silvarolla M.B., Mistro J.C., Gallo P.B. (Brazil)

- Use of Pichloram for Somatic Embryogenesis Induction in Coffee (*Coffea arabica* L.) Leaf Explants
  Ferreira M.R. de A., Ferreira J.M., Teixeira J.B. (Brazil)

- Optimization of Somatic Embryogenesis in Coffee (*Coffea arabica* L.) Leaf Explants
  Teixeira J.B., Junqueira C.S., Carvalho C.H.S. (Brazil)

- Effect of Peg on the Somatic Embryogenesis of *Coffea arabica* Genotypes
  Almeida J.A.S., Machado D.F.S.P., Silvarolla M.B., Machado E.C. (Brazil)

- Comparative Proteomic Analysis of *Coffea arabica* Somatic Embryos in the Different Stages of Embryogenesis
  Sato J.H., Teixeira J.B., Eira M.T.S., Franco O.L., Mehta A. (Brazil)

- Exsudate Coloring Test to Evaluate the Physiological Quality of Coffee Seeds
  Hilst P.C., Dias D.C.F.S., Mantovani-Alvarenga E., Dias L.A.S. (Brazil)

- Genetic Diversity of *Colletotrichum* Species Associated to Coffee in Colombia and Their Relationship to CBD Causing Isolates
  Rodriguez A., Loureiro A., Varzea V., Gaitan A. (Colombia)

- Differential Induction of Superoxide Dismutase in *Coffea arabica* - *Hemileia vastatrix* Interactions
  Guerra-Guimarães L., Cardoso S., Martins I., Loureiro A., Bernardes da Silva A., Varzea V.M.P., Silva M.D.C. (Portugal)

- Histological and Ultrastructural Characterization of Coffee Resistance to *Colletotrichum kahawae*
  Loureiro A., Silva M.D.C., Varzea V.M.P., Moncada P., Bertrand B., Nicole M. (Portugal)

- Defense-Related Gene Expression in Response to Leaf-Miner Infection
  Silvestrini M., Ramiro D.A., Guerreiro-Filho O., Maluf M.P. (Brazil)

- Biochemical Characterization of Resistant and Susceptible Coffee Plants to the Coffee Leaf Miner (*Leucoptera coffeella*)
  Maratarello F.H.H., Guerreiro-Filho O., Salva T.J.G., Maluf M.P. (Brazil)

- Peroxidase Activity as a Biochemical Marker for Resistance to the Coffee Wilt Disease
  Nakendo S., Lubega G.W., Kangire A.O., Musoli P. (Uganda)

Agronomy & Ecophysiology

Communications

- The Impact of Climatic Variability in Coffee Crop
  Camargo M.B.P., Marcelo B.P. (Brazil)

- Influence of Soil Properties on Cup Quality of Wild Arabica Coffee in Coffee Forest Ecosystem of SW Ethiopia
  Yadessa A., Burkhardt J., Denich M., Woldemariam T., Bekele E., Goldbach (Germany)

- Carbon Sequestration in an Agroforestry System of Coffee and *Mimosa scabrella* (Bracatinga) in Southern Brazil
  Caramori P.H., Carneiro Leal A., da Silva W., Morais H., Suano de Souza F., Androcioli Filho A. (Brazil)

- Relative Humidity of Coffee Canopy under Different Types of Pruning in Mococa, SP, Brazil
  Rolim G.S., Souza P.S., Pedro-Junior M.J., Gallo P.B., Pezzopane J.R.M. (Brazil)

- Water Relations of Ethiopian wild Coffee Populations: Genetic Fixation and Phenotypic Plasticity
  Beining A., Burkhardt J., Fetene M. (Germany)

- Life-Cycle Based Sustainability Indicators of the Brazilian Coffee
  Coltro L., Mourad A.L. (Brazil)

Posters

- Nutrient Utilizations of *Coffea arabica* Seedlings Under Integrated Use of Organic and Mineral Fertilizers
  Kufa T. (Ethiopia)

- Development and Evaluation of a Container with Perforated Wall in the Quality of the Coffee Seedling
  Silva V.A., Durante A.D., Poli L., Monteiro J.M., Ferreira E.C.(Brazil)

- Effect of Plant Density and Pruning on Yield of Compact Disease Resistant Coffee Variety
  Odeny D.A., Kimemia J.K. (Kenya)

- Advances in Coffee Nursery Management in Ethiopia
  Netsere N, Taye E., Kufa T., Shimber T. (Ethiopia)

- Regulating Quality of Shade Tree Mulch Into Soil to Control Nitrification in a Coffee Agroforestry
  Bako Baon J.B., Purwanto (Indonesia)

- Arbuscular Mycorrrhiza and Copper Uptake and Toxicity in Coffee Plants
  Andrade S.A.L., Mazzafera P., Schiavinato M.A., Silveira A.P. (Brazil)

- Arboreal Leguminous Windbreak as Bank of Beneficial Mites for Coffee Plants
  Rebelle Reis P., Zacarias M.S., de Carvalho T.M.B. (Brazil)

- Farmers' Empowerment for Sustainable Coffee Productivity and Quality: a Case Study of Kagera Region, Tanzania
  Ng'Homa N.M., Kilambo D.L., Teri J.M., Kaiza P.M.L. (Tanzania)

- Irrigated Coffee Flowering and Grain Maturation Uniformity as Affected by Controlled Water Stress in the Brazilian Cerrado Region
  Guerra A.F., Rodrigues G.C., Rocha O.C., Sanzonowicz C., Jerke C., Mera A.C., Cordeiro A. (Brazil)

- Improvement of Coffee Production System by Using Controlled Water Stress and Phosphorous Application in the Cerrado and South of Minas Gerais State Regions
  Guerra A.F., Rocha O.C., Rodrigues G.C., Sanzonowicz C., Ribeiro Filho G.C., Mera A.C., Cordeiro A. (Brazil)

- Water Deficit in Arabica Coffee Trees as Affected by Irrigation Regimes in the Cerrado Region
  Rocha O.C., Guerra A.F., Rodrigues G.C., Sanzonowicz C., Jerke C., Mera A.C., Cordeiro A. (Brazil)

- Temporary Shading of Coffee (*Coffea arabica*) Plantations with Pigeonpea (*Cajanus cajan*) in Southern Brazil
  Morais H., Caramori P.H., Hugo R.G., Androcioli Filho A. (Brazil)

- Technological Change and Development of Coffee Plantation: a Case Study of Kodagu District
  Kiran K.B. (India)

- Determining the Critical Period of Weed Competition in Young Clonal Robusta Coffee in Ghana
  Oppong F.K., Opoku-Ameyaw K., Owusu-Ansah F. (Ghana)

- Search for Suitable Coffee Planting Materials for Coffee Farmers in Ghana: Effects of Improved Robusta Coffee Clones on Soil Properties
  Ofori-Frimpong K., Afrifa A.A., Acquaye S., Anim-Kwapong E. (Ghana)

- Evaluation of Some Robusta Coffee (*Coffea canephora* Pierre ex A. Froehner) Clones for High Density Planting in Ghana
  Anim Kwapong G.J., Anim-Kwapong E., Oppong F.K. (Ghana)

- Characteristics of *Coffea canephora* and *Coffea arabica* Production in West Region of Sao Paulo State, Brazil
  Bliska F.M.M., Vegro C.L.R., Pereira S.P., Giomo G.S. (Brazil)

- Application of Urea with Urease Inhibitor in Young Coffee Plants
  Alvarenga Garcia A.L., Garcia A.W.R., Padilha L. (Brazil)

- Weed Control on Coffee Crop by Glyphosate Applied Through Controlled Droplet Application System (Micron-Herbi)
  de Alcântara E.N., Cunha R.L., (Brazil)

- Recycled Coffee Wastes as Potential Replacements of Inorganic Fertilisers for Coffee Production
  Chemura A., Mandhlazi R, Mahoya C. (Zimbabwe)

- Use of Precision Agriculture Techniques to Produce High Quality Coffee
  Queiroz D.M., Pinto F.A.C., Alves E.A. (Brazil)

- Arborized Coffee Crop in São Paulo State, Brazil: Microclimatic, Phenological and Agronomic Evaluations
  Pezzopane J.R.M., Pedro Junior M.J., Gallo P.B., de Camargo M.B.P., de Souza P.S., Rolim G.S. (Brazil)

- Evaluation of Productivity and Quality of Coffee Growing in Cerrado (Brazilian Savannah) Conditions and Irrigated Using the Drip System
  Fernandes A.L.T., Rabelo P.V., Drumond L.C.D., de Oliveira Silva R. (Brazil)

- Effective Intercropping for Rehabilitating Old Unproductive *Coffea arabica* (Linneaus) on Mambilla Plateau, Nigeria
  Omolaja S.S., Iremiren G.O. (Nigeria)

- Effect of Training and Pruning Practices on Yield and Growth Parameters of Coffee (*Coffea arabica* L.) in Southwestern Ethiopia
  Taye E., Yilma A., Netsere A., Kufa T., Gebrewold S., Shimber T., Asaw F. (Ethiopia)

- Effect of Different Indigenous Shade Trees on the Quality of Wild Arabica Coffee in the Afromontane Rainforests of Ethiopia
  Yadessa A., Burkhardt J., Denich M. Woldemariam T., Bekele E., Goldbach H. (Ethiopia)

- Use of Shade Trees in Coffee as a Green House Gas Mitigation Strategy
  Kimemia J.K., Odeny D.A. (Kenya)

- Test of an Agrometeorological Model for Estimating the Beginning of the Flowering Period for Coffee Crop
  Zacharias A.O., Camargo M.B.P. (Brazil)

- Climate Change and Coffee Sustainability in Tanzania: Use of Historical Data Trends for Impact Assessment
  Maro G.P., Teri J.M. (Tanzania)

- Air Temperature in *Coffea arabica* Microclimate Arborized with Dwarf Coconut Palm and Rubber Tree in Mococa, SP, Brazil
  Camargo M.B.P., Rolim G.S., Souza P.S., Gallo P.B. (Brazil)

- Maximum Global Irradiance and Photosynthetically Active Radiation Intercepted by Coffea arabica Canopy Under Different Types of Pruning in Mococa SP, Brazil
  Rolim G.S., Souza P.S., Pedro-Junior M.J., Gallo P.B., Pezzopane J.R.M. (Brazil)

- Air Temperature of Coffee Canopy Before and After Different Types of Pruning in Mococa SP, Brazil
  Rolim G.S., Souza P.S., Pedro-Junior M.J., Gallo P.B., Pezzopane J.R.M. (Brazil)

- Relative Humidity in *Coffea arabica* Microclimate Arborized with Dwarf Coconut Palm and Rubber Tree at Mococa, SP, Brazil
  Rolim G.S., Camargo M.B.P., Souza P.S., Gallo P.B. (Brazil)

- Global Solar Radiation in *Coffea arabica* Microclimate Arborized with Dwarf Coconut Palm and Rubber Tree in Mococa, SP, Brazil
  Rolim G.S., Souza P.S., Camargo M.B.P., Gallo P.B., Slava T.J.G. (Brazil)

- Evapotranspiration Components and Dual Crop Coefficients of Coffee Trees during Crop Production
  Flumignan D.L., Faria R.T. (Brazil)

- Estimation of Duration of the Flowering-Maturation Stage for Different Arabic Coffee Cultivars
  Nunes F.L.N., Camargo M.B.P., Fazuoli L.C., Pezzopane J.R.M. (Brazil)

- Vegetative Development and Leaf Water Potential of Irrigated Coffee Tree, cv Catuaí, Cultivated under Different Densities of Plantation in Mococa, SP
  Sakai E., Queiroz V., Kobayashi E.S., Souza P.S., Silveira J.M.C., Pires R.C.M., Arruda F.B. (Brazil)

- Agrometeorological Parameters for Prediction of Sucrose Content in Developing Fruits of Different Cultivars of Arabica Coffee
  Pezzopane J.R.M., Salva T.J.G., de Lima V.B., Fazuoli L.C. (Brazil)

- Certification of Forest Coffee in South-Western Ethiopia: Local Level Performance and Challenges
  Stellmacher T., Volkmann J., Grote U. (Germany)

- Commercialization Analysis of Organic and Traditional Coffee Farming in Londrina – PR, Brazil
  Miglioranza R., Massarutti J., Miglioranza E. (Brazil)

- Implementation of an Institutional Coffee Quality Assurace Strategy at the Farm Level in the Quindío Region of Colombia
  Jaramillo O., Duque J. (Colombia)

- Coffee Seed Conservation in Penta Packing
  Santanna L.A.R., Guimaraes G.O.F., Reis M. (Brazil)

- Morphological Aspects of *Coffea arabica* L. cv. Mundo Novo Seed Development
  Crisafulli P., Silizio F., Mistro J.C., Micali F., Pallavicini A., Dentan E., Graziosi G. (Italy)

- Coffee Quality Assessment: the Case of Two Kenyan Cultivars, Ruiru 11 and Sl 28
  Omondi C.O. (Kenya)

Pathology Management & Plant Protection

Communications

- Recent Advances in Genetic and Biological Solutions to Reducing Biotic Stress Factors to Coffee Production in Latin America
  Bustamante A.G. (Colombia)

- White Stem Borer Resistance in Coffee: Perspectives on Breeding, Management and Consumption
  Ram A.S., Sabir R.K., Mythrasree S.R., Seetharama H.G., Rao R.V., Jayarama (India)

- Characterization of Variability on *Colletotrichum kahawae* Isolates from Angola and Search for Resistance on Angolan Coffee Germplasm
  Manuel L., Neves-Martin J.M., Talhinas P., Varzea V.M.P. (Portugal)

- Variation of *Colletotrichum kahawae* Isolates from Diseased Cherries of Montane Rainforest Coffee in Ethiopia
  Zeru A., Assefa F., Adugna G., Hindorf H. (Ethiopia)

- Disease Resistance and Cup Quality in Arabica Coffee: the Persistent Myths in the Coffee Trade versus Scientific Evidence
  Van der Vossen H. (The Netherlands)

- Coffees of Brazil Network: Participative Building of Knowledge within the Agroindustrial Coffee System
  Pereira S.P., de Aguiar C.M.G., Leme P.H. (Brazil)

Posters

- Coffee Leaf Rust (*Hemileia vastatrix*) in Wild Forest Coffee Accessions and Released Varieties in Southwestern Ethiopia
  Jefuka C., Adugna G., Hindorf H., Fininsa C.H. (Ethiopia)

- Effects of Climate Changes on Coffee Diseases in Minas Gerais State, Brazil
  Chalfoun S.M., Xavier E.P., Pereira M.C. (Brazil)

- Leaf Rust Resistance Heterosis and Gene Accumulation in Arabica Coffee Hybrids
  Fazuoli L.C., Braghini M.T., Mistro J.C., Petek M.R. Silvarolla M.B. (Brazil)

- Simultaneous Control of Pests and Diseases in Coffee Plants
  Rebelles Reis P., de Souza J.C. (Brazil)

- Evaluation of Natural Products for the Control of Coffee Leaf Rust, *Hemileia vastatrix* Berkeley & Brues
  Kutywayo D., Mtetwa N., Nzarayebani D.H. (Zimbabwe)

- Evaluation of the Effect of the Fungicide Priorixtra (Azoxystrobin + Cyproconazole) on the Control of Leaf Rust and Cercospora of Irrigated Coffee Fields, Irrigated by the Net Sprinkler Irrigation System
  Fernandes A.L.T., Becker C.A.V., de Oliveira Silva R., Becker G. (Brazil)

- Efficiency of Cyproconazole + Thiamethoxan, Within a Program of Use, Applied Via Drip Irrigation Water in the Control of Coffee Leaf Rust
  Fernandes A.L.T., Becker C.A.V., de Oliveira Silva R. (Brazil)

- Early Haustoria Development in Coffee (*Coffea arabica*) - Rust (*Hemileia vastatrix*) Interaction
  Ramiro D.A., Escoute J., Petitot A.-S., Maluf M., Fernandez D. (France)

- Effectiveness of the Brocap Trap in Controlling the Coffee Berry Borer (*Hypothenemus hampei* Ferr.) in Indonesia
  Wiryadiputra S., Cilas C., Morin J.-P. (Indonesia)

- The Coffee Berry Borer (*Hypothenemus hampei*) Distribution and the Effect of Coffee Management Practices in Kenya
  Mugo H.M. (Kenya)

- Resistance to Leaf Rust on Coffee Cultivars Developed at Instituto Agronômico do Paraná
  Sera G.H., Sera T., Ito D.S., Fonseca I.C. de B., Kanayama F.S., Del Grossi L., Marana F.L., Shigueoka L.H. (Brazil)

- Heterosis and Simultaneous Resistance to Rust and Leaf Miner in Hybrids Carrying *Coffea racemosa* Genes
  Ito D.S., Sera T., Sera G.H., Santagio D.C., Del Grossi L., Marana F.L., Shigueoka L.H., Kanayama F.S. (Brazil)

- Coffee Disease Risk Analysis: How Epidemiology Knowledge Could Help in Assessing and Preventing Disease Invasion
  Bieysse D., Cilas C., Mouen J., Musoli P., Avelino J. (France)

Breeding for Disease Resistance

Communications

- The Use of Sarchimor Derivatives in Coffee Breeding Resistance to Leaf Rust
  Varzea V.M.P.,Marques D.V., Pereira A.P., Silva M.C. (Portugal)

- Pathogenicity of *Colletotrichum kahawae* Strains and their Effect on Resistant Arabica Coffee Varieties in Tanzania
  Kilambo, D.L., Guerra-Guimarães L., Varzea V., Loureiro A., Silvia M., Mabagala R.B., Mtenga D.J., Masumbuko L., Teri J.M. (Tanzania)

- Effect of Temperature and Rainfall Variations on Coffee Berry Disease (*Colletotrichum kahawae*)
  Mouen Bedimo J.A., Bieysse D., Cilas C., Notteghem J.-L. (Cameroon)

- Recent Leads in Breeding and Field Strategies Adopted for Achieving Durable Rust Resistance in Coffee (Coffea arabica) in India
  Surya Prakash N., Sundaresha, Roohe K., Muniswamy B., Bhaskar Reddy K., Bhat S.S., Sreenath H.L., Santaram A., Srinivasan C.S., Ramamurthy N., Jayarama (India))

- Multiple Resistances to Coffee Berry Disease, Coffee Wilt and Leaf Rust in *Coffea arabica* Populations
  Adugna G., Jefuka C., Teferei D. Zeru A. (Ethiopia)

- Variation in Resistance to Coffee Berry Disease (*Colletotrichum kahawae*) Among Germplasm Progenitors in Tanzania
  Mtenga D.J., Reuben S.O.W.M., Varzea V.M.P., Pereira P., Kilambo D.L., Teri J.M. (Tanzania)

- Towards a Variety Resistant to Coffee Wilt Disease (CWD): a Case for Robusta Coffee (*Coffea canephora*) in Uganda
  Musoli P., Kangire A., Leroy T., Nabaggala A., Nakendo S., Olal S., Ochugo J., Kabole C., Pande J., Cilas C., Charrier A., Bieysse D., Ogwang J.A., Kyetere D.T. (Uganda)

Posters

- Variability of *Hemileia vastatrix* from Coffee Ecosystems and Resistance of Hibrido de Timor Derivatives to Aggressive Races
  Kilambo D.L., Varzea V.M.P., Mtenga D.J., Pereira P.P., Jamaes E.T., Masumbuko L.I. (Tanzania)

- Cultivars Evaluation *Coffea arabica* to the Parasitism of *Meloidogyne exigua*
  Garcia A.L.A., Campos V.P., Padilha L. (Brazil)

Breeding & Diversity

Communications

- Coffee Genetic Resources Under Severe Threat from Genetic Erosion in the Centres of Origin and Diversity: an Urgent Need for Conservation Measures
  Bayetta B., Sacko J.C. (Ivory Coast)

- Chemometric Discrimination of Coffee (*Coffea arabica* L.) Genotypes and Growing Origins
  Villareal D., Bertrand B., Laffargue A., Posada H., Lashermes P., Dussert S. (Colombia)

- Yield Stability Over Several Years in *Coffea canephora*: Definition of Synthetic Traits and Longitudinal Data Analyses
  Cilas C., Montagnon C. (France)

- Assessment of the Genetic Diversity of Wild Coffee Populations from Southeastern Ethiopia Using ISSR Marker
  Tesfaye K., Balemi S., Bekele E., Govers K., Borsch T. (Ethiopia)

- Heterosis and Combining Ability for Yield and Yield Related Traits in Arabica Coffee (*Coffea arabica* L.)
  Wassu M., Bayetta B., Singh H.. (Ethiopia)

- Evaluation of Conilons for Genetic Diversity, Cup Quality and Biochemical Composition
  Lambot C., Crouzillat D., da Fonseca A.F.A.,, Leloup V., Haiduc A., Broun P., Pétiard V., Ferrão M.G.A., Ferrão R.G. (France)

- Analysis of Organic and Traditional Coffee Farming in Londrina
  Miglioranza R., Massarutti J., Miglioranza E. (Brazil)

Posters

- Morpho-Agronomic Characterization of Seedlings of Wild *Coffea arabica* Accessions Under Controlled Conditions in Southwestern Ethiopia
  Kufa T., Burkhardt J. (Ethiopia)

- Study on the *Coffea arabica*/*Colletotrichum kahawae* Pathosystem. Impact of a Natural Elicitor (FEN 560) on this Pathosystem
  Durand N., Bertrand B., Guyot B., Guiraud J., Fontana-Tachon A. (France)

- Development of Selection Indices Based on Morphological Traits and Average of the First Two Years Yield at Early Bearing Stage of *Coffea arabica* L.
  Ermias H., Bayetta B., Singh H. (Ethiopia)

- Summary of a Decade of South Ethiopian Coffee Improvement Activity at Awada Coffee Research Center: Fruit of the Landrace Arabica Coffee Variety Development Strategy
  Mesfin K., Addisu T., Ketema K. (Ethiopia)

- Agronomic Characterization of *Coffea canephora* Germplasm
  Ferrão M.A.G., Ferrão R.G., da Fonseca A.F.A., Riva-Souza E.M., Volph P.S., Verdin Filho A.C. (Brazil)

- Agronomic Performance of Short Plant Arabica Coffee Progenies
  Mistro J.C., Fazuoli L.C., Petek M.R., Oliveira A.C.B., Braghini M.T., Gallo P.B. (Brazil)

- Selection of Robusta Coffee Stock Plants at Mococa, Brazil
  Fazuoli L.C., Mistro J.C., Braghini M.T., Gallo P.B. (Brazil)

Advanced Biology Applied to Coffee Research - Current Status and Future Perspectives

A.C. ANDRADE

Laboratory of Molecular Genetics (LGM-NTBio), Embrapa Genetic Resources and Biotechnology, C.P. 02372, 70770-900 Brasília-DF, Brazil

SUMMARY

The coffee sector has faced innumerous challenges in the past decades and the outcome just highlighted its great economic and social importance worldwide, as well as, its strength. Many other challenges are ahead, which poses impacts on coffee production and may affect quality, such as the potential climate changes, the competition for restricted arable lands with other non-food crops (*e.g.* bioenergy) and the limited availability of natural resources (*e.g.* water) to sustain productivity, among others. Therefore, to couple with these new challenges there is a need for development of advanced genomic tools to support and accelerate breeding in order to ensure a sustainable coffee production in the near future. Rapid genome sequence information provided by the new generation sequencing platforms coupled with the advancement of other technologies for transcription profiling, proteomics and metabolomics, may lead coffee researchers to a better understanding of important biological processes such as abiotic and biotic stress resistance, coffee fruit development and the determinism of quality. This improved knowledge might allow coffee breeders the access to previously uncharacterized sources of genetic variation and the use of these coffee genetic resources for the rapid development of improved cultivars in terms of quality and other agronomical characteristics, providing coffee production with added value, as well as reduced economic and environmental costs. Some results highlighting the current status of coffee genomics research and future perspectives will be presented and discussed.

INTRODUCTION

Amongst more than one hundred coffee species identified, *Coffea arabica* is the only tetraploid and the major cultivated coffee species (Maurin et al., 2007). This ploidy level has hampered the exploitation of the existing genetic diversity in Arabica breeding programs as the other cultivated (*C. canephora*) and non-cultivated wild-species are diploid and self-incompatible. As a result, the transfer of genetic traits from wild outbred species of the genus *Coffea* to *C. arabica* cultivars is quite difficult. Furthermore, coffee is a perennial crop with a lengthy period for fruit development and bean-to-bean generation time and thus, a new coffee cultivar takes about 28 years to be generated by conventional breeding (Carvalho, 1998). Establishing more efficient methods to support coffee breeding is a key component to ensure the sustainability of the coffee sector. Advances in genomic technologies may provide the tools to shorten the time required for coffee improvement and may offer feasible strategies to decipher the genetic and molecular basis of important biological traits in coffee species that are relevant to growers, processors, and consumers.

COFFEE EST RESOURCES

Since the first repot of a coffee cDNA sequence encoding a metallothionein I-like protein (Moisyadi and Stiles, 1995), the number of entries of coffee nucleotide sequences on public databases has significantly increased. Only recently, large sets of coffee EST data have become available and the large expressed sequence tag (EST) collections (single-pass cDNA sequences) that have been generated for *C. arabica* (>300,000 ESTs) and *C. canephora* (>55,000 ESTs), by different groups, indicates that considerable information has been generated in the last few years. The largest set of ESTs (46,914) released so far, was produced mainly from developing seeds of *C. canephora* (Lin et al., 2005). In addition, two other *C. canephora* EST sequence sets were developed from mRNA isolated from leaves and fruits at different development and maturation stages with 8,778 valid EST sequences (Poncet et al., 2006). All together, a significant set of 55,692 ESTs is already publicly available for *C. canephora*. Large-escale EST sequencing from *C. arabica* and other species has also been reported (Vieira et al., 2006). The Brazilian Coffee EST Project has generated single-pass sequences of a total of 214,964 randomly picked clones from 37 cDNA libraries of *C. arabica*, *C. canephora*, and *C. racemosa*, representing specific stages of cells and plant development that after trimming resulted in 130,792, 12,805, and 10,510 good quality sequences for each species, respectively (Vieira et al., 2006). Coffee EST resources have also been developed by the Cenicafe research group in Colombia, which have in their database, to date, 32,000 coffee EST sequences from 22 libraries organized in 9,257 *C. arabica* and 1,239 *C. liberica* unigenes (Cristancho et al., 2006). In addition, the Cenicafe database contains 6,000 *Beauveria bassiana* and 4,000 *H. hampei* (coffee berry borer) EST sequences. Additionally, around 4,000 coffee EST sequences from leaves infected with *H. vastatrix* (Fernandez et al., 2004), from leaves and embryonic roots (De Nardi et al., 2006) and for EST-SSR mining (Aggarwal et al., 2007), have also been reported. All these coffee EST data are organized in different databases with different bioinformatics resources (Lashermes et al., 2008).

These large EST data from coffee are valuable tools for discovering genes (several novel genes have already been already identified) and developing EST-SSR and SNP markers, providing the basis for mapping and the establishment of breeding programs based on marker-assisted selection (MAS). Furthermore, an EST data set allows the development of novel tools such as microarrays (Alba et al., 2004). Initiatives for the generation of these arrays have already begun and some results have started to appear (De Nardi et al., 2006; Privat et al., 2008). New generation sequencing technologies might also be an interesting approach to perform transcriptome profiling of coffee (Shendure and Ji, 2008). Similarly to SAGE (serial analysis of gene expression) shotgun libraries derived from mRNA or small RNAs are deeply sequenced using these novel sequencing technologies, and, the counts (tags) corresponding to individual genes can be used for quantification.

The access to the available coffee EST sequence information has allowed researches to identify candidate genes of interest, transposable elements (Lopes et al., 2008) and perform expression studies by conventional techniques such as Northern Blot or real-time quantitative qRT-PCR. These studies resulted in the identification of genes involved in response to biotic stress such as infection to the rust fungus (*H. vastatrix*) (Fernandez et al., 2004; Ganesh et al., 2006; Petitot et al., 2008; Andrade, 2008) and coffee leaf miner (Mondego et al., 2005); to abiotic stress such as drought (Marraccini et al., 2008); fruit development and maturation (Hinniger et al., 2006; Simkin et al., 2006; Bustamante et al., 2007; Salmona et al., 2008), and several studies of particular biosynthetic pathways such as sugar (Geromel et al., 2006, 2008, 2008a; Privat et al., 2008a), chlorogenic acids (Campa et al., 2003; Mahesh et al., 2006, 2007;

Lepelley et al., 2007), caffeine (Koshiro et al., 2006), carotenoids (Simkin et al., 2008), storage proteins and galactomannans (Rogers et al., 1999; Marraccini et al., 1999, 2001, 2005; Acuña et al., 1999, Pré et al., 2008). As the cDNA clones were available heterologous expression for structure determination of key proteins also became possible (McCarthy et al., 2008). Further characterization of gene networks in coffee plants will help us to identify new targets for manipulation of physiological, biochemical, and developmental processes of this very important crop species.

MARKER DEVELOPMENT AND GENETIC MAPPING

The detection of polymorphisms at the coffee DNA level, has been performed using a variety of techniques, including randomly amplified polymorphic DNA (RAPD) (Orozco-Castilho et al., 1994; Lashermes et al., 1996; Agwanda et al., 1997; Anthony et al., 2001; Aga et al., 2003; Silvestrini et al., 2008), cleaved amplified polymorphisms (CAP) (Lashermes et al., 1996a; Orozco-Castilho et al., 1996), restriction fragment length polymorphisms (RFLP) (Paillard et al., 1996; Dussert et al., 2003; Lashermes et al., 1999), amplified fragment length polymorphism (AFLP) (Lashermes et al., 2000; Anthony et al., 2002; Coulibaly et al., 2003; Prakash et al., 2004, 2005), inverse sequence-tagged repeat (ISR) (Aga and Bryngelsson, 2006), and simple sequence repeats or microsatellites (SSR) (Mettulio et al., 1999; Combes et al., 2000; Baruah et al., 2003; Moncada and McCouch, 2004; Poncet et al., 2004). As coffee expressed sequence tag (EST) databases became available, the number of markers has been considerably increased by SSR mining (Bhat et al., 2005; Poncet et al., 2006, 2007; Aggarwal et al., 2007; Pinto et al., 2007; Cubry et al., 2008) in these databases. The identification of these molecular markers are already generating applied results with markers linked to important traits such as leaf rust resistance (Mahe et al., 2008), resistance to coffee leaf miner (Pinto et al., 2007) and caffeine content (Priolli et al., 2008).

Due to the allogamous nature of *C. canephora* and the higher polymorphic populations as compared to *C. arabica*, the first attempt to develop a linkage map was based on Canephora doubled haploid (DH) segregating populations (Paillard et al., 1996). However the first genetic map of *C. canephora* spanning the eleven linkage groups was reported latter (Lashermes et al., 2001). This genetic linkage map comprised more than 40 specific STS markers, either single-copy RFLP probes or SSRs that constituted an initial set of standard landmarks of the coffee genome which have been used as anchor points for map comparison (Herrera et al., 2002) and coverage analysis of bacterial artificial chromosome (BAC) libraries (Leroy et al., 2005, Noir et al., 2004). More recently, Crouzillat et al. (2004) reported the development of a Canephora consensus genetic map. Backcross genetic maps were established for each parent (i.e., elite clones BP409 and Q121) and then a consensus map was elaborated. More than 453 molecular markers such as RFLP and SSRs were mapped covering a genome of 1,258 cM. Recently, this map was used to map COS (i.e. Conserved Orthologous sequence) markers and perform comparative mapping between coffee and tomato (Wu et al., 2006).

The low polymorphism of *C. arabica* has been a major hurdle for developing genetic maps. Hence, only partial maps have been reported so far (Prakash et al., 2004; De Oliveira et al., 2007). Nevertheless, Pearl et al. (2004) recently obtained a genetic map from a cross between Catimor and Mokka cultivars of *C. arabica*. In parallel several diploid interspecific maps were built. Those maps are based on either an F1 hybrid population resulting from a cross between coffee diploid species (López and Moncada, 2006) or progenies obtained by backcrossing of hybrid plants to one of the parental species (Ky et al., 2000; Coulibaly et al., 2003).

BAC LIBRARIES AND PHYSICAL MAPPING

As an essential tool for genome research, bacterial artificial chromosome (BAC) libraries have been reported for both cultivated *C. arabica* and *C. canephora* species. Arabica BAC libraries were constructed from the cultivar IAPAR 59 (Noir et al., 2004) and Timor Hybrid 832/2 (Pereira et al., 2008). A Canephora BAC library (Leroy et al., 2005) was developed on a relatively good cup quality genotype (e.g. clone 126). In addition, two large insert BAC libraries (Eco RI and Hind III) for the doubled haploid *C. canephora* genotype that was previously genetically mapped, are under construction at the Arizona Genomics Institute (AGI) with the aim of making it available for the whole coffee scientific community at a cost recovery base (Lashermes, personal communication). Integration of physical and genetic mapping information has been limited so far and restricted to localized physical maps based on BAC contigs corresponding to agronomical important disease resistance genes (Lashermes et al., 2004).

MOLECULAR CYTOGENETICS

The use of fluorescent methods to study coffee chromosomes has also been reported. In particular, genomic *in situ* hybridization (GISH) and fluorescence *in situ* hybridization (FISH) were successfully applied. The origin of the *C. arabica* genome was confirmed by GISH using simultaneously labeled total genomic DNA from the two potential genome donor species as probes (Raina et al., 1998; Lashermes et al., 1999). Recent cytogenetic analyses have confirmed that *C. arabica* is not a segmental allopolyploid, but a true allotetraploid species (Clarindo and Carvalho, 2008). Furthermore, FISH and BAC-FISH procedure was used as a tool for introgression analysis and chromosome identification (Barre et al., 1998; Lombello and Pinto-Maglio, 2004; Herrera et al., 2007).

THE CHLOROPLAST GENOME

The complete sequencing of the coffee chloroplast genome reported recently (Samson et al., 2007), is another very important achievement for the coffee scientific community. The genome is 155 189 bp in length, including a pair of inverted repeats of 25 943 bp. Of the 130 genes present, 112 are distinct and 18 are duplicated in the inverted repeat. The coding region comprises 79 protein genes, 29 transfer RNA genes, four ribosomal RNA genes and 18 genes containing introns (three with three exons). The available sequence provides the tools for the molecular characterization of the existing genetic diversity of coffee species and evolutionary studies (Maurin et al., 2007). Certainly, chloroplast sequence data will be crucial for accessing the *Coffea* diversity (Bremer and Jansen, 1991; Cros et al., 1998; Maurin et al., 2007) and the accurate determination of the true mother plant of the natural hybrid *C. arabica* (Tesfaye et al., 2007). In addition, the availability of the complete chloroplast genome of coffee provides regulatory and intergenic spacer sequences that might be used in chloroplast genetic engineering, opening a new venue up for coffee improvement (Samson et al., 2007).

GENETIC TRANSFORMATION

The first genetic transformation of coffee cells reported (Barton et al., 1991) was by protoplast electroporation. Genetic transformation with *Agrobacterium* sp. has also been reported (Feng et al., 1992; Freire et al., 1994). The regeneration of transgenic coffee trees was first obtained by the transformation of somatic embryos via *A. rhizogenes* (Spiral et al., 1993; Sugiyama et al,. 1995; Kumar et al., 2006), and later on via *A. tumefaciens* (Hatanaka et al., 1999, Leroy et al., 2000; Cruz et al., 2004). Transient expression of genes was also

achived by particle bombardment (Van Boxtel et al., 1995; Fernandez-Da Silva and Yuffá, 2003; Rosillo et al., 2003). More recently, stable transformation of *Coffea canephora* was also obtained by particle bombardment of embryogenic tissue (Cunha et al., 2004; Ribas et al., 2005). The first report of a genetically modified coffee plant for expression of an agronomic trait (resistance to coffee leaf miner) was obtained using a synthetic *cry*1Ac gene which was introduced into three coffee genotypes from the two cultivated species, *C. arabica* and *C. canephora*, by means of *A. tumefaciens* transformation. A pluriannual field experiment was established in French Guiana with some of these transformed plants aiming at evaluating the resistance of the transformed *C. canephora* 126 to *L. coffeella*. This was the first field trial of a transformed *Coffea* sp. and one of the first involving a transformed perennial tropical crop after *Carica papaya*. Results indicated that the strategy used was able to confer a stable resistance against the coffee leaf miner (*L. coffeella*) in the majority of independent transformed clones of *C. canephora* differing by the copy number and the location of the insert (Perthuis et al., 2005). Nowadays, genetic transformation of coffee plants has been successfully achieved by several research groups (Ogita at al., 2003, 2004; Ashihara et al., 2006; Ribas et al., 2006; Canche-Moo et al., 2006; Kumar et al., 2007; Arroyo-Herrera at al., 2008). However, despite significant advances over the last 15 years, coffee transformation is still very laborious, with bottlenecks in the methodology that make it far from a routine laboratory technique (Berthouly and Etienne, 2000; Etienne, 2005). Recent efforts have been dedicated to the establishment of efficient and rapid regeneration of transformed roots with *A. rhizogenes* for quick validation and functional analysis of nematode resistance genes (Alpizar et al., 2006, 2008).

MASS SPECTOMETRY

The recent advances in the mass spectrometry technologies are already revolutionizing the field of proteomics and metabolomics. These new mass spectrometers have provided scientists the required tools to identify and characterize proteins and metabolites at an unprecedented pace and accuracy. Application of these technologies on coffee research will make possible to scientists the dissection of complex biochemical pathways, the nature and importance of post-transcriptional modifications related to a range of different biological processes. Furthermore, the integrated analysis of transcription, protein and metabolite profiling of important biological processes of coffee such as fruit development and maturation is an area of required fundamental research to uncover key proteins, pathways and metabolites that will be essential for further studies on genotype assessment at different environments. This knowledge is a building block for fast development of superior cultivars with not only desired agronomic characteristics such as tolerance to abiotic and biotic stress, but also with appropriate chemical composition for good quality. The use of these advanced technologies on coffee research has just begun (Silva et al., 2006; Perrone at al., 2008; Mendonça et al., 2008) and its application as a high-throughput phenotyping platform for future genetic association studies of quality and other important traits has an enormous potential to support precision breeding on coffee.

NEW GENERATION SEQUENCING TECHNOLOGIES

Over the past three years, massively parallel DNA sequencing platforms have become widely available and are rapidly evolving, increasing the output of data and reducing the cost of DNA sequencing by several orders of magnitude. Next-generation DNA sequencing has the potential to dramatically accelerate biological and biomedical research, by enabling the comprehensive analysis of genomes, transcriptomes and interactomes to become inexpensive, routine and widespread, rather than requiring significant production-scale efforts (Shendure

and Ji, 2008). Next-generation sequencing is applied for a variety of goals. Important applications include: (i) full-genome resequencing or more targeted discovery of mutations or polymorphisms; (ii) mapping of structural rearrangements, which may include copy number variation, balanced translocation breakpoints and chromosomal inversions; (iii) 'RNA-Seq', analogous to expressed sequence tags (EST) or serial analysis of gene expression (SAGE), where shotgun libraries derived from mRNA or small RNAs are deeply sequenced and the counts corresponding to individual species can be used for quantification. (iv) large-scale analysis of DNA methylation, by deep sequencing of bisulfite-treated DNA; (v) 'ChIP-Seq', or genome-wide mapping of DNA-protein interactions, by deep sequencing of DNA fragments pulled down by chromatin immunoprecipitation. Over the next few years, the list of applications will undoubtedly grow, but coffee research will benefit most with the use of these technologies to fully explore existing natural variability for polymorphism discovery and genotyping of segregating populations for mapping and association studies. In addition transcription profiling using these novel technologies seems the method of choice in the near future. However, for most applications of the new-generation sequencing technologies mentioned above, a reference sequence of the complete coffee genome is required and achieving this goal should have high priority of the entire coffee community.

THE ICGN INITIATIVE TOWARDS SEQUENCING THE COFFEE GENOME

The International Coffee Genome Network (ICGN) is a worldwide network of scientists from universities, research institutes and industry within the coffee producing and coffee consuming countries (http://www.coffeegenome.org/). The ICGN is committed to advancing coffee genomic research through international partnerships for sustainable coffee production worldwide. This collaborative network is focused on building the foundation for advancing agricultural research for coffee by developing genomic tools and resources to further our understanding of the coffee genome at the molecular, biochemical, and physiological levels. The ICGN was established to facilitate and to coordinate international efforts and to integrate existing international resources, as well as to develop a comprehensive strategy to sequence the coffee genome and to explore international funding to support this important Initiative for the coffee scientific community. A white paper describing this initiative will be available at the ICGN website (http://www.coffeegenome.org/communications/).

PERSPECTIVES

We are in a transition period for research into plant genetics and metabolism in which the ongoing development and integration of various "omics-technologies" with established biochemical, molecular and genetic approaches have created a near perfect storm for plant biology research. These combined tools through integrated efforts of genomics research and breeding will certainly be essential to quickly overcome practical problems faced by the coffee agribusiness such as control of pre- and post-harvest physiological factors involved in quality, disease, and pest control and management of plant responses to environmental changes (e.g., limited water availability and adverse temperatures). However, an important requirement to perform these integrated analyses is the full knowledge of the genome sequence (Committee on the National Plant Genome Initiative: Achievements and Future Directions, National Research Council, 2008) and with the aid of the new-generation sequencing technologies, projects on a number of crop species are on the way to achieve this goal (http://www.ncbi.nlm.nih.gov/genomes/PLANTS/PlantList.html). In the same direction, the recent efforts to set an international commitment up, with support and endorsement of all segments of the coffee sector through ICGN (http://www.coffeegenome.org/), in order to work jointly for the development of pre-competitive common sets of genomic tools, plant

populations, and concepts are timely needed and welcome for such an economically and socially important perennial crop like coffee.

REFERENCES

Acuña R., Bassüner R., Beillinson V., Cortina H., Cadena-Gómez G. et al. (1999). Coffee seeds contain 11S storage proteins. Physiol Plant 105:122-131

Aga E., Bryngelsson T., Bekele E., Salomon B. (2003). Genetic diversity of forest arabica coffee (*Coffea arabica* L.) in Ethiopia revealed by random amplified polymorphic DNA (RAPD) analysis. Hereditas 138:36-46

Aga E., Bryngelsson T. (2006). Inverse sequence-tagged repeat (ISTR) analysis of genetic variability inforest coffee (*Coffea arabica* L.) from Ethiopia. Genetic Res Crop Evol 53:721-728

Aggarwal R.K., Hendre P.S., Varshney R.K., Bhat P.R., Krishnakumar V. et al. (2007). Identification, characterization and utilization of EST-derived genetic microsatellite markers for genome analyses of coffee and related species. Theor Appl Genet 114:359–372

Agwanda C.O., Lashermes P., Trouslot P. et al. (1997). Identification of RAPD markers for resistance to coffee berry disease, *Colletotrichum kahawae*, in arabica coffee. Euphytica 97: 241-248

Alba R., Fei Z., Payton P., Liu Y., Moore S.L. et al. (2004). ESTs, cDNA microarrays, and gene expression profiling: tools for dissecting plant physiology and development. Plant J 39:697-714

Alpizar E., Dechamp E., Espeout S., Royer M., Lecouls A.-C. et al. (2006a). Efficient production of *Agrobacterium rhizogenes*-transformed roots and composite plants for studying gene expression in coffee roots. Plant Cell Rep 25:959-967

Alpizar E., Dechamp E., Bertrand B., Lashermes P., Etienne H. (2006b). Transgenic roots for functional genomics of coffee resistance genes to root-knot nematodes. Proc Intl Sci Colloquium on Coffee 21:653–659, ASIC (http://www.asic-cafe.org)

Andrade A.C. (2008). The Coffee Genome Project and plant disease resistance. Tropical Plant Pathology 33(Sup): 22-25.

Anthony F., Bertrand B., Quiros O., Wilches A., Lashermes P. et al. (2001). Genetic diversity of wild coffee (*Coffea arabica* L.) using molecular markers. Euphytica 118:53–65

Anthony F., Combes M.C., Astorga C., Bertrand B., Graziosi G. et al. (2002). The origin of cultivated *Coffea arabica* L. varieties revealed by AFLP and SSR markers. Theor Appl Genet 104:894–900

Ashihara H., Zheng X.Q., Katahira R. et al. (2006). Caffeine biosynthesis and adenine metabolism in transgenic *Coffea canephora* plants with reduced expression of N-methyltransferase genes. Phytochemistry 67: 882-886

Arroyo-Herrera A, Gonzalez AK, Moo RC, et al. (2008) Expression of WUSCHEL in *Coffea canephora* causes ectopic morphogenesis and increases somatic embryogenesis. Plant Cell Tiss Org Cult 94: 171-180

Barre P., Layssac M., D'Hont A., Louarn J., Charrier A. et al. (1998). Relationship between parental chromosomic contribution and nuclear DNA content in the coffee interspecific

hybrid: *C. pseudozanguebariae* x *C. liberica* var. *dewevrei*. Theor Appl Genet 96:301–305

Barton C.R., Adams T.L., Zarowitz M. (1991). Stable transformation of foreign DNA into *Coffea arabica* plants. Proc Intl Conf Coffee Sci 14:460-464

Baruah A., Naik V., Hendre P.S., Rajkumar R., Rajendrakumar P. et al. (2003). Isolation and characterization of nine microsatellite markers from *Coffea arabica* L., showing widecross-species amplifications. Mol Ecol Notes 3:647–650

Berthouly M., Etienne H. (2000). Somatic embryogenesis of coffee. In: Coffee biotechnology and quality. Proc Intl Seminar Biotechnology Coffee Agro-industry 13:71-90

Bhat P.R., Krishnakumar V., Hendre P.S., Rajendrakumar P.,Varshney R.K. et al. (2005). Identification and characterization of gene-derived EST–SSR markers from robusta coffee variety 'CxR' (an interspecific hybrid of *Coffea canephora* and *Coffea congensis*). Mol Ecol Notes 5:80–83

Bremer B., Jansen R.K. (1991). Comparative restriction site mapping of chloroplast DNA implies new phylogenetic relationships within *Rubiaceae*. Am J Bot 78:198–213

Bustamante J., Campa C., Poncet V., Noirot M., Leroy T. et al. (2007). Molecular characterization of an ethylene receptor gene (*CcETR1*) in coffee trees, its relationship with fruit development and caffeine content. Mol Genet Genomics 277: 701-712

Campa C., Noirot M., Bourgeois M. et al. (2003). Genetic mapping of a caffeoyl-coenzyme A 3-O-methyltransferase gene in coffee trees. Impact on chlorogenic acid content. Theor Appl Genet 107: 751-756

Canche-Moo R.L.R., Ku-Gonzales A., Burgeff C., Loyola-Vargas V.M., Rodríguez-Zapata L.C. et al. (2006). Genetic transformation of *Coffea canephora* by vacuum infiltration. Plant Cell Tiss Org Cult 84:373-387

Carvalho A. (1988). Principles and practice of coffee plant breeding for productivity and quality factors: *Coffea arabica*. In: Clarke RJ, Macrae R (eds) Coffee, volume 4: Agronomy. Elsevier Applied Science, London, pp 129–165

Clarindo W.R. and Carvalho C.R. (2008). First *Coffea arabica* karyogram showing that this species is a true allotetraploid. Plant Systematics And Evolution 274: 237-241

Combes M.C., Andrzejewski S., Anthony F., Bertrand B., Rovelli P. et al. (2000). Characterization of microsatellite loci in *Coffea arabica* and related coffee species. Mol Ecol 9:1178-1180

Committee on the National Plant Genome Initiative (2008) Achievements of the National Plant Genome

Initiative and New Horizons in Plant Biology. National Academies Press, 182 p. http://www.nap.edu/catalog/12054.html

Coulibaly I., Revol B., Noirot M., Poncet V., Lorieux M. et al. (2003). AFLP and SSR polymorphism in a *Coffea* interspecific backcross progeny [(*C. heterocalyx* x *C. canephora*) x *C. canephora*]. Theor Appl Genet 107:1148–1155

Cristancho M.A., Rivera C., Orozco C., Chalarca A., Mueller L. (2006) Development of a bioinformatics platform at the Colombia National Coffee Research Center Proc Intl Sci Colloquium Coffee 21: 638-643, ASIC (http://www.asic-cafe.org)

Cros J., Combes M.C., Trouslot P., Anthony F., Hamon S. et al. (1998). Phylogenetic relationships of *Coffea* species: new evidence based on the chloroplast DNA variation analysis. Mol Phylo Evol 9:109-117

Crouzillat D., Rigoreau M., Bellanger L., Priyono S., Mawardi S., Syahrudi, McCarthy J., Tanksley S., Zaenudin I., Petiard V. (2004). A Robusta consensus map using RFLP and microsatellites markers for the detection of QTL. Proc Intl Sci Colloquium Coffee 20: 546-553, ASIC (http://www.asic-cafe.org)

Cruz A.R.R., Paixao A.L.D.,Machado F.R., Barbosa M.F.F., Junqueira C.S. et al. (2004). Metodologia para obtenção de plantas transformadas de *Coffea canephora* por co-cultivo e calos embriogênicos com *A. tumefaciens*. Boletim de Pesquisa e Desenvolvimento. Embrapa, Brasilia, Brasil, vol.58:15 p

Cubry P., Musoli P., Legnate H. et al. (2008). Diversity in coffee assessed with SSR markers: Structure of the genus *Coffea* and perspectives for breeding. Genome 51: 50-63

CunhaW.G.,Machado F.R.B., Vianna G.R., Texteira J.B., Barros E.V.S.A. (2004). Obtenção de *Coffea arábica* geneticamente modificado por bombardeio de calos embriogênicos. Boletim de Pesquisa e Desenvolvimento. Embrapa, Brasilia, Brasil, vol. 73:15 p

De Oliveira A.C.B., Sakiyama N.S., Caixeta E.T., Zambolim E.M., Rufino R.J.N., Zambolim, L. (2007). Partial map of *Coffea arabica* L. and recovery of the recurrent parent in backcross progenies. Crop Breeding and Applied Biotechnology 7:196-203

De Nardi B., Dreos R., Del Terra L., Martellossi C., Asquini E. et al. (2006). Differential responses of *Coffea arabica* L. leaves and roots to chemically induced systemic acquired resistance. Genome 49:1594–1605

Dussert S., Lashermes P., Anthony F., Montagnon C., Trouslot P. et al. (2003). Coffee (*Coffea canephora*). In: Hamon P, Seguin M, Perrier X, Glaszmann JC (eds) Genetic diversity of cultivated tropical plants. Science Publishers Inc., Plymouth, pp 239–258

Etienne H. (2005). Protocol of somatic embryogenesis: Coffee (*Coffea arabica* L. and *C. canephora* P.). In: Protocols for somatic embryogenesis in woody plants. Series: Forestry Sci Vol. 77, Jain SM, Gupta PK (Eds). Springer, the Netherlands. ISBN: 1-4020-2984-5, pp. 167-179

Feng Q., Yang M.Z., Zheng X.Q., Zhen X.S., Pan N.S., Chen Z.L. (1992). Agrobacterium-mediated transformation of coffee (*Coffea arabica* L.). Chin J Biotechnol 8 :255-260

Fernandez D., Santos P., Agostini C., Bon M.C., Petitot A.S. et al. (2004). Coffee (*Coffea arabica* L.) genes early expressed during infection by the rust fungus (*Hemileia vastatrix*). Mol Plant Pathol 5:527–536

Fernandez-Da Silva R., Menéndez-Yuffá A. (2003). Transient gene expression in secondary somatic embryos from coffee tissues electroporated with genes *gus* and *bar*. Electronic J Biotech 6:29-35

Freire A.V., Lightfoot D.A., Preece J.E. (1994). Genetic transformation of coffee (*Coffea arabica* L.) by *Agrobacterium* spp. Hortsc 29:454

Ganesh D., Petitot A.-S., Silva M., Alary R., Lecouls A.C. et al. (2006). Monitoring of the early molecular resistance responses of coffee (*Coffea arabica* L.) to the rust fungus (*Hemileia vastatrix*) using real-time quantitative RT-PCR. Plant Sci 170:1045-1051

Geromel C., Ferreira L.P., Guerreiro S.M.C., Cavalari A.A., Pot D. et al. (2006). Biochemical and genomic analysis of sucrose metabolism during coffee (*Coffea arabica*) fruit development. J Exp Bot 57:3243-3258

Geromel C., Ferreira L.P., Bottcher A. et al. (2008). Sucrose metabolism during fruit development in *Coffea racemosa*. Annals of Applied Biology 152: 179-187

Geromel C., Ferreira L.P., Davrieux F. et al. (2008a). Effects of shade on the development and sugar metabolism of coffee (*Coffea arabica* L.) fruits. Plant Physiol Biochem 46: 569-579

Hatanaka T., Choi Y.E., Kusano T., Sano H. (1999). Transgenic plants of *Coffea canephora* from embryogenic callus via *Agrobacterium tumefaciens*-mediated transformation. Plant Cell Rep 19:106-110

Herrera J.C., Combes M.C., Anthony F., Charrier A., Lashermes P. (2002). Introgression into the allotetraploid coffee (*Coffea arabica* L.): segregation and recombination of the *C. canephora* genome in the tetraploid interspecific hybrid (*C. arabica* x *C. canephora*). Theor Appl Genet 104:661-668

Herrera J.C., D'Hont A., Lashermes P. (2007). Use of fluorescence in situ hybridization as a tool for introgression analysis and chromosome identification in coffee (*Coffea arabica* L.). Genome 50:619-626

Hinniger C., Caillet V., Michoux F., Ben Amor M., Tanksley S. et al. (2006). Isolation and characterization of cDNA encoding three dehydrins expressed during *Coffea canephora* (Robusta) grain development. Ann Bot 97:755-765

Koshiro Y., Zheng X.-Q., Wang M.-L., Nagai C., Ashihara H. (2006). Changes in content and biosynthetic activity of caffeine and trigonelline during growth and ripening of *Coffea arabica* and *Coffea canephora* fruits. Plant Science 171: 242-250

Kumar V., Satyanarayana K.V., Sarala Itty S., Indu E.P., Giridhar P. et al. (2006). Stable transformation and direct regeneration in *Coffea canephora* P ex. Fr. *Agrobacterium rhizogenes* mediated transformation without hairy-root phenotype. Plant Cell Rep 25:214-222

Kumar V., Satyanarayana K.V., Ramakrishna A. et al. (2007). Evidence for localization of N-methyltransferase (MMT) of caffeine biosynthetic pathway in vacuolar surface of *Coffea canephora* endosperm elucidated through localization of GUS reporter gene driven by NMT promoter. Current Science 93: 383-386

Ky C.L., Barre P., Lorieux M., Trouslot P., Akaffou S. et al. (2000). Interspecific genetic linkage map, segregation distortion and genetic conversion in coffee *Coffea* sp. Theor Appl Genet 101:669-676

Lashermes P., Trouslot P., Anthony F., Combes M.C., Charrier A. (1996). Genetic diversity for RAPD markers between cultivated and wild accessions of *Coffea arabica*. Euphytica 87:59–64

Lashermes P, Cros J, Combes MC, Trouslot P, Anthony F, et al. (1996a) Inheritance and restriction fragment length polymorphism of chloroplast DNA in the genus *Coffea* L. Theor Appl Genet 93:626–632

Lashermes P., Combes M.C., Robert J., Trouslot P., D'Hont A. et al. (1999). Molecular characterization and origin of the *Coffea arabica* L. genome. Mol Gen Genet 261:259-266

Lashermes P., Andrzejewski S., Bertrand B., Combes M.C., Dussert S. et al. (2000). Molecular analysis of introgressive breeding in coffee (*Coffea arabica* L.). Theor Appl Genet 100:139-146

Lashermes P., Combes M.C., Prakash N.S., Trouslot P., Lorieux M. et al. (2001). Genetic linkage map of *Coffea canephora*: effect of segregation distortion and analysis of recombination rate in male and female meioses. Genome 44:589-595

Lashermes P., Noir S., Combes M.C., Ansaldi C., Bertrand B. et al. (2004). Toward an Integrated Physical Map of the Coffee Genome. Proc 20th Intl Sci Colloquium Coffee 20: 554-559, ASIC (http://www.asic-cafe.org)

Lashermes P., Andrade A.C., Etienne H. (2008). Genomics of Coffee, One of The World´s Largest Traded Commodities. In: Paul H. Moore; Ray Ming. (Org.). Genomics of Tropical Crop Plants. New York: Springer, 2008, v. 1, p. 203-226.

Lepelley M., Cheminade G., Tremillon N., Simkin A., Caillet V. et al. (2007). Chlorogenic acid synthesis in coffee**:** An analysis of CGA content and real-time RT-PCR expression of HCT, HQT, C3H1, and CCoAOMT1 genes during grain development in *C. canephora*. Plant Sci 172: 978-996

Leroy T., Henry A.-M., Royer M., Altosaar I., Frutos R. et al. (2000). Genetically modified coffee plants expressing the *Bacillus thuringiensis cry1Ac* gene for resistance to leaf miner. Plant Cell Rep 19:382-389

Leroy T., Marraccini P., Dufour M., Montagnon C., Lashermes P. et al. (2005). Construction and characterization of a *Coffea canephora* BAC library to study the organization of sucrose biosynthesis genes. Theor Appl Genet 111:1032-1041

Lin C., Mueller L.A., McCarthy J., Crouzillat D., Pétiard V. et al. (2005). Coffee and tomato share common gene repertoires as revealed by deep sequencing of seed and cherry transcripts. Theor Appl Genet 112:114-130

Lombello R.A., Pinto-Maglio C.A.F. (2004). Cytogenetic studies in *Coffea L.* and *Psilanthus* Hook. Using CMA/DAPI and FISH. Cytologia 69:85–91

Lopes F.R., Carazzolle M.F., Pereira G.A., Colombo C.A., Carareto C.M. (2008). Transposable elements in *Coffea* (Gentianales: Rubiacea) transcripts and their role in the origin of protein diversity in flowering plants. Mol Genet Genomics 279:385-401.

López G., Moncada M.D.P. (2006). Construction of an interspecific genetic Linkage map from a *Coffea liberica* x *C. eugenioides* F1 Population. Proc Intl Sci Colloquium Coffee 21: 644-652, ASIC (http://www.asic-cafe.org)

Mahe L., Combes M.C., Varzea V.M.P. et al. (2008). Development of sequence characterized DNA markers linked to leaf rust (*Hemileia vastatrix*) resistance in coffee (*Coffea arabica* L.). Molecular Breeding 21: 105-113

Mahesh V., Rakotomalala J.J., Le Gal L. et al. (2006). Isolation and genetic mapping of a *Coffea canephora* phenylalanine ammonia-lyase gene (CcPAL1) and its involvement in the accumulation of caffeoyl quinic acids. Plant Cell Reports 25: 986-992

Mahesh V., Million-Rousseau R., Ullmann P. et al. (2007). Functional characterization of two p-coumaroyl ester 3 '-hydroxylase genes from coffee tree: evidence of a candidate for chlorogenic acid biosynthesis. Plant Mol Biol 64:145-159

Marraccini P., Deshayes A., Petiard V. et al. (1999). Molecular cloning of the complete 11S seed storage protein gene of *Coffea arabica* and promoter analysis in transgenic tobacco plants. Plant Physiol Biochem 37: 273-282

Marraccini P., Rogers W.J., Allard C. et al. (2001). Molecular and biochemical characterization of endo-beta-mannanases from germinating coffee (*Coffea arabica*) grains. Planta 213: 296-308

Marraccini P., Rogers W.J., Caillet V. et al. (2005). Biochemical and molecular characterization of alpha-D-galactosidase from coffee beans. Plant Physiol Biochem 43: 909-920

Marracini P., Ramos H., Vieira L.G.E., Ferrão M.A.G., Da Silva F.R., Bloch Jr. C., Andrade A.C. (2008). Integrated analysis of drought stress responses in coffee plants. Book of The XVI International Conference of Plant and Animal Genome Research .p 28. XVI PAG (http://www.intl-pag.org/16/16-pag.html).

Maurin O., Davis A.P., Chester M. et al. (2007). Towards a phylogeny for *Coffea* (Rubiaceae): Identifying well-supported lineages based on nuclear and plastid DNA sequences. Ann Bot 100: 1565-1583

McCarthy A.A., McCarthy J.G. (2008). The structure of two N-methyltransferases from the caffeine biosynthetic pathway. Plant Physiol 144: 879-889

Mendonca J.C.F., Franca A.S., Oliveira L.S. et al. Chemical characterisation of non-defective and defective green arabica and robusta coffees by electrospray ionization-mass spectrometry (ESI-MS). Food Chemistry 111: 490-497

Mettulio R., Rovelli P., Anthony F., Anzueto F., Lashermes P. et al. (1999). Polymorphic microsatellites in *Coffea arabica*. Proc Intl Sci Colloquium Coffee 18:344–347

Moisyadi S. and Stiles J.I. (1995). A cDNA encoding a metallothionein I-like protein from coffee leaves (*Coffea arabica*). Plant Physiol 107:295-6.

Moncada P., McCouch S. (2004). Simple sequence repeat diversity in diploid and tetraploid *Coffea* species. Genome 47:501-509

Mondego J.M.C., Guerreiro-Filho O., Bengtson M.H. et al. (2005). Isolation and characterization of *Coffea* genes induced during coffee leaf miner (*Leucoptera coffeella*) infestation. Plant Science 169: 351-360

Noir S., Patheyron S., Combes M.C., Lashermes P., Chalhoub B. (2004). Construction and characterization of a BAC library for genome analysis of the allotetraploid coffee species (*Coffea arabica* L.). Theor Appl Genet 109:225-230

Ogita S., Uefuji H., Yamaguchi Y., Sano H. (2003). RNA interference: producing decaffeinated coffee plants. Nature 423:823

Ogita S., Uefuji H., Morimoto M., Sano H. (2004). Application of RNAi to confirm theobromine as the major intermediate for caffeine biosynthesis in coffee plants with potential for construction of decaffeinated varieties. Plant Mol Biol 54:931-941

Orozco-Castillo C., Chalmers K.J., Waugh R., Powell W. (1994). Detection of genetic diversity and selective gene introgression in coffee using RAPD markers. Theor Appl Genet 87:934-940

Orozco-Castillo C., Chalmers K.J., Powell W., Waugh R. (1996). RAPD and organelle specific PCR re-affirms taxonomic relationships within the genus *Coffea*. Plant Cell Rep 15:337-341

Paillard M., Lashermes P., Pétiard V. (1996). Construction of a molecular linkage map in coffee. Theor Appl Genet 93:41-47

Pearl H.M., Nagai C., Moore P.H., Steiger D.L., Osgood R.V. et al. (2004). Construction of a genetic map for arabica coffee. Theor Appl Genet 108:829-835

Pereira L.F.P., Cação S.M.B., Silva N.V., Alves G.S.C., Vinecky F., Caixeta E.T., Andrade A.C., Vieira L.G.E. (2008). Using a *Coffea arabica* bacterial artificial chromossome

library for gene cloning and integrative mapping approaches. p 276. XVI PAG (http://www.intl-pag.org/16/16-pag.html

Perrone D., Donangelo C.M., Farah A. (2008). Fast simultaneous analysis of caffeine, trigonelline, nicotinic acid and sucrose in coffee by liquid chromatography–mass spectrometry. Food Chemistry 110:1030-1035

Perthuis B., Pradon J., Montagnon C., Dufour M., Leroy T. (2005). Stable resistance against the leaf miner *Leucoptera coffeella* expressed by genetically transformed *Coffea canephora* in a pluriannual field experiment in French Guiana. Euphytica 144:321-329

Petitot A.S., Lecouls A.C., Fernandez D. (2008) .Sub-genomic origin and regulation patterns of a duplicated WRKY gene in the allotetraploid species *Coffea arabica.* Tree Genetics & Genomes 4:379-390

Pinto F.D., Maluf M.P., Guerreiro O. (2007). Study of simple sequence repeat markers from coffee expressed sequences associated to leaf miner resistance. Pesquisa Agropecuaria Brasileira 42: 377-384

Pinto-Maglio C.A.F. and Da Cruz N.D. (1998). Pachytene chromosome morphology in *Coffea* L. II. *C. arabica* L. complement. Caryologia, 51:19-35

Poncet V., Hamon P., Minier J., Carasco C., Hamon S. et al. (2004). SSR cross-amplification and variation within coffee trees (*Coffea* spp.). Genome 47:1071-1081

Poncet V., Rondeau M., Tranchant C., Cayrel A., Hamon S. et al. (2006). SSR mining in coffee tree EST databases: potential use of EST-SSRs as markers for the *Coffea* genus. Mol Genet Genomics 276:436-449

Poncet V., Dufour M., Hamon P. et al. (2007). Development of genomic microsatellite markers in *Coffea canephora* and their transferability to other coffee species. Genome 50: 1156-1161

Prakash N.S.,Marques D.V., Varzea V.M.P., Silva M.C., Combes M.C. et al. (2004). Introgression molecular analysis of a leaf rust resistance gene from *Coffea liberica* into C*offea arabica* L. Theor Appl Genet 109:1311–1317

Prakash N.S., Combes M.C., Dussert S., Naveen S., Lashermes P. (2005). Analysis of genetic diversity in Indian robusta coffee genepool (*Coffea canephora*) in comparison with a representative core collection using SSRs and AFLPs. Genetic Res Crop Evol 52: 333-343

Priolli R.H.G., Mazzafera P., Siqueira W.J. et al. (2008). Caffeine inheritance in interspecific hybrids of *Coffea arabica* x *Coffea canephora* (Gentianales, Rubiaceae). Genetics And Molecular Biology 31: 498-504

Privat I., Bertrand B.S., Lashermes P. (2008). The coffee microarray project: a new tool to discover candidate genes correlated to coffee traits. Abstract Book of The XVI International Conference of Plant and Animal Genome Research .p 29. XVI PAG (http://www.intl-pag.org/16/16-pag.html).

Privat I., Foucrier S., Prins A. et al. (2008a). Differential regulation of grain sucrose accumulation and metabolism in *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta) revealed through gene expression and enzyme activity analysis. New Phytologist 178: 781-797

Pre M., Caillet V., Sobilo J. et al.(2008). Characterization and expression analysis of genes directing galactomannan synthesis in coffee. Annals Of Botany 102: 207-220

Raina S.N., Mukai Y., Yamamoto M. (1998). *In situ* hybridisation identifies the diploid progenitor of *Coffea arabica* (Rubiaceae). Theor Appl Genet 97:1204-1209

Ribas A.F., Kobayashi A.K., Pereira L.F.P., Vieira L.G.E. (2005). Genetic transformation of *Coffea canephora* by particle bombardment. Biol Plant 49:493-97

Ribas A.F., Kobayashi A.K., Pereira L.F.P., Vieira L.G.E. (2006). Production of herbicide-resistance coffee plants (*Coffea canephora* L.) via *Agrobacterium tumefaciens*-mediated transformation. Braz Arc Biol Technol 49:11-19

Rogers W.J., Bezard G., Deshayes A. et al. (1999). Biochemical and molecular characterization and expression of the 11S-type storage protein from *Coffea arabica* endosperm. Plant Physiology And Biochemistry 37: 261-272

Rosillo A.G., Acuna J.R., Gaitan A.L., de Pena M. (2003). Optimized DNA delivery into *Coffea arabica* suspension culture cells by particle bombardment. Plant Cell Tiss Org Cult 74:75-79

Salmona J., Dussert S., Descroix F. et al. (2008). Deciphering transcriptional networks that govern *Coffea arabica* seed development using combined cDNA array and real-time RT-PCR approaches. Plant Molecular Biology 66: 105-124

Samson N., Bausher M.G., Lee S.B. et al. (2007). The complete nucleotide sequence of the coffee (*Coffea arabica* L.) chloroplast genome: organization and implications for biotechnology and phylogenetic relationships amongst angiosperms. Plant Biotechnology Journal 5: 339-353

Shendure J. and Ji H. (2008). Next-generation DNA sequencing. Nature biotech 26:1135-1145

Silva L.P., Vinecky F., Barbosa E.A., Andrade A.C., Bloch Jr.C. (2008). Imaging Mass Spectrometry as an innovative tool to study coffee fruit development. Proc Intl Sci Colloquium Coffee 21:173-178, ASIC (http://www.asic-cafe.org)

Silvestrini M., Maluf M.P., Silvarolla M.B. et al. (2008). Genetic diversity of a *Coffea* Germplasm Collection assessed by RAPD markers. Genetic Resources And Crop Evolution 55: 901-910

Simkin A.J., Qian T., Caillet V.,Michoux F., Ben Amor M. et al. (2006). Oleosin gene family of *Coffea canephora*: quantitative expression analysis of five oleosin genes in developing and germinating coffee grain. J Plant Physiol 163:691-708

Simkin A.J., Moreau H., Kuntz M. et al. (2008). An investigation of carotenoid biosynthesis in *Coffea canephora* and *Coffea arabica*. J Plant Physiol 165: 1087-1106

Spiral J., Thierry C., Paillard M., Pétiard V. (1993). Obtention de plantules de *Coffea canephora* Pierre (Robusta) transformées par *Agrobacterium rhizogenes*. C. R. Acad Sci Paris 3:1-6

Sugiyama M., Matsuoka C., Takagi T. (1995). Transformation of *Coffea* with *Agrobacterium rhizogenes*. Proc Intl Conf Coffee Sci 16:853-859

Tesfaye K., Borsch T., Govers K., Bekele E. (2007). Characterization of *Coffea* chloroplast microsatellites and evidence for the recent divergence of *C. arabica* and *C. eugenioides* chloroplast genomes. Genome 50:1112-1129

van Boxtel J., Berthouly M., Carasco M., Dufour M., Eskes A. (1995). Transient expression of ÿ-glucuronidase following biolistic delivery of foreign DNA into coffee tissue. Plant Cell Rep 14:748-752

Vieira L.G.E., Andrade A.C., Colombo C.A., Araujo A.H., Metha A. et al. (2006). Brazilian coffee genome project: an EST-based genomic resource. Braz J Plant Physiol 18:95-108

Wu F., Mueller L.A., Crouzillat D., Pétiard V., Tanksley S.D. (2006). Combining bioinformatics and phylogenetics to identify large sets of single-copy orthologous genes (COSII) for comparative, evolutionary and systematic studies: A test case in the euasterid plant clade. Genetics 174:1407-1420

Towards the Development of Sequence Based Markers for Resistance to Coffee Berry Disease (*Colletotrichum kahawae*)

E.K. GICHURU[1], M.-C. COMBES[2], E.W. MUTITU[3], E.C.K. NGUGI[3], C.O. OMONDI[1], B. BERTRAND[4], P. LASHERMES[2]

[1]Coffee Research Foundation (CRF), P O Box 4, Ruiru 00232, Kenya
[2]Institut de Recherche pour le Développement (IRD), UMR DGPC, Résistance des Plantes, 911, Av. de Agropolis, BP 64501, F-34394, Montpellier, France
[3]University of Nairobi, P O Box 29053, Nairobi, Kenya
[4]Centre de coopération Internationale en Recherche Agronomique pour le Développement, (CIRAD-CP). UMR-DGPC, IRD, 911 Av. de Agropolis, BP 64501, F-34394, Montpellier, France

SUMMARY

Coffee Berry Disease which affects green Arabica coffee (*Coffea arabica*) berries is caused by the fungus *Colletotrichum kahawae* and is a major problem in Arabica coffee production in African countries. Breeding for resistance to this disease is therefore to a major priority in these countries avoid intensive chemical usage for its control. Recently, microsatellite and Amplified Fragment Length Polymorphisms (AFLP) markers for a gene conferring resistance to the disease were identified and mapped onto the chromosomal region carrying the gene. To improve the repeatability of the AFLP markers, four of the marker bands were selected for cloning and sequencing to facilitate specific primers to be designed. Three of the resultant primers did not amplify products that exhibited polymorphism characteristic of the parent AFLP bands; but one primer pair amplified a product that dominantly identified the presence of the parent AFLP marker at an optimum temperature of 62 °C followed by electrophoresis in agarose. The reliability of the designed primers was confirmed by analysis in 95 plants from a F_2 population previously used to map the chromosomal fragment carrying the resistance. The importance of the results in enhancing the utility of the parent AFLP marker in relation to analytical costs and position on the chromosomal fragment is discussed.

INTRODUCTION

Coffee is an important export crop and a major foreign currency earner for many countries located in the tropical areas of Africa, Asia and Latin America. Arabica coffee (*Coffea arabica* L.) accounts for about 75% of the total world coffee production and the rest is mainly Robusta coffee (*Coffea canephora* Pierre). One of the major constraints of coffee production includes disease epidemics. Disease management is an especially limiting factor to economic coffee production by smallholders due to limitation of financial and technical capabilities (Masaba and Waller, 1992). Among the most important coffee diseases is Coffee Berry Disease (CBD) which currently occurs only in Africa. This is an anthracnose of coffee berries that is caused by the fungus *Colletotrichum kahawae.* Infection of green immature fruits by the fungus can cause up to 80% crop loss if not controlled when conditions are favourable (Griffiths *et al.*, 1971). An alternative strategy for its management by breeding for resistance is highly desirable due to low cost to producer and environmental safety.

Inheritance studies by Van der Vossen and Walyaro, (1980) identified three genes of resistance carried in varieties Rume Sudan (*R* and *k* genes), Hibrido de Timor (*T* gene) and K7

(*k* gene). Hibrido de Timor (HDT) is natural cross between Arabica and Robusta (*C. canephora*) coffees and is widely used for breeding programmes especially for pest and disease resistance including Coffee Leaf Rust, CBD and nematodes (Lashermes et al., 2000; Silva et al., 2006). For example in Kenya, a breeding programme involving HDT led to the release of an Arabica coffee cultivar (cv Ruiru 11) that combines resistance to CBD and CLR with high yields, fine cup quality and compact growth (Nyoro and Sprey, 1986). This cultivar is a composite of about 60 hybrids, each derived from a cross between a specific female and male population (Omondi et al., 2001). The population is therefore not genetically uniform, raising a need to conduct molecular studies to identify markers that can help in tracking the genes in breeding programmes (marker assisted selection: MAS).

Recent work by Gichuru et al. (2006, 2008) mapped the *T* gene within a chromosomal fragment of about 10 cM that is introgressed into *C. arabica* from *C. canephora* through HDT. The molecular markers identified included two microsatellites and nine Amplified Fragment Length Polymorphism (AFLP) bands. AFLP markers are not very specific to DNA sequences of the genome under study and are therefore not highly repeatable over time and between laboratories (Rafalski et al., 1996). The reproducibility of AFLP markers can be improved by converting them into Sequence Characterised Amplified Regions (SCARs). This technique involves sequencing of markers (DNA fragments) and designing primers that are specific to the parent loci. The subsequently amplified products can be analysed under more stringent PCR conditions, may maintain the polymorphism of their parental markers, may exhibit different polymorphism like co-dominance while the parent markers were dominant, or even loose the polymorphism (Shan et al., 1999; Zhang and Stommel, 2001). Another reason for conversion of markers such AFLPs to SCARs is the possibility of the resultant markers being easier and cheaper to analyse than the more sophisticated procedures such as AFLP. The objective of this study was therefore to develop SCAR markers from AFLP markers linked to resistance to CBD.

MATERIALS AND METHODS

Extraction of DNA from AFLP bands

Four AFLP markers linked to resistance to CBD (Gichuru et al., 2008) were selected for this study based on size (more than 100bp) and clear separation from other bands. Genomic DNA from seven plant samples consisting of a susceptible parent cultivar (SL28), a resistant parent cultivar (Catimor) and five F2 progeny of their cross was amplified with the appropriate selective AFLP primers used by Gichuru et al. (2008). During electrophoresis, a 62-well comb with alternate teeth removed to give double sized wells was used. Ten micro-litres of each sample were loaded and after electrophoresis, the dried gels were stapled onto the films before placing them into the cassettes to avoid movements between the two. To extract the DNA from the bands of interest, the developed films and the gels were re-matched, pieces of the dry gel were cut from at least two of the F2 plants with the target band and soaked PCR grade water as detailed in Gichuru (2007). The resultant DNA solution was amplified in 25 µl reaction mixtures containing 2 µl of the DNA solution, 2.5 µl of 10X buffer, 2.0 µl of $MgCl_2$ (25 mM), 0.5 µl of dNTPs (5 mM), 0.6 µl of each of the primers used to amplify the particular bands during AFLP (10 µM), 0.1µl of *Taq* DNA polymerase and 16.7 µl of PCR water. The PCR programme consisted of an initial denaturation step of 5 minutes at 95 °C followed by 35 cycles of denaturation at 94 °C for 45 sec, primer annealing at 50 °C for 45 sec, elongation at 72 °C for 45 sec and a final extension step of 10 min at 72 °C. Two micro-litres of the amplification products were electrophoresed in 2% agarose gel and revealed in ethidium bromide. Only samples with one clear band were judged to be good for cloning and two of the samples with high intensity bands were selected for cloning.

Cloning DNA extracted from AFLP bands

The fresh PCR products were cloned using TOPO TA Cloning® kit with pCR® 2.1-TOPO® vector and chemically competent cells (Invitrogen, Life Technologies) according to the manufacturer's instructions. The cultures were then tested for inserts by PCR using 2 µl of the liquid culture and the same reaction mix as the one used to amplify the AFLP DNA before cloning. The PCR programme was also the same as during amplification of extracted AFLP DNA but the initial denaturation step was increased to 10 min, to ensure adequate rupturing of the bacterial cells to release the plasmids. To assess the inserts, 2 µl of the PCR products were electrophoresed in 2% agarose gel alongside a sample of the PCR product used for cloning to ascertain that the size of the cloned fragments were the ones targeted. Clones with inserts of different sizes from the targeted fragments and those without inserts were discarded. A maximum of four and a minimum of two clones with the right size of insert per individual cloning reaction (depending on availability) were selected for extraction of plasmid DNA for sequencing commercially by Genome Express, France. In all cases, the sequenced bands included two different plants samples.

The actual plant DNA sequences were identified from the entire sequences to exclude AFLP primers and vector sequences. Replicate sequences of the same band were aligned using CLUSTAL W 1.82 programme (European Bioinformatics Institute, http://www.ebi.ac.uk/clustalw). Only sequences that were highly similar were considered as allelic. Sequence specific primers were designed from one of the alleles of the same band using Primer3 programme (Whitehead Institute, USA, http://frodo.wi.mit.edu/cgi-bin/primer3/primer3www.cgi). The parameters considered in designing the primers targeted sizes between 18 and 22bp and optimum annealing temperature of 55 °C or 60 °C, so that they could all be analysed under the same PCR conditions.

The primers (synthesised by Eurogentec, Belgium) were first tested for performance and possible polymorphism in 2% agarose gels as described by Poncet et al. (2005). Where amplification was successful but without polymorphism in agarose, further amplification was done using a radioactive nucleotide ($\alpha dATP^{33}$) followed by electrophoresis in 6% denaturing acrylamide gel as described by Combes et al. (2000). Once polymorphism was observed, reliability of the marker was confirmed by analysis on the 95 plants used by Gichuru et al. (2008) to map the chromosomal fragment.

RESULTS

Four AFLP markers linked to resistance to CBD were cloned, sequenced and loci-specific primers designed. SCARs were amplified using the primers and tested for polymorphism related to CBD resistance in agarose and denaturing poly-acrylamide gels. One primer pair amplified non-specific products (a smear) and two primer pairs amplified products that did not exhibit any polymorphism between plants with and without the parental AFLP band. However, the fourth one designed from the AFLP marker AGC-CTG-c (Gichuru *et al*., 2008) appeared to be more intense in samples with the parent AFLP marker than in samples without the marker when amplified at 60 °C (Figure 1A). More tests at different annealing temperatures and electrophoresis in denaturing poly-acrylamide gel were done to confirm if the difference was due to primer mismatch which can be exploited by altering the annealing temperature or due to multiple products in plants with the parent marker. Reduction of the annealing temperature to 55 °C resulted into PCR products that did not appear to have differences between plants with and without the parent AFLP band (Figure 1B). At an annealing temperature of 62 °C, only samples with the parent AFLP marker were amplified (Figure 1C). At a higher temperature of 64 °C, the intensity of the bands decreased (Figure

1D). The marker was designated 'ScAGC-CTG-c' by adding 'Sc' to denote SCAR. Its reliability was tested by amplification in all the 95 plants from the F2 population used to map the resistance and it amplified as expected. The sequences of the primers are ACCTATCAGAGGGGAATTTG (forward) and GGTGATGAGGTACAGTT GCT (reverse).

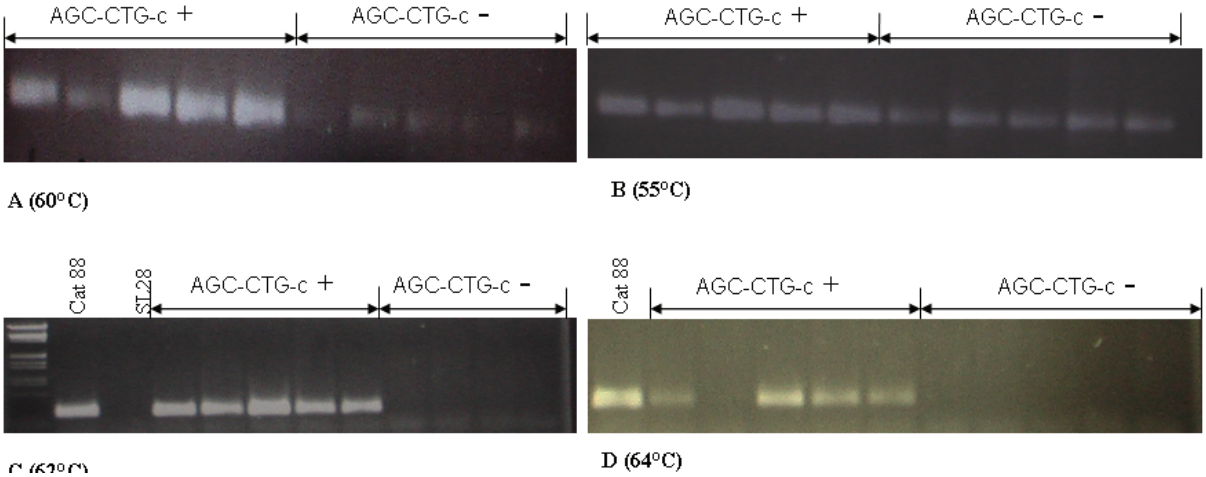


Figure 1. PCR products of the ScAGC-CTG-c SCAR from plants with and without the parent AFLP marker (AGC-CTG-c +) and those without the marker (AGC-CTG-c) at different annealing temperatures.

DISCUSSION

Four AFLP markers for resistance to CBD were sequenced and specific primers designed. One pair of primers amplified non-specific products that were revealed as a smear in 2% agarose. Two other pairs amplified products which were not polymorphic between plants with and without the parent AFLP bands. The fourth pair from the AFLP band AGC-CTG-c (Gichuru et al., 2008) amplified a SCAR (ScAGC-CTG-c) that identified the presence of the parent marker in a dominant manner. The amplification of the SCAR was sensitive to annealing temperature and the optimum temperature to detect the polymorphism in this study was 62 °C (Figure 1). The conversion of AFLP markers into SCAR markers is not often successful as also observed other workers such as Shan *et al*. (1999) in wheat and barley and Diniz *et al*. (2005) in coffee. The most effective parameters in optimization of PCR results are annealing temperatures and concentration of Mg^{++} ions (Zhang and Stommel, 2001). The success of achieving polymorphism by alteration of annealing temperature depends on the degree of mismatch between the primers and DNA sequences. An optimum is achieved between low temperatures that amplify all samples and higher temperatures that lead to unreliable results as observed in this study. For successful use of this marker, pre-testing to optimise and ascertain the difference in amplification is due to genetic factors and not due to technical factors is recommended. This is because the temperature regimes of different thermocyclers in different conditions might affect the results. It is also important to ensure that lack of amplification is not due to technical attributes of the DNA sample such as its quality. Currently the analysis of ScAGC-CTG-c has been successfully demonstrated in Coffee Reaseach Foundation, Kenya

The marker is dominant and therefore less informative than co-dominant markers but is as informative as the parent AFLP marker. However it has the advantage of being analysed in agarose and revealed by ethidium bromide which makes it suitable for use in laboratories lacking in the higher skills and equipments/reagents required for AFLP analysis. The utility of ScAGC-CTG-c is especially high in breeding programmes due to its position in the

chromosomal fragment carrying the CBD resistance gene in relation to two microsatellites mapped onto the same chromosomal fragment (Gichuru et al., 2008). Use of the three markers will enable selection of recombinant plants on both sides of the gene, which will be useful both for MAS breeding and collection of recombinant plants for finer mapping. The markers will be of importance for breeding even in regions which do not have CBD.

ACKNOWLEDGEMENTS

This study was supported financially by IRD, France, EU (CBDRESIST Project) and CRF, Kenya. Technical assistance from IRD, CRF and CIRAD staff is gratefully appreciated. The presentation of this paper was supported financially by CTA through the ASIC 2008 Organisers (Contract CTA/ASI-Project No 2-8-23-308-8). This paper is published with the permission of the Director of Research, CRF.

REFERENCES

Combes M.C., Andrzejewski S., Anthony F., Bertrand B., Rowelli P., Graziosi G. and Lashermes P. (2000). Characterization of microsatellite loci in *Coffea arabica* and coffee species. Mol Ecol 9: 1178-1180.

Diniz L.E.C., Sakiyama N.S., Lashermes P., Caixeta E.T., Oliveiera, Zambolin E.M., Loureiro M. E., Pereira A. A. and Zambolin L. (2005). Analysis of AFLP markers associated to the *Mex*-1 locus in Icatu progenies. Crop Breed Applied Biotech 5: 387-393.

Gichuru E.K., Combes M.-C., Mutitu E.W., Ngugi E.C.K., Bertrand B., Lashermes P. (2006) Characterisation and genetic mapping of a gene conferring resistance to coffee berry disease (*Colletotrichum kahawae*) in Arabica coffee (*Coffea arabica* L.) *21st International Scientific Colloquium on Coffee. (Montpellier, France, 11-15th September)*

Gichuru E.K. (2007) Characterization of genetic resistance to Coffee Berry Disease (*Colletotrichum kahawae* Waller and Bridge) in Arabica coffee (*Coffea arabica* L.) that is introgressed from *Coffea canephora* Pierre. PhD Thesis University of Nairobi, Kenya, 225p.

Gichuru E.K., Agwanda C.O., Combes M.C., Mutitu E.W., Ngugi E.C.K., Bertrand B. and Lashermes P. (2008). Identification of molecular markers linked to a gene conferring resistance to coffee berry disease (*Colletotrichum kahawa*e) in C*offea arabica* L. Pl. Path. (Online puplication)

Griffiths E., Gibbs J.N. and Waller J.M. (1971). Control of coffee berry disease. Ann Appl Biol. 67: 45-74

Lashermes P., Andrzejewski S., Bertrand B., Combes M.C., Dusert S., Graziosi G., Trouslot P. and Anthony F. (2000). Molecular analysis of introgressive breeding in coffee (*Coffea arabica* L.) Theor Appl Genet 100: 139-146.

Masaba D.M. and Waller J.M. (1992). Coffee Berry Disease: The current status. *In*: Bailey, J. A. and Jerger, M. J. (Eds), *Colletotrichum*; Biology, pathology and control. CABI Wallingford. UK. pp 237-249.

Nyoro J.K. and Sprey L.H. (1986). Introducing Ruiru 11 to estates and smallholders. Kenya Coffee 51: 7-28.

Omondi C.O., Ayiecho P.O., Mwang'ombe A.W. and Hindorf H. (2001). Resistance of *Coffea arabica* cv Ruiru 11 tested with different isolates of *Colletotrichum kahawae*, the causal agent of coffee berry disease. Euphytica 121: 19-24

Poncet V., Hamon P., Sauvage de Saint Marc M.B., Bernard T., Hamon S. and Noirot M. (2005). Base composition of *Coffea* AFLP sequences and their conservation within the genus. Journal of Heredity 96: 59-65.

Rafalski J.A., Vogel J.M., Morgante M., Powell W. Andre C. and Tingey S.V. (1996). Generating and using DNA markers in Plants. In: Non-mammalian genomic analysis: A practical guide (Biren, B. and Lai, E., Eds), Academic Press Inc. New York p 75-134.

Shan X., Blake T.K. and Talbert L.E. (1999). Conversion of AFLP markers to sequence specific PCR markers in barley and wheat. Theoretical and Applied Genetics 98: 1072-1078.

Silva M.C., Varzea V., Guerra-Guimaraes L., Azinheira H.G., Fernandez D., Petitot A.-S., Bertrand B., Lashermes P. and Nicole M.(2006). Brazilian Journal of Plant Physiology 18: 119-147.

Van der Vossen H.A.M. and Walyaro D.J. (1980). Breeding for resistance to coffee berry disease in *Coffea arabica* L. Inheritance of the resistance. Euphytica 29: 777-791.

Zhang Y. and Stommel J.R. (2001). Development of SCAR and CAPS markers linked to the *Beta* gene in tomato. Crop Science 41: 1602-1608.

Two Critical Factors: *Agrobacterium* Strain and Antibiotics Selection Regime Improve the Production of Transgenic Coffee Plants

M.K. MISHRA[1,2*], H.L. SREENATH[1], JAYARAMA[1], A.C. McCORMAC[2], S. DEVI [2], M.C. ELLIOTT[2] AND A. SLATER[2]

[1]Central Coffee Research Institute, Coffee Research Station- 577117,
Chikmagalur –Dist, Karnataka, INDIA
[2]The Norman Borlaug Institute for Research and Training in Plant Science,
De Montfort University, The Gateway, Leicester, LE1 9BH, UnitedKingdom.
[*]E mail: manojmishra.m@gmail.com

SUMMARY

The development of an efficient method for plant genetic transformation is critical to the success of genetic engineering. Among various transformation methods used, *Agrobacterium* mediated transformation is the method of choice for many crop plant species including coffee. However large scale use of this technology in coffee transformation has been limited due to low transformation efficiency and genotype specificity. To improve *Agrobacterium tumefaciens* transformation of coffee, we examined the effect of different factors on t-DNA transfer using *pBECKS 2000* vector constructs incorporating two different reporter (*GUS* and *SGFP*) genes. Improved transformation frequencies were obtained with *Agrobacterium* strain *EHA 101* and EHA 105 compared to AGL1 and LBA 4404. Optimized co-cultivation was performed at 22 °C in an acidic co-cultivation medium (pH 5.4) in the presence of acetosyringone. Two to three weeks old somatic embryogenic calli obtained from the hypocotyls and leaf explants were most receptive to t-DNA transfer. Selection of transformed tissue was carried out at monthly intervals in Murashige and Skoog (MS) salts and vitamins supplemented with 0.1 mg/l 2,4-D, 1 mg/l IAA and 4 mg/l kinetin and further supplemented with 20 mg/l hygromycin. By combining the optimized parameters an efficient and transformation procedure was established for three commercial cultivars of coffee. The production of non transformed 'escapes' has been completely eliminated. A large number of independently transformed plants were regenerated and established in the glass house Regenerated plants exhibit normal growth and development.

INTRODUCTION

Genetic engineering has many potential applications in fields of agriculture and medicine. In agriculture, plant genetic transformation is used as a core research tool for genetic improvement of crop plants by incorporating desirable traits such as disease and insect resistance, drought and salt tolerance and herbicide resistance. Besides, transgenic technology has also been used for increasing photosynthetic efficiency, nitrogen fixing ability, increasing nutritional value and production of hybrid crops for molecular farming (Suzuki et al., 2000; Daniel 2001; Aharon et al., 2003).

The success of genetic engineering depends on the development of an efficient gene transfer and regeneration system. However, there is no universally applicable method of culture, generation and transformation systems for all crop plants as different plants respond

differently to *in vitro* culture. Among various gene transfer methods currently employed, *Agrobacterium* – mediated gene transfer is considered as the method of choice because of several advantages which includes (1) the ability to transfer large segments of DNA (2) precise integration of transgene with fewer copies and (3) simple and low cost technology. However *Agrobacterium* transformation is influenced by many variables such as plant genotype, target tissue, *Agrobacterium* strains, binary vector system, antibiotics selection and culture conditions (Pniewski and Kapusta, 2005; De Clercq, 2002; Mishra et al., 2002; Mishra and Sreenath, 2004; Mohamed et al., 2004). In coffee, *Agrobacterium* mediated transformation is achieved in both arabica and robusta using various target tissue and different antibiotics selection (Ribas et al., 2006). However the efficiency of transformation reported was very low and the routine transformation in coffee is very cumbersome (Hatanaka et al., 1999; Leroy, 2000; Ribas et al. 2006). Recently Mishra and Sreenath (2004) have reported a highly efficient transformation protocol in robusta coffee using hypocotyls explants. The goal of the investigation reported here was to further improve the transformation efficiency and reproducibility in both arabica and robusta coffee. Therefore, the influence of various *Agrobacterium* strains, co cultivation conditions and the antibiotics regime was assessed using both *uidA* and *Sgfp* reporter system. Secondly, the optimized transformation conditions were shown to facilitate the production of large number of phenotypically normal transgenic plants and transgene integration was confirmed through molecular analysis.

MATERIALS AND METHODS

Plant material:

Three commercial coffee cultivars belonging to *C. canephora* (var. S.274 and C x R) and *C. arabica* were used for transformation experiments. Both leaves and mature seeds were used as start up material to establish *in vitro* culture. Seeds were dehusked and disinfected for 2 min in 70% (v/v) ethanol for 2 min followed by dipping in 30% Domestos for 15 min. and rinsing four times with sterile distilled water at 10 min intervals and finally rinsed with 1 % ascorbic and citric acid solution. The sterilized seeds were dried in filter paper for 30 min following which they were inoculated in Culture bottles containing germination medium. The germination medium consists of half strength Murashige and Skoog (MS) salts solution (Murashige and Skoog, 1962) together with 0.2 mg/l thiamine hydrochloride, 1 mg/l pyridoxine hydrochloride, I mg/l nicotinic acid, 2% sucrose and 2.5 g/l phytagel with the pH of the medium adjusted to 5.8. The media was autoclaved at 121 °C for 20 min.

Leaf explants were collected both from the green house as well as the field grown plants. The disinfection protocol of leaf explants is same as described earlier. The leaf explants were incubated in medium containing Full strength MS medium supplemented with various growth regulators 2,4D (0.1-0.5 mg/l), IAA (1 mg/l) and kinetin (4 mg/l) with 3% sucrose and gelled with 2.5 g/l phytagel for callus induction.

All the culture bottles were kept in dark till seed germination following which they are transferred to light with a 16-h photo period at a 40-50 µE m^{-2} s^{-1} provided by cool fluorescents tubes. The leaf explants were however incubated continuously in dark till the development of globular somatic embryos.

Bacterial strains and plasmids

Four *Agrobacterium tumefaciens* strains were used in this study: LBA 4404, AGL1, EHA 101, and EHA 105 (Table.1). Binary vectors pBECKS2000 series which incorporates the clean gene facilities (Mc Cormac et al., 1999) were introduced in to all four *Agrobacterium*

strains by electroporation. All the binary vectors contain various reporters and selectable marker genes (Table.1) of which GUS, HPH and GFP genes are under the transcriptional control of 35S promoter and selectable marker NPTII gene under the control of nopaline synthase (nos) promoter.

Engineered *Agrobacterium* strains were grown on Lauria-Bertani medium (10 g l^{-1} Bacto-tryptone, 5 g l^{-1} Bacto-yeast extract, 1 g l^{-1} Nacl, 1g l^{-1} glucose; pH 7.0) solidified with 15 g l^{-1} Bacto-agar with appropriate antibiotics (Table.1). For co-cultivation, a single colony was inoculated in to 20 ml of LB medium with appropriate antibiotics, incubated for with 16 hours with constant agitation (180 rpm). Overnight cultures were centrifuged at 3500 rpm for 10 min and resuspended on 20 ml of bacterial suspension medium consisting of MS salts, 3% (w/v) sucrose, 0.1 mg/l 2,4-D, 0.5 mg/l IAA, and 4 mg/l kinetin with 50µM acetosyringone , pH5.4. Cultures in bacterial suspension were shaken (180 rpm) for 2 hours before use.

Transformation procedure

Embryogenic calli attached to the hypocotyl and leaf explants were transferred to the pre culture medium consists of MS salts and vitamins supplemented with 0.5 mg/l 2,4-D, 1 mg/l IAA and 4 mg/l kinetin and gelled with phytagel 2.5 g/l for three days. Following pre culture, embryogenic calli were immersed in *Agrobacterium* suspension for 20 min, bacterium suspension drained out and the calli were transferred to solid co cultivation medium with 50µM acetosyringone and incubated in the dark at 25 °C for three days to inhibit A*grobacterium* over growth as well as to have the calli recovered from co-cultivation shock.

Transformants selection and regeneration

Following co-culture with *Agrobacterium* for four days, the infected embryogenic calli were washed first with sterile distilled water two times of 10 min duration following which they were washed with basal medium (half strength MS salts and vitamins) containing 250 mg/l cefotaxim for two times of 15 min duration to supress the *Agrobacterium* growth. The calli were then dried on a filter paper and devided in to three equal parts for transformants selection. All the transformation procedure were carried out in dark for six weeks.

In the first experiment, co-cultivated embryogenic calli were transferred to 30 mg/l hygromycin for transformants selection and this dose of hygromycin was maintained in subsequent subculture. In the second experiment 10 mg/l hygromycin was supplemented to the selection medium. After two rounds of selection at 4 weeks interval, hygromycin concentration was doubled and maintained at 20 mg/l till transformants regeneration. In the third experiment the proceure of Hatanaka et al. (1999) was followed in which both hygromycin and kanamycin was employed in transformants selection.

Molecular analysis

High molecular weight genomic DNA was isolated from the leaves of control and transgenic plants using the protocol of Mishra et al. (2008) with slight modification. After RNase A treatment the DNA was re-suspended in TE buffer. PCR was performed in a MJ Research thermocycler. The primer sequences of *hpt, gusA and nptII* genes and the amplification conditions were same as mentioned earlier (Mishra and Sreenath, 2003).

Southern blot hybridization was carried out to confirm the integration of transgene in coffee genome. Genomic DNA was digested with *EcoRI* and *HindIII* separated on 0.8% agarose gels and blotted to nylon membrane (Hybond N^+, Amersham) and redivue α-32p) –dCTP

(Amersham). The filter was hybridized with 32 p-dCTP probe as described by Sambrook et al. (1989).

Table 1. *Agrobacterium* strains, vectors, selection and marker genes used for coffee transformation.

Bacterial strain	Origin and Helper plasmid	Binary plasmid	Marker genes	selection	Opine type	reference
LBA4404	Ach5 pAL4404	pBECKS 2000	NPTII, HPH, GUS/ NPTII, HPH, GFP	Spectinomycin ^{200}	Octopine	Hoekema et al. 1983
AGL1	C58 with pTiBO542T from A281	pBECKS 2000	NPTII, HPH, GUS/ NPTII, HPH, GFP	Spectinomycin ^{200}	Agropine	Lazo et al. 1991
EHA101	pTi EHA101	pBECKS 2000	NPTII, HPH, GUS/ NPTII, HPH, GFP	Spectinomycin ^{200}	Agropine	Hood et al. 1986
EHA105	pTi EHA101	pBECKS 2000	NPTII, HPH, GUS/ NPTII, HPH, GFP	Hygromycin^{50}	Agropine	Hood et al. 1993

RESULTS AND DISCUSSION

Comparison of four *A. tumefaciens* strains

This study for the first time demonstrates the transfer and expression of T-DNA from four different *A. tumefaciens* strains to coffee embryogenic cells. Two different opine type *A. tumefaciens* strains (LBA 4404, AGL1, EHA 101 and EHA105) with various chromosomal background all harbouring pBECKS2000 vector constructs were compared (Figure 1) using same infection protocol. The expression of gus and sgfp genes in embryogenic tissue were evaluated after five days of inoculation showed high variability in transient expression. Variability gene transfer efficiency using different *Agrobacterium* strains were reported in soybean (Ko et al., 2003) Medicago (Chabaud et al., 2003) and pinus (Humara et al., 1999) and the present observation is in agreement with the earlier reports.

As observed from the transisent gene expression assay of gusA gene, the most efficient *A. tumefaciens* strain for the transfer of T-DNA to coffee embrygenic cells was EHA 105 and EHA 101 both belonging to agropine type of bacterial strains (Fig.1). AGL1 which is also an agropine type of bacterial strain carrying the *vir A* and *vir G* genes is comparatively less effective than EHA 105 and EHA 101 in transferring genes. *Agrobacterium* LBA 4404 which is an octopine strain is least effective. Although, no significant variation was noticed in transient expression between EHA 101 and EHA 105, higher regeneration of transgenic plants were achieved using EHA 105 compared to EHA 101 irrespective of the reporter gene constructs in four repetitive experiments. All the three bacterial strains i.e. AGL1, EHA 101 and EHA 105 are agropine types with identical chromosomal background derived from A281, it is surprising to see their differences in agro-infection efficiency. A combination of factors such as plasmid background, *vir* genes and their induction, *Agrobacterium* growth phage,

culture medium compositions, temperature and target tissue could determine the gene transfer efficiency in plants and it could be possible that majority of the combinations are favourable for vir gene induction in EHA 105 leading to its hypervirulence.

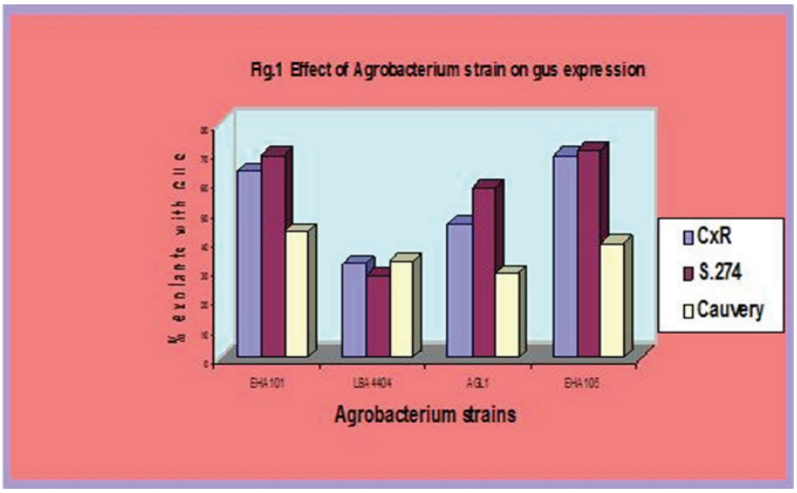


Figure 1.

Evaluation of factors influencing T-DNA transfer

Various factors influencing the T-DNA delivery efficiency were evaluated using pBECKS2000 gene construct incorporating both gusA and sgfp reporter genes. Both acetosyringone concentration and co-cultivation period was tested independently for all the *Agrobacterium* strains.

Effect of acetosyringone

In our protocol, agrobacteria were pre-cultured for 4 hour with different concentration (0, 25, 50, 75, 100 and 200 µm) of acetosyringone (AS) and the same concentration of AS was also added to co-cultivation medium. This experiment was repeated three times and always gave consistent results. The number of sgfp and gus zones increased with the increase in AS concentration and reached maximum when 50 µm AS was used in case of agrobacterial strains EHA 105 and EHA 101 following which there was a sharp decline in transient expression. However, in case of Agrobacterium strains like AGL1 and LBA4404 maximum transient expression of gusA and gfp was obtained when 75 µm AS was used and further increase in AS concentration had negative effect on transient expression. Variation in AS concentration requirement by different Agrobacterial strains could be related to their differences in vir gene activation. The beneficial role of acetosyringone has been demonstrated in several woody plants such as apple (James et al., 1993), kiwifruit (Jansen and Gardner 1993) Christmas tree (Tang and Newton, 2005) and coffee (Hatanaka et al., 1999; Mishra et al., 2002) and the present study further confirmed the requirement of AS for coffee transformation.

Effect of co-cultivation period

In many plants, higher transient expression was obtained by increasing the co- cultivation period. In citrange, transient transformation frequency increased reaching maximum after five days of co-cultivation (Cervera et al., 1998).In the present study, the transient transformation frequency increased progressively and reached maximum after 5 days using all the *Agrobacterium* strains. However severe bacterial over growth was noticed in calli co-

cultivated with AGL1, EHA101 and EHA105 agrobacterial strains. These embryogenic calli could not be separated from the bacterial out growth in spite of repeated washing with antibiotics. However, calli transformed with LBA4404 could be controlled using cefotaxime. Therefore, maximum of 4 days of co-cultivation was found to be optimum for coffee transformation.

Antibiotics selection regime

In any transformation protocol, antibiotics selection is critical to the regeneration of transgenic plants. The type of antibiotics, quantity and time of application influence the regeneration process. In the present study three different antibiotics selection protocols were compared and it was observed that selection of transformants with low level of hygromycin (10 mg/l) for 6 weeks followed by selection at 20 mg/l hygromycin not only improved the recovery of transgenic plants but also completely eliminated the escapes. Selection of transformants at high level of hygromycin (30 mg/l) immediately after co-cultivation although completely eliminated the escapes but reduced the differentiation of embryogenic calli which resulted in the production of lower number of transgenic plants. The antibiotics selection regime of Hatanaka et al. (1999) resulted in the production of many escapes as well as lower transformants recovery as both kanamycin and hygromycin were used for transformants selection.

Transgene expression and molecular analysis

The expression of both uidA and gfp genes were monitored soon after co-cultivation. Expression of the *sgfp-S65T* gene driven by the CaMV35S promoter (signified by green fluorescence) was observed in co-cultivated calli just 2 d after co-cultivation. During this period often green fluorescence was obscured by background fluorescence at other wave lengths. In majority of the cases, yellow and red fluorescence was commonly intermixed with green fluorescence. Initially, green fluorescence appeared as discrete spots but subsequently, calli that showed green fluorescence increased with bright fluorescence mass after 15 days co-cultivation. This pattern of expression was similar to results obtained using the gusA marker gene in the same system (Mishra et al, 2002).

The expression of both *gusA* and *sgfp* was intense in globular and torpedo shaped embryos till the development of cotyledonary leaves. In older leaves, green florescence was week due to the interference of chlorophyll which emits red fluorescence at the same wave length. Transgene expression was much more pronounced in the leaf veins and root tips and in vascular zones. This expression pattern of transgene is characteristics feature of CaMV35S promoter. The transgenic plants were morphologically normal in growth and development.

Transgenic plants were subjected to PCR analysis which revealed the integration of transgenes. Southern blot hybridization was carried out revealed the integration of transgene in coffee genome. The site of integration and transgene copy number was detected which indicated maximum of two copies of transgene to coffee genome.

In conclusion, we have developed a simple and highly efficient *Agrobacterium* mediated transformation protocol in coffee and regenerated a large number of transgenic plants and transgene integration was confirmed through molecular analysis.

ACKNOWLEDGEMENT

The senior author is thankful to the Department of Biotechnology, Govt. of India for an overseas associateship to NBIPSR, UK for carrying out a part of the work mentioned here. We thank Jen sheen for gifting the plasmid SGFP (S65T) used in this work.

REFERENCES

Aharon R., Shahak Y., Wininger S., Bendov R., Kapulnik., Galili G. (2003) Over expression of aplasma membrane aquaporin in transgenic tobacco improves plant vigor under favourable growth conditions but not under drought or salt stress. Plant Cell 15: 439-447.

Cervera M., Pina J.A., Juarez J., Navarro L., Pena L. (1998) *Agrobacterium* mediated transformation of citrange: gactors affecting transformation and regeneration Plant Cell Rep. 18: 271-278.

Chabaud M., Carvalho-Nibel F. de, Barker D.G. (2003) Efficient transformation of Medicago truncalata cv. Jemalong using the hypervirulent *Agrobacterium tumefaciens* strain AGL1 Plant Cell Rep 22: 46-51.

Daniell H. (2001) Medical molecular farming: production of antibodies, biopharmaceuticals and edible vaccines in plants. Trends in Plant Sci., 6: 219-226.

De Clercq J., Zambre M., Van Montagu M., Dillen W., Angenon G. (2002) An optimized Agrobacterium mediated transformation protocol for phaseolus A. Gray Plant Cell Rep 21: 333-340.

Hatanaka T., Choi Y.E., Kusano T., Sano H. (1999) Transgenic plant of coffee. *Coffea canephora* from embryogenic callus via-*Agrobacterium tumefaciens* mediated transformation. Plant Cell Rep. 19:106-110

Hoekema A., Hirsch P.R., Hooyka P.K., Schilperoort R.A. (1983) A binary plant vector strategy based on separation of vir- and T- region of the *Agrobacterium tumefaciens* Ti-plasmid. Nature 303:179-80.

Hood E.E., Gelvin S.B., Melchers L.S., Hoekema A. (1993) New *Agrobacterium* helper plasmids for gene transfer to plants. Transgene Res 2:208-218.

Hood E.E., Helmer G.L., Fraley R.T., Chilton M.D. (1986) The hypervirulence of *Agrobacterium tumefaciens* A281 is encoded in a region of pTiB0542 outside the T-DNA J.Bacteriol. 169: 1291-1301.

Humara J.M., Lopez M., Ordas R.J. (1999) Agrobacterium tumefaciens – mediated transformation of *Pinus pinea* L. cotyledons: an assessment of factors influencing the efficiency of *uidA* gene transfer Plant Cell Rep19: 51-58.

James D.J., Uratsu S., Cheng J., Negri P., Viss P., Dandekar A.M. (1993) Acetosyringone and osmoprotectants like betaine or praline synergistically enhance Agrobacterium mediated transformation of apple. Plant Cell Rep. 12: 559-563.

Jansen B.J., Gardner R.C. (1993) The use of transient GUS expression to develop an Agrobacterium – mediated gene transfer system for kiwifruit. Plant Cell Rep.13: 28-31

Ko T.S., Lee S., Krasnyanski S., Korban S.S. (2003) Two critical factors are required for efficient transformation of multiple soybean cultivars: *Agrobacterium* strain and orientation of immature cotyledonary explant. Theor Appl Genet 107: 439-447

Lazo G.R., Stein P.A., Ludwig R.A. (1991) A DNA transformation competent *Arabidopsis* genomic library in *Agrobacterium*. Biotechnology 9: 963-967.

Leroy T., Henry A.M., Royar M., Altosar Frutos R., Duris D., Philippe R. (2000) Genetically modified coffee plants expressing the *Bacillus thuringiensis cry* 1Ac gene for resistance to leaf minor. Plant Cell Rep 19: 382-389.

McCormac A.C., Elliott M.C., Chen D.F. (1999) pBECKS2000: a novel plasmid series for the facile creation of complex binary vectors, which incorporates clean gene facilities Mol Gen Genet 261: 226-235.

Mishra M.K., Sandhyarani N., Santaram A., Sreenath H.L., Jayarama (2008) A simple method of DNA isolation from coffee seeds suitable for PCR analysis. African Journal of Biotechnology 7: 409-413.

Mishra MK, Sreenath HL (2004) High- efficiency *Agrobacterium* –mediated transformation of coffee (*Coffea canephora* Pierre ex. Frohner) using hypocotyls explants Proceedings of the 21st International Association for Coffee Science (ASIC) , Bangalore, India, pp 792-796.

Mishra M.K., Sreenath H.L., Srinivasan C.S. (2002) *Agrobacterium* mediated transformation of coffee : an assessment of factors affecting gene transfer efficiency. PLACROSYM XV, edited by K.Sreedharan, P.K.Vinod Kumar, Jayarama & B.M.Chulaki pp 251-255

Mohamed Sh., Boehm R., Binsfeld P.C., Schnabl H. (2004) *Agrobacterium* – mediated transformation of two high oleic sunflower (*Helianthus annuus* L.) Genotypes: assessment and optimization of important parameters. Helia. 27. Nr.40.pp 25-40.

Murashige T., Skoog F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture Physiol Plantarum 15: 473-479

Pniewski P., Kapusta J. (2005) Efficiency of transformation of polish cultivars of pea (*Pisum sativum* L.) with various regeneration capacity by using hypervirulent *Agrobacterium tumefaciens* strains. J. Appl. Genet 46(2) 139-147.

Ribas A.F., Pereira L.F.P., Vieira L.G. (2006) Genetic transformation of coffee Braz J.Plant Physiol 18(1) 83-96.

Sambrook J., Fritsch E.F., Maniatis T. (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory press, Cold Spring Harbor, NY

Suzuki S., Murai N., James N., Burnell R., Arai R. (2000) Changes in photosynthetic carbon flow in transgenic rice plants that express C4 –Type phosphoenolpuruvate carboxylase from *Uroclhoa panicoides*. Plant Physiol., 124: 163-172.

Tang W., Newton R.J. (2005) Transgenic Christmas trees regenerated from Agrobacterium tumefaciens mediated transformation of zygotic embryos using the green fluorescence protein as a reporter Mol. Breeding 16: 235-246.

Sensing Low Temperature in *Coffea* sp. through Photosynthesis and Gene Expression Analysis

P. EICHLER[1,2,*], J.C. RAMALHO[1,3], F. PARTELLI[4], A. FORTUNATO[1], M.M. CHAVES[2,5], A. RIBEIRO[1,3]

[1]Centro de Ecofisiologia, Bioquímica e Biotecnologia Vegetal / Inst. Inv. Científica Tropical, Av. República, Quinta do Marquês, 2784-505 Oeiras, Portugal. [*]E-mail: peichler@iict.pt
[2]Inst. Superior de Agronomia/UTL, Tapada da Ajuda, 1349-017 Lisboa, Portugal
[3]Unid. Biotecnologia Ambiental/FCT/UNL, 2829-516 Monte de Caparica, Portugal
[4]Universidade Federal de Goiás - UFG, Escola de Agronomia e Engenharia de Alimentos. Campus II Samambaia. Caixa Postal 131, 74001-970, Goiânia - GO, Brazil
[5]Inst. Tecnol. Quím. Biológica/UNL, Quinta do Marquês, 2781-901 Oeiras, Portugal

SUMMARY

Photosynthesis is a highly integrated and regulated process which is sensitive to environmental conditions. Low temperatures leads usually to an imbalance between energy captured and its use through metabolic sinks, thus requiring adjustments of photosynthesis to maintain the balance of energy flow. In this way, photosynthesis acts as a sensor of that imbalance, namely, through the redox state of photosynthetic electron-transport components and interacts with other processes to regulate plant acclimation to low temperatures. Despite the known cold sensitivity of coffee plant to low temperatures, previous works reported appreciable differences in cold sensitivity and acclimation ability in *Coffea* sp. In order to contribute to the understanding of the molecular basis of the chilling acclimation process, particularly in what concerns the photosynthetic apparatus, gene expression analysis by quantitative RT-PCR was performed, using plants of *C. arabica*, *C. canephora* and an interspecific hybrid. The plants were submitted to a progressive temperature decline before the exposure to chilling (4 °C), giving them the opportunity to express differential acclimation abilities. Post stress effects were also analysed during a 14 day recovery period after the stress ending. Our results, integrated with physiological data, suggest that the transcriptional activity of some of the studied genes could be related to the degree of cold tolerance/susceptibility of coffee genotypes.

INTRODUCTION

Plants have adapted to respond to various stresses at the molecular and cellular levels as well as at the physiological and biochemical levels, thus enabling them to survive. Low temperature is a major limiting factor for plant metabolism and is often associated to a reduction in crop production, especially in tropical and sub-tropical plants. Low-positive temperatures (chilling) can impair cell metabolism, with strong impacts on membranes, respiratory and photosynthetic machinery. Among the cell structures, the chloroplast is usually the one that is more rapidly and deeply affected, influencing the photosynthetic process (Wise and Naylor, 1987).

The potential for an energy imbalance between photochemistry, electron transport and metabolism is exacerbated under conditions of cold temperatures, which lead to increased excitation pressure over the photosystems (PS). On a time scale of minutes, some plants can attempt to compensate for exposure to high PSII excitation pressure, namely, by reducing

energy transfer efficiency to PSII either by diverting energy from PSII to PSI through the state transitions mechanism or by thermal energy dissipation through non-photochemical quenching (NPQ) and some xanthophylls action. On a longer time scale, photosynthetic acclimation may also involve a reduction on PSII antenna size (Huner et al., 1998) and qualitative and quantitative changes of pigments and lipid constituents of chloroplast membranes.

Previous work performed with *Coffea* sp. plants reported the existence of appreciable differences in cold sensitivity and acclimation ability (Campos et al., 2003; Ramalho et al., 2003), thus suggesting some genetic variability that could be exploited in breeding programmes.

The expression of a variety of genes is induced by cold in various plants (Tomashow, 1999; Shinozaki and Yamaguchi-Shinozaki, 2000; Yamaguchi-Shinozaki and Shinozaki, 2006), allowing them to cope with this environmental constraint. The PSII light-harvesting complex contains a number of highly conserved gene products, like the CP24. This is an important molecule in the structure and function of PSII, providing the link for association of M-trimer into PSII complex, what allows a specific macroorganization that is necessary both for maximum quantum efficiency and for photoprotective dissipation of excess excitation energy (Kovács et al., 2006). Furthermore, it has also been suggested that plant acclimation involves an increased NPQ capacity through abundance adjustment of a gene encoding a *PsbS* (CP22) protein (Rorat et al., 2001).

Since cold acclimation is a complex mechanism involving numerous changes in gene expression, metabolism and morphology, we have examined the effect of cold stress in photosynthesis and the expression patterns of some genes related to the photosynthetic apparatus in order to better understand the basis for acclimation ability of coffee plants.

MATERIALS AND METHODS

Plant material and growth conditions

The experiments were carried out using 1.5 years old plants from the genotypes *C. canephora* cv. Apoatã (IAC 2258), *C. arabica* cv. Catuaí (IAC 81) and Piatã (IAC 387 - *C. dewevrei* x *C. arabica*). After growing under greenhouse semi-controlled conditions, the plants were transferred into a walk in growth chamber (10000 EHHF, ARALAB, Portugal) and submitted successively to: 1) a gradual temperature decrease (0.5 °C/day) from 25/20 °C (day/night) to 13/8ºC over 24 days, 2) a 3 day chilling cycle (13/4 °C), where plants were subjected to 4 °C during the night and in the first 4 h of the morning, followed by a rise to 13ºC applied throughout the rest of the diurnal period, 3) a rewarming period up to 15 days at 25/20 °C, in order to allow recovery. Photoperiod was set to 12 h, RH to 65-70% and PPFD to 800-900 μmol m^{-2} s^{-1}. Determinations were made in the 2 top pairs of recent mature leaves from each branch in 8-10 plants per genotype.

Gas exchange measurements

The net photosynthetic rate, P_n (external CO_2 set to 380 ppm), stomatal conductance to water vapour, g_s, and the internal CO_2 concentration (C_i), were measured in growth chambers during the experimental period, under photosynthetic steady state conditions after *ca*. 2 h of light exposure, using a CO_2/H_2O porometer (CIRAS I, PP Systems, UK).

Chlorophyll fluorescence parameters

For analysis of non-photochemical quenching NPQ (Adams III and Demig-Adams, 1995), the fluorescence was measured on leaves under photosynthetic steady-state conditions (PPFD 400 μmol m^{-2} s^{-1}), using a PAM 2000 system (H. Walz, Effeltrich, Germany).

Gene expression studies

cDNA clones corresponding to *caCP24, cacytf, cadhar, caPII10a* and *caPlip* genes (Table 1) were isolated from subtractive cDNA libraries obtained from coffee leaves (Fernandez et al., 2004). Based on the cDNA sequences, specific primers were designed (data not shown) in order to perform the mRNA expression studies by quantitative RT-PCR (Livak and Schmittgen, 2001; Ramalho et al., 2006).

Table 1. Genes and homologies.

Gene	Homology
caCP24	Chlorophyll a/b-binding protein CP24
caCytf	Cytochrome *f*
caPII10a	Photosystem II 10 Kd polypeptide precursor
caDhar	Dehydroascorbate reductase
caPlip	Phospholipase-like protein

RESULTS AND DISCUSSION

Gas exchange measurements

The gradual temperature decrease provoked reductions in P_n and g_s in all genotypes (Figure 1). At the moderate low temperature of 18/13 °C Catuaí showed already a 39% decrease of P_n, but Piatã and Apoatã had 48% and 54% reductions, respectively. At 13/8ºC and during chilling conditions further reductions of P_n were observed, reaching negligible values at 13/4ºC in all genotypes. The observation that the impact on P_n was clearly stronger than in g_s during cold imposition, together with the fact that internal CO_2 concentration (C_i) gradually increased until 13/4 °C, points out for photosynthesis limitation by other reasons than restriction in CO_2 supply to carboxylation sites.

After chilling exposure, the analysis of the after effects is quite important to evaluate the extension of cold stress impairments and the plant recovery ability. The P_n in Catuaí recovered somewhat faster until the 5th day of the rewarming period. However, after 15 days only Piatã showed a complete P_n recovery, while Catuaí and Apoatã presented, respectively, 74% and 59% of their control values. At this point all genotypes recovered g_s levels. These results reflect differences in cold impact of the studied genotypes as previous reported (Ramalho et al., 2003; 2006). In fact, Apoatã was the most affected genotype at the beginning of the acclimation and during the recovery period (particularly visible after 15 days). Furthermore, together with such incomplete P_n recovery, Apoatã showed a strong shed of leaves between 10 and 20 days after rewarming, leading in most plants to a total loss of leaves, a fact also observed in our group in two *C. canephora* cv. Conilon clones submitted to similar cold conditions (data not shown).

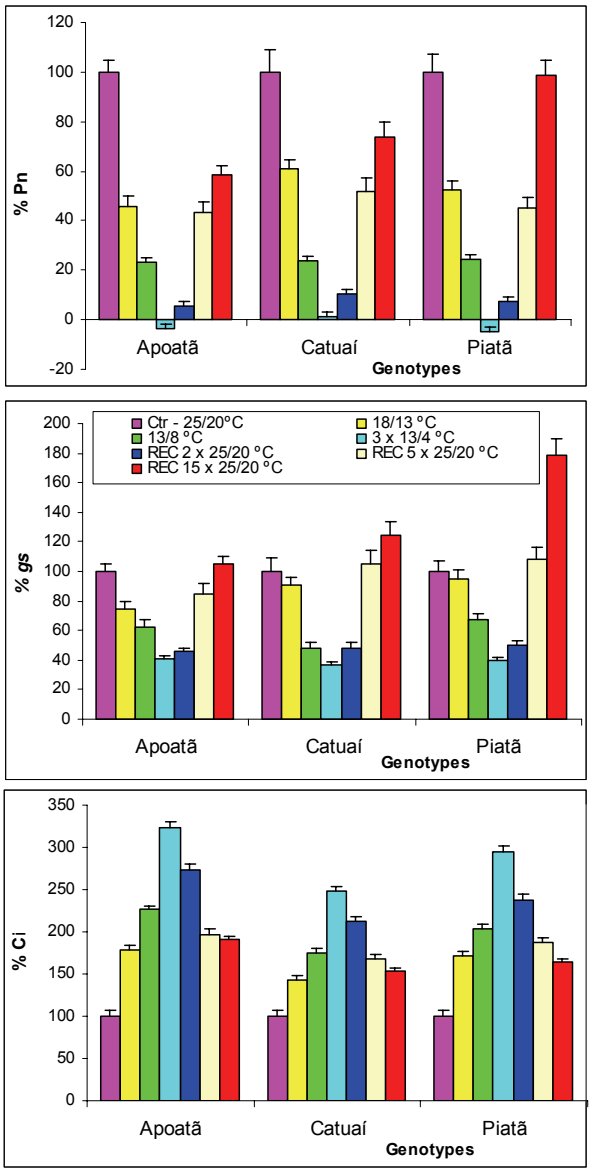


Figure 1. Variation in percentage of the net photosynthetic rate (P_n), the stomatal conductance rate to water (g_s) and the estimated internal CO_2 concentration (C_i), as compared to their respective control value. Each value represents the mean +SE (n = 15).

Gene expression studies

The expression of *caCP24* gene (Figure 2) presented progressive decreases in all genotypes along the acclimation period, reaching minimum values after chilling conditions (more than 75% reduction, as compared to control). Upon re-warming conditions Catuaí did not reach the control values, while Piatã and especially Apoatã presented a complete recovery of the expression of this gene.

On the other hand, *caCytf* expression was strongly suppressed in Apoatã during the gradual temperature decrease and during the recovery period. An opposite tendency was observed in Catuaí and Piatã with some increases higher than 60% during cold imposition. As for *caCP24*, minimum expression levels were observed under chilling conditions, but after that, Piatã and especially Catuaí presented an enhanced transcriptional activity. The accumulation

of thylakoid proteins, which are the products of chloroplast genes, (*e.g.*, cyt *f*) is repressed as growth temperature is lowered (Nie and Baker, 1991). That probably occurred in a more pronounced way in Apoatã, as reflected in the stronger impacts on *caCytf* expression observed in Apoatã over the all experiment.

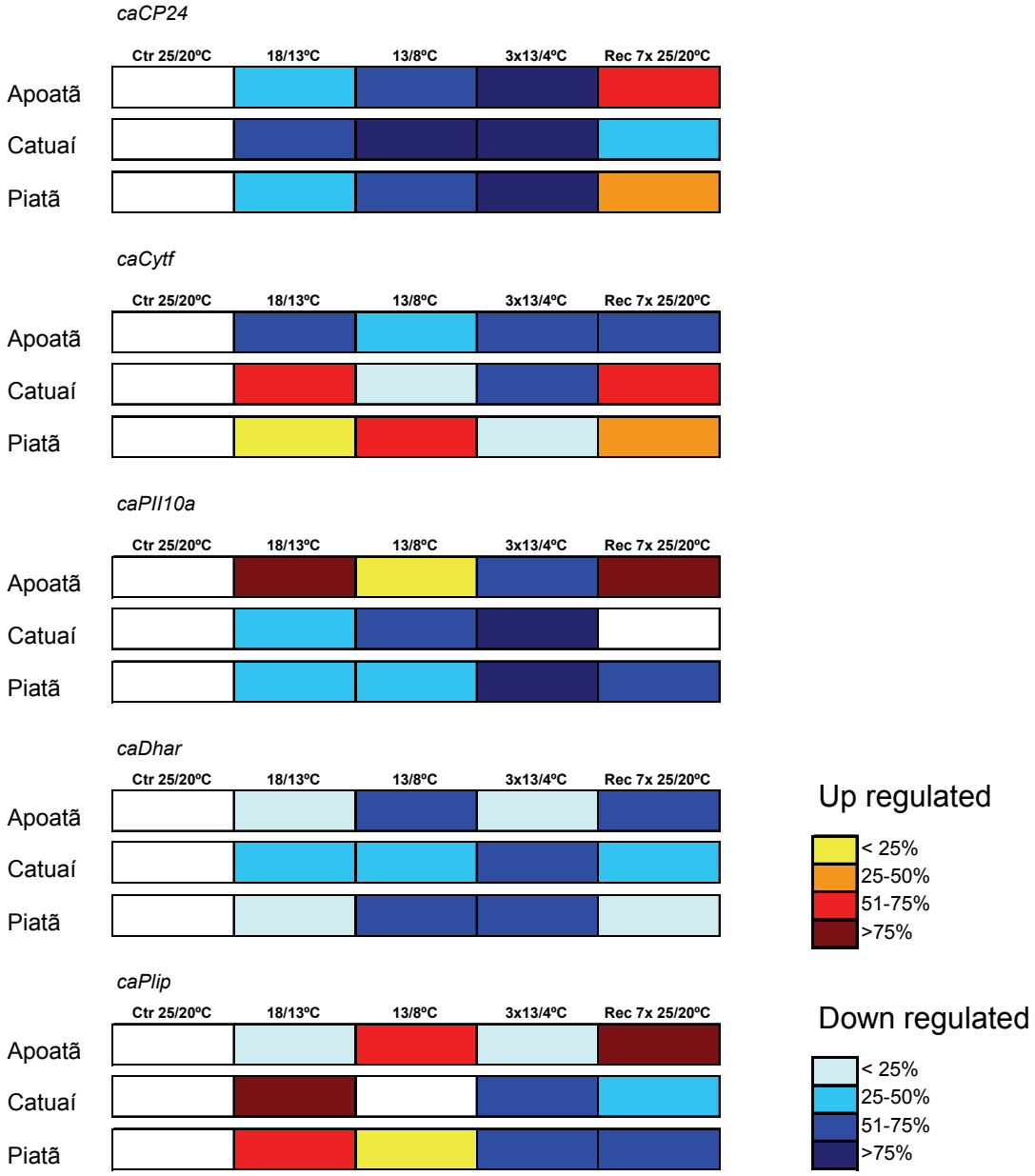


Figure 2. Relative expression level of *caCP24*, *caCytf, caPII10a, caDha* and *caPlip* genes evaluated by real-time PCR in *Coffea* sp. genotypes under control conditions (25/20 °C), by the middle (18/13 °C) and at the end of the gradual temperature decrease (13/8 °C), after 3 cycles at 13/4 °C (3x13/4 °C) and upon 7 days of recovery (Rec) at 25/20 °C. Each value represents a mean value of 3 independent experiments. All changes are expressed relative to control (25/20 °C) that was considered to represent 100%.

During cold exposure the expression of the gene *caPII10a,* encoding the 10 kd protein of the water-splitting system of PSII, highly increased in Apoatã (more than 75%) at 18/13 °C, but declined after that. In the other genotypes a down regulation pattern was observed during cold exposure. All genotypes showed the strong decreases after the chilling exposure. Upon re-

warming expression differences were observed again, with Piatã not recovering while Catuaí and especially Apoatã presented a complete recovery. In potato plants the elimination of the 10 kd protein retarded the reoxidation of Q_A and introduced a general disorder into the PS II complex (Stockhaus et al., 1990). However, since Catuaí and Piatã showed the best P_n recoveries, despite the down regulation of *caPII10a* expression, PSII components were probably preserved/repaired without the need of *de novo* synthesis of this constituent.

In relation to this set of genes, related to constituents of PSII structure (*caCP24, caPII10a*) and thylakoid electron transport (*caCytf*), it seems that under cold conditions their expression changes did not show clear trends or differences, being strongly affected under chilling conditions. Therefore they seem not to constitute good candidates as cold-tolerance markers in coffee.

However, a more detailed analysis involving *caCP24* expression and NPQ formation (Figure 3) was performed, since some studies have demonstrated that the xanthophyll cycle pigments (involving violaxanthin, anteraxanthin and zeaxanthin) are enriched in the minor CPs (as CP24), where they could play an important role in photoprotection under conditions of excess of light energy (Bassi et al., 1993; Gilmore, 1997).

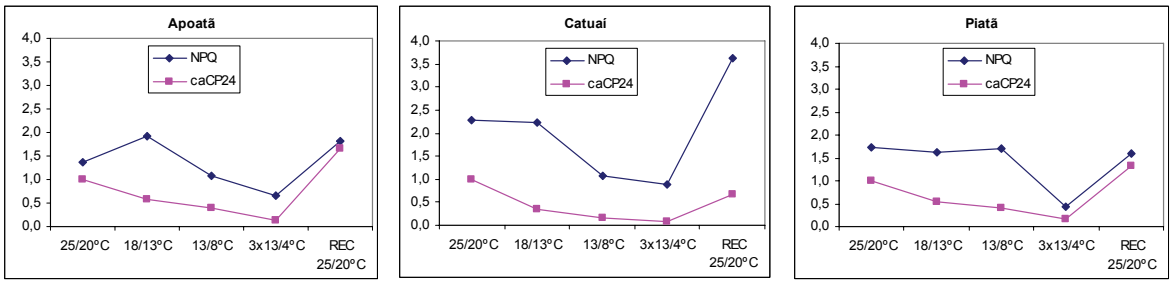


Figure 3. Evolution of NPQ formation and *caCP24* gene expression levels determined in *Coffea* sp. genotypes under control conditions(25/20 °C), by the middle (18/13 °C) and at the end of the gradual temperature decrease (13/8 °C), after 3 cycles at 13/4ºC (3x13/4 °C) and upon 5-7 days of recovery (Rec) at 25/20 °C. Each value represents the mean + SE (n = 3 for gene expression and n = 25 for NPQ determinations).

With the gradual temperature decrease NPQ and *caCP24* expression levels tended to be reduced in all genotypes, reaching its minimum values after chilling exposure. Upon recovery there was an increase in the levels of both parameters for all genotypes. This was particularly evident for Catuaí that presented increases in NPQ levels superior than 50% of the control values. Assuming that the *caCP24* expression is related to the presence of the corresponding protein, our results are in agreement with those of (Kovács et al., 2006) that reported a dramatic reduction in NPQ upon removal of the antenna complex, CP24, in higher plants. Based on these observations the authors suggested that CP24 is also involved in the build up of NPQ, providing the site of interaction with *Psbs* (CP22) and zeaxanthin.

Following the expression studies on photosynthesis-related genes, we have further examined another set of genes that could be involved in the acclimation process. The expression of the gene *caDhar* encoding a DHAR was down regulated in all genotypes over the experiments. Only Piatã showed a value close to control after the re-warming period.

Exposition to cold stress can cause cellular damage and secondary oxidative stress through the formation of ROS (Wang et al., 2003). In this way, the presence of reactive oxygen species (ROS) should be kept under control and the presence of oxidative scavengers, such as

those involved in the ascorbate/glutathione cycle, as is the case of DHAR, is of great importance. The decreases of *caDhar* expression observed could reflect a lower production of this enzyme. Previously reported by Ramalho et al. (2006), Apoatã showed higher levels of $OH^{\bullet}$ than Catuaí. Such differential control can result from several contributions, but DHAR seems not to be a component that distinguishes the differential control ability between these 2 genotypes.

In what concerns *caPlip* expression, at the beginning of gradual temperature decrease, Apoatã presented a slightly reduced expression, followed by a rise at 13/8 ºC. Piatã and Catuaí showed *caPlip* expression increases (*ca*. 75%) at 18/13 °C, decreasing after that. At chilling conditions all genotypes presented lower transcript levels than the control. Upon rewarming Catuaí and Piatã maintained the reduced transcript levels, while Apoatã more than doubled the control value. In general, the studied genotypes presented some increased *caPlip* gene expression during part of the acclimation period. Concomitantly, total phospholipids content increased in these genotypes during the gradual temperature decrease (data not shown). Phospholipases are lipid-degrading enzymes that could be involved in signal transduction events during drought stresses (Katagiri et al., 2001). A concomitant increase in total phospholipid content and transcript levels of some phospholipase coding genes was previously observed in wheat plants submitted to cold (Skinner et al., 2005). The authors suggested that this observation reflected a re-engineering of membranes in order to change their physical properties, such as elasticity and tensile strength, as well as the relations with other associated components.

CONCLUSIONS

All genotypes presented low transcript levels after chilling exposure, compromising eventual repair needs. However, differences occurred during the acclimation and recovery periods, justifying the different *Pn* impacts.

PSII components seem to be targets of cold stress. *CaCP24* expression and NPQ seemed to present a good relationship to be exploited in futur works.

Further integrated physiological, biochemical and molecular studies are needed to better understand the cold acclimation process in coffee.

ACKNOWLEDGEMENTS

Thanks are due to Dr.s Joel Fahl, M. Luíza Carelli and Luiz C. Fazuoli (IAC) for access to seed material. This work was supported by Fundação para a Ciência e a Tecnologia, through the projects POCTI/AGG/43101/2001 and PTDC/AGR-AAM/64078/2006 (partially financed by the European Fund FEDER) and by the grants SFRH/18361/2004 (P. Eichler) and SFRH/BPD/21884/2005 (A. Fortunato) co-financed by the Portuguese PIDDAC program and European Social Fund, under the 3rd framework program.

REFERENCES

Adams III W.W., Demig-Adams B. (1995). Plant Cell Environ., 18:117-127.

Bassi R., Pineau B., Dainese P., Marquardt J. (1993). Eur J Biochem 212: 297-303.

Campos P.S., Quartin V., Ramalho J.C., Nunes M.A. (2003). J. Plant Physiol., 160: 283-292.

Fernandez D., Santos P., Agostini C., Bon M.C., Petitot A.S., Silva M.C., Guerra-Guimarães L., Ribeiro A., Nicole M. (2004). Mol. Plant Pathol., 5(6), 527-536.

Gilmore A.M. (1997). Physiol Plant 99**:** 197-209.

Huner N.P.A., Öquist G., Sharhan F. (1998). Trends Plant Sci. 3:224-230.

Katagiri T., Takahashi S., Shinozaki K. 2001. Plant J., 26:595-605.

Kovács L., Damkjær J., Kereïche S., Ilioaia C., Ruban A.V., Boekema E.J., Jansson S., Horton P. (2006). The Plant Cell, 18: 3106-3120.

Livak K.J., Schmittgen T.D. (2001). Methods, 25:402-408.

Nie G.Y., Baker N.R. (1991). Plant Physiol., 95: 184-191.

Ramalho J.C., Quartin V., Fahl J.I., Carelli M.L., Leitão A.E., Nunes M.A. (2003). Plant Biol., 5: 631-641.

Ramalho J.C., Lidon F.C., Leitão A.E., Eichler P., Santos P., Pais I.P., Campos P.S., Rodrigues A.P., Chaves M.M., Branco P., Ribeiro A.I. 2006. Proceedings of the 21st ASIC Conference, Montepellier, France.

Rorat T., Havaux M., Irzykowski W., Cuiné S., Becuwe N., Rey P. (2001). Physiol. Plant, 113: 72-78.

Shinozaki K., Yamaguchi-Shinozaki K. (2000). Curr. Opin. Plant Biol. 3:217-323.

Skinner D.Z., Bellinger B.S., Halls S., Baek K.-H, Garland-Campbell K., Siems W.F. 2005. Crop Sci. 45:1858-1867.

Stockhaus J., Hofer M., Renger G., Westhoff P., Wydrzynski T., Willmitzer L. 1990. EMBO J. 9: 3013-21.

Tomashow M.F. (1999). Annu. Rev. Plant Biol. 50:571-599.

Wang W., Vinocur B., Altman A. (2003). Planta 218:1-14.

Wise R.P., Naylor A.W. (1987). Plant Physiol. 83:272-277.

Yamaguchi-Shinozaki K., Shinozaki K. (2006). Annu. Rev. Plant Biol. 57:781-803.

Anti Sense Expression of an Ethylene Receptor Gene from *Coffea canephora* Induces Tolerance to Abiotic Stress in Transgenic Arabidopsis Plants

J. BUSTAMANTE[1], V. PONCET[2], C. CAMPA[2], V. VAISSAYRE[3], S. DUSSERT, S. HAMON[2], A. DE KOCHKO[2]

[1]Instituto Nacional de Investigaciones Agrícolas (INIA), Bramón, Rubio, Táchira, Venezuela
[2]GECOFA
[3]Casuarina ; IRD, UMR DIAPC, BP 64501, 34394 Montpellier Cedex 5, France

SUMMARY

Knowing the major role of the hormone ethylene in fruit maturation and ripening and its implication in biotic and abiotic stress response, this work was undertaken in order to determine the structure, expression and role of the different ethylene receptor genes in the climacteric coffee trees. The *Coffea canephora* cDNA and corresponding genomic sequence of one ethylene receptor gene (*CcEIN4*) was cloned and characterized. This gene is present as a single copy in the genome of this species. *CcEIN4* is homologue with *LeETR5* from *Solanum lycopersicum* and it is constantly expressed during fruit development but its expression increases during the last fruit maturation stage. Over expression of *CcEIN4* was performed in *Arabidopsis thaliana,* no evident phenotype was observed. Gene silencing was also investigated using the antisense strategy. *CcEIN4* expression in anti sense orientation induces a tolerance to salinity in *A. thaliana* (up to 150 mM NaCl in the germinating medium). All these findings, suggest an important role of this gene both in the fruit development and maturation of coffee-trees as in various aspects of plant development.

INTRODUCTION

Ethylene (C2H4) is a simple gaseous hydrocarbon that has profound effects upon plant and developmental processes, such as germination, growth, flower initiation, senescence of leaves and flowers, organ abscission and fruit ripening (Abeles et al., 1992). It is also a major signal, mediating responses to a range of both biotic and abiotic stresses. At the level of gene expression, ethylene has been shown to induce transcription of a wide range of genes involved in wound signalling (O'Donnell et al., 1996) and defence against pathogens (Ecker and Davis, 1987; Bleecker and Kende, 2000).

A family of five receptors mediates ethylene perception in Arabidopsis: ETR1, ERS1, ETR2, ERS2, and EIN4 (reviewed in Guo and Ecker, 2004;Wang et al., 2002). The five genes belong to two sub families: the first consists of ETR1 and ERS1. Their corresponding proteins have three hydrophobic transmembrane domains and a conserved hystidine kinase domain, whereas the second sub family of ETR2, EIN4 and ERS2 contains additionally a putative signal sequence in the aminoterminal region that could target the proteins to the secretory pathway. They have a degenerated hystidine kinase domain. Two of the receptors (ERS1 and ERS2) lack a receiver domain at the C terminus.

At least two receptors interact with CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1), which is homologous to the Raf family of Ser/Thr kinases and negatively regulates ethylene

signalling (Kieber et al., 1993; Huang et al., 2003). Inactivation of CTR1 leads to activation of EIN2, an integral membrane protein, with is homologue to NRAMP family of metal ion transporters (Roman et al., 1995; Alonso et al., 1999). The biochemical function of EIN2 remains unknown, but apparently all ethylene responses described to date are transduced through this signalling intermediate (Hall and Bleecker, 2003). Downstream EIN2 act a plant-specific family of transcription factors encoded by *EIN3* and *EIL* (*EIN3-like*) that binds to defined target in promoter region of a downstream transcription factor, ERF1 (Solano et al., 1998); which in turn activate ethylene-response target genes. The over expression of this gene and silencing by antisense construction was studied in *Arabidopsis thaliana* wild-type (Columbia).

MATERIAL AND METHODS

Plant material

Identification and characterisation of the ethylene receptor gene was carried out on *Coffea canephora* (CAN) and *C. pseudozanguebaiae* (PSE). The trees were grown under tropical conditions in a green house at the IRD centre in Montpellier, France. Leaf samples were collected and total DNA was immediately extracted using the DNeasy Plant Maxi kit® (Qiagen GmbH, Germany) following the manufacturer's instructions.

Amplification of cDNA and full length gene

Degenerated primers, based on sequence alignments of homologue genes from different species available in international databases (Lasergene, DNASTAR Inc) were used. By PCR amplification on PSE genomic DNA and CAN leaves cDNA library, two specific fragments of 413 and 386 pb in size were amplified. These fragments were cloned in TOPO4 cloning® vector (Invitrogen) and sequenced (MWG-Biotech (Ebersberg, Germany). Then, using sequence specific primers from these blasted sequences, we amplified a big fragment of cDNA. Finally, the full length cDNA was amplified from fruits cDNA library using sequence specific primers and T3 and T7 universal primers from the CAN cDNA library (Bustamante-Porras et al., 2007). Sequence assembly was performed using seqMan and MEGALING programs from DNSTAR (Madison, WI, USA). The corresponding gene was amplified by PCR in CAN genomic DNA using sequence specific primers.

Plasmid construction and plant Transformation

From the cDNA full-length clone previously amplified by PCR (CcEIN4/C18), was amplified the *BamH*I/*Sma*I cDNA with the forward primer 5'-CGCGGATCCATGGTTCAAGAT TAAGGGAT-3' and the reverse primer 5'- CGCCCCGGGTCAAAAACCATCACCTGC TCG- 3'. This cDNA was constitutively expressed under the control of Cauliflower Mosaic Viris (CaMV) 35S promoter. To construct pCcEIN4; the pCAMBIA 1305.1 was digested with *Bgl*II/*Pml*I and the gene GUS was removed by rescue the specific band. A 2298 bp. *BamH*I/*Sma*I PCR fragment, including the full-length CcEIN4 was digested from TOPO and re-ligated both in the sense orientation and antisense orientation in that plasmid.

Generating of transgenic plants

Agrobacterium tumefaciens (strain GV3101) containing the 35S::CcEIN4 in sense and anti sense orientation were used to transform *Arabidopsis thaliana* Columbia wild-type by floral-dip method (Clough and Bent, 1998) with modifications described by Bustamante-Porras (2007). With the construction in sense or in anti sense orientation was transformed the *A.*

thaliana. Independent homozygous lines for each transformation were obtained based on segregation of the acquired antibiotic resistance to 50 $mg.L^{-1}$ hygromycine on Murashige ang Skoog (MS) agar medium.

Seedling Growth-Response Assays

To examine the tolerance to abiotic stress, seeds were grown on squared petri dishes containing one-half-strength Murashige and Skoog basal media with Gamborg's vitamins (pH 5.65; Sigma, St. Louis) and 0.8% (w/v) plant agar, supplemented with 0, 50, 100 µM; NaCl. Seedlings root length size was measured one week later.

RESULTS

Characterisation of *CcEIN4*

A full-length cDNA corresponding to the *CcEIN4* gene was isolated from CAN fruit cDNA library. It was 2,906 bp; in length, it contain a putative open reading frame of 2,298 bp translated *in silico* into 765 amino acids. The 5'-UTR was 114 bp long and its 3'-UTR was 313 bp. The *CcEIN4* predicted polypeptide had a molecular weight of 85.63 kDa. Sequence at amino acid level is 74.4% identical to LeETR5 of *Solanum lycopersicun* and 60.8% identical to *AtEIN4* of *Arabidopsis thaliana.* This sequence is only 35.3 % identical to *CcETR1* of *Coffea canephora* (Bustamante et al., 2007). N-terminal segment (432 bp.) is conserved. It is 85.7% identical to *LeETR5.*

Arabidopsis plants were transformed with *CcEIN4* coding sequences in sense but in antisense orientation controlled with CaMV 35S promoter. At least ten (10) T2 Arabidopsis plants resistant to hygromycine and coming from independent transforming events have been obtained for each genetic construction. All transformed plants have been tested in half MS medium supplemented with different concentration of NaCl. Plants transformed with construction *CcEIN4* in sense orientation no evident phenotype was observed; but in anti-sense, transgenic plants show tolerance to salinity in concentration of 50 and 100 mM of NaCl. Figure 1 shows the root growing in transgenic and wild type *A. thaliana* at different salt concentration.

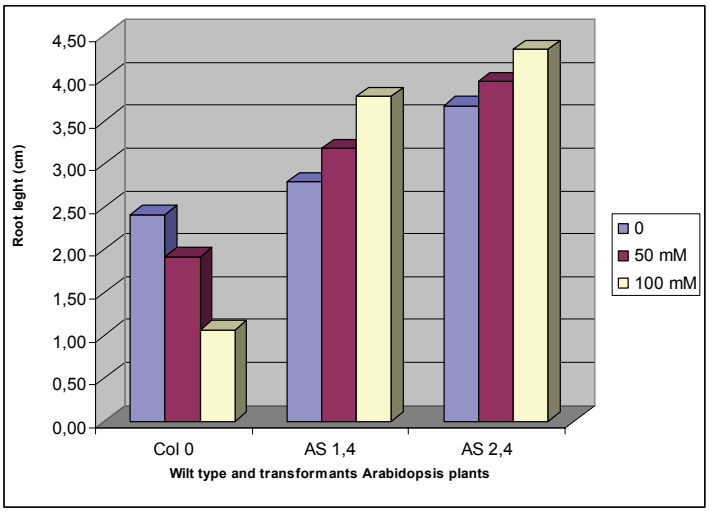


Figure 1. Root growing in wild type (Col 0) and two transgenic lines anti-sense *CcEIN4* and *A. thaliana* at different salt concentration.

REFERENCES

Abeles F., Morgan P. and Saltveit, M. (1992). Ethylene in plant biology. (San Diego: Academic Press.).

Alonso J.M., Hirayama T., Roman G., Nourizadeh S. and Ecker J.R. (1999). EIN2, a bifunctional transducer of ethylene and stress responses in Arabidopsis. Science 284, 2148-2152.

Bleecker A.B. and Kende H. (2000). Ethylene: A Gaseous Signal Molecule in Plants. Annual Review of Cell and Developmental Biology 16, 1-18.

Bustamante J., Poncet V., Campa C., Noirot M., Hamon S. and Kochko A. (2007). Characterization of three ethylene receptor genes in *Coffea canephora* Pierre. In Advances in Plant Ethylene Research (Springer), pp. 53-56.

Bustamante-Porras J., Campa C., Poncet V., Noirot M., Leroy T., Hamon, S. and de Kochko A. (2007). Molecular characterization of an ethylene receptor gene (CcETR1) in coffee trees, its relationship with fruit development and caffeine content. Mol Genet Genomics 277, 701-712.

Clough S.J. and Bent A.F. (1998). Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J 16, 735-743.

Ecker J. and Davis R. (1987). Plant defense genes are regulated by ethylene. Proceedings of the National Academy of Sciences of the USA 84, 5202-5206.

Guo H. and Ecker J.R. (2004). The ethylene signaling pathway: new insights. Current Opinion in Plant Biology 7, 40-49.

Hall A.E., and Bleecker,A.B. (2003). Analysis of Combinatorial Loss-of-Function Mutants in the Arabidopsis Ethylene Receptors Reveals That the ers1 etr1 Double Mutant Has Severe Developmental Defects That Are EIN2 Dependent. Plant Cell 15, 2032-2041.

Huang Y., Li H., Hutchison C.E., Laskey J. and Kieber J.J. (2003). Biochemical and functional analysis of CTR1, a protein kinase that negatively regulates ethylene signaling in Arabidopsis. Plant J 33, 221-233.

Kieber J.J., Rothenberg M., Roman G., Feldmann K.A. and Ecker J.R. (1993). CTR1, a negative regulator of the ethylene response pathway in Arabidopsis, encodes a member of the raf family of protein kinases. Cell 72, 427-441.

O'Donnell P., Calvert C., Atzorn R., Wasternack C., Leyser H. and Bowles D.J. (1996). Ethylene as a signal mediating the wound response of tomato plants. Science 274, 1914-1917.

Roman G., Lubarsky B., Kieber J.J., Rothenberg M., and Ecker J.R. (1995). Genetic Analysis of Ethylene Signal Transduction in Arabidopsis thaliana: Five Novel Mutant Loci Integrated into a Stress Response Pathway. Genetics 139, 1393-1409.

Solano R., Stepanova A., Chao Q. and Ecker J.R. (1998). Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. Genes Dev. 12, 3703-3714.

Wang K.L.-C., Li H. and Ecker J.R. (2002). Ethylene Biosynthesis and Signaling Networks. Plant Cell 14, S131-151.

Differential Gene Expression Response of *Coffee arabica* and *C. liberica* to Coffee Berry Borer Attack*

C. GONGORA[1], S. IDARRAGA[1], A. SANCHEZ[1], A. GAITAN[1], L. RIVERA[1], M. CRISTANCHO[1], M. YEPES[2] AND R. BUELL[3]**

[1]Centro Nacional de Investigaciones de Café, CENICAFE, Chinchina, Colombia.
[**]E-mail: carmenza.gongora@cafedecolombia.com
[2]Department of Plant Pathology, Cornell University, NY, USA
[3]Department of Biology, Michigan State University, MI, USA
[*]Research Co-sponsored by the Colombian Ministry of Agriculture

SUMMARY

The coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: *Curculionidae*) is the most important coffee pest in Colombia. No sources of natural resistance have been identified in *Coffea sp.* against the insect, except for some levels of antibiosis present in *C. liberica*. A coffee cDNA microarray was developed to do transcriptional analysis and conduct functional genomics studies of the molecular interaction between coffee and CBB, characterize the response of coffee plants under CBB attack, and establish the bases of the *C. liberica* antibiosis. A normalized library of the susceptible genotype *Coffea arabica* var. Caturra (mixed tissues) was constructed, as well as two *C. liberica* normalized libraries: one from leaves and one from fruits infested with CBB. A total of 33,263 cDNAs were sequenced and arrayed including: 19,074 from *C. arabica* and 14,189 from the two *C. liberica* libraries. The array was hybridized with RNA extracted from *C. arabica* and *C. liberica* fruits either untreated or after 24 or 72 h of infestation with CBB. Four biological replicas were done per treatment. Overall a total of 2,585 genes were differentially expressed following CBB infestation. Statistical analysis revealed shifts in expression with 25 different patterns. Interestingly, 520 clones showed significantly higher induction in *C. liberica* versus the susceptible *C. arabica* var. Caturra, at both 24 h and/or 72 h. Many of the up-regulated genes were related to enzymes involved in stress response, insect defense, and transcriptional regulation. There were also a few clones related to cellular maintenance and photosynthesis. In this group, there were genes involved in the jasmonic acid pathway, supporting our previous findings on the activation of this metabolic route in genotypes exhibiting CBB antibiosis. The expression patterns observed indicate differential transcriptional responses against CBB in the two species which can be used to generate new sources of resistance that are absent in the known genetic pool of cultivated varieties.

INTRODUCTION.

Coffee is an important crop commodity in the world and the coffee bery borer CBB, *Hypothenemus hampey* (Ferrari) (Coleoptera: Curculionidae: Scolitidae) is the most important pest of commercial coffee and it is present in all the coffee growing region of the world (Le Pelley, 1968; Reid , 1983; Bustillo, 2002). Colombia is the most important producer of coffee arabica, with approximately 850.000 Ha planted. Most of the economic losses in Colombian coffee plantations are due to the attack of this pest. The female beetle borers a hole into the coffee berries, where she deposits her eggs. The larvae hatch and feed on the bean, lowering the quality of the coffee berry and often causing the fall of small fruits, loss of grain weight and diminishing the quality of the grain (Duque et al., 1997). Because most of the time the

insect is inside of the berry the control of the insect is difficult. An strategy of integrated pest management that includes cultural, biological and chemical control should be done for controlling the insect (Bustillo, 2005).

Until now no sources of natural resistance in cultivated or non-cultivated coffee plants have been identified. However, previous researches found differences in the susceptibility of different coffee species to the insect (Koch, 1973; Villagran, 1991; Duarte, 1992). Romero and Cortina (2004; 2007) reported differences in the intrinsic growth rate of insect feeding on *Coffea arabica* var. Caturra comparing with insects growing on *C. liberica*. The net reproductive rate of insects that fed on *C. liberica* was 15 $\pm$ 2 eggs and the ones feeding on *C. arabica* var. Caturra was 25 $\pm$ 1 eggs, also a delay in growth was observed in the insects feeding in *C. liberica*. The variations in oviposition can be due to the presence of antimetabolic substances, the lack of appropriated nutrients or to differences in cell wall structure that affect digestibility.

With the purpose of understanding the differences between *C. arabica* and *C. liberica* and to establish the bases of the *C. liberica* antibiosis, cDNA normalized libraries and a coffee cDNA microarray were developed to investigate the response to the coffee berry borer at the molecular level. We want to identify the transcripcional changes in coffee elicited by attack from the coffee berry borer over time, and to compare the responses between the two coffee species against *H. hampei*. Finally, we want to identify insect-specific genes or pathways that might be useful in the development of CBB resistance cultivars.

MATERIAL AND METHODS

From field plants of *C. liberica*, berries susceptible to insect attack in active transcriptional stages – 120 days post flowering – were artificially infested with CBB in a 3:1 insect:berry proportion using cages. The leaves and the infested berries were collected at 24 hours and stored in liquid nitrogen. From *C. arabica* var. Caturra leaves, roots, berries, calli and flower primordiums were collected.

Total RNA from the *C. liberica* berries, *C. liberica* leaves and each one of the *C. arabica* tissues was extracted using the RNeasy Plant Maxi Kit (Qiagen, USA). With each of the Total RNA samples: berries, leaves and the mixture of *C. arabica* RNA tissues, three normalized libraries were constructed at Evrogen (Rusia).

From the libraries, 37.632 spots were registered on the array, distributed as follows: 19,074 spots corresponded to a normalized library of *C. arabica* (RNA mixture from 5 tissues). 7,169 to the *C. liberica* normalized berry library and 7,020 to the *C. liberica* normalized leaf library, for a total of 33,000 effective spots. 3217 corresponded to sequences that were discarded out of the transcript assembly pipeline.

cDNA clones were printed onto Corning Ultra Gaps Slides (Cat# 40015). Equal volumes of purified cDNA and DMSO were mixed into 384 well printing plates (Corning; Cat # 3656) using the Biomek FX (Beckman Coulter). Dust was removed by blowing the slides with high-pressure air, and the slides were placed in the arrayer. Microtiter printing plates were loaded into the arrayer and PCR/DMSO products were spotted onto the slides at 72 °F and 45% relative humidity. DNA was spotted with Stealth Microspotting Pins (SMP-2.5, Telechem). Following printing, the slides were allowed to dry and spotted DNA was bound to slide by UV-crosslinking at 120 °mJ using a Stratalinker™ (Stratagene, Cat# 400071). Printed slides were stored in a light-tight box in a bench-top dessicator at room temperature until they were used for hybridization.

The probes for the coffee microarray were synthesized from equal amounts of total RNA extracted with the RNeasy Plant Maxi Kit (Qiagen, USA) from: 1. *C. liberica* berries from plants established in the field infested for 24 h or 72 h with CBB as well as from non infested berries, and 2. *C. arabica* berries subjected to the same treatments and non infested ones. The berries were infested artificially using cages with an insect berry relation of 3:1. Four replicas for each treatment were done.

Slides were scanned using an Axon 4000B scanner (Axon Instruments, Union City, CA). Both the 635 nm (red, Cy5) and 532 nm (green, Cy3) channels were scanned simultaneously at 100% laser power. Images were saved in a non-compressed TIFF file format for both channels. The TIFF images were quantified using Genepix 5.1 (Axon Instruments, Union City, CA). Both the Cy3 and Cy5 image were analyzed simultaneously. Using a GAL-file (Gene Array List) the grid is overlaid on the image. For background correction and data normalization for each hybridization the raw intensities were loaded into the limma package of Bioconductor (www.bioconductor.org) using the read.maimages function.

Normalized Cy3 and Cy5 intensities were calculated for each spot. A transcript was defined as being differentially regulated if both of the following criteria were fulfilled: (1) It consistently showed the same expression pattern along the 4 biological replicas according to the Spearman test; (2) The two intensities were significantly different as evaluated by t-tests.

The assembly and annotation of transcripts was done using the Bioinformatics Pipe line-developed at Cenicafé. Sequence quality was determined using Phred values. Sequence assembly was done using an identity percentage of 95% and an overlapping of 50. The Codoncode aligner software was also used. Gene annotation was done using ESTscan for prediction of the reading frame and Blast (-Blastx -NCBI), InterProScan (EMBL-EBI) and Gene Ontology databases to find conserved domains and motifs in protein sequences.

RESULTS AND DISCUSSION

From a total of 33,000 spots, 2585 (7%) had consistent expression patterns under insect attack in all biological replicas, summarized in 25 expression patterns. Hot maps showed that *C. liberica* had an earlier gene expression that *C. arabica* and a larger number of genes expressed at 24 h comparing to *C. arabica*. At 72 h. there are more genes expressed in *C. arabica* compared to *C. liberica* and there are differential transcriptional responses.

With respect to the *C. liberica* response, seven of these 25 clusters (Figure 1), containing 520 transcripts, showed over expression in *C. liberica* at 24 h and/or at 72 h after insect attack when compared to *C. arabica*.

Many of the transcripts correspond to enzymes in metabolic pathways related to stress responds, high transcription, and catalytic and metabolic processes. A few number of transcripts related to cellular maintenance and photosynthesis. Also under-regulation of some transcripts was observed in both *C. arabic*a and *C. liberica*.

Two of the clusters, 25 and 22 (Figure 1) showed genes with the highest level of expression in *C. liberica* at 24 and 72 h. Those transcripts seem to be silenced in *C. arabica*. In this group there are transcripts related to jasmonic acid or linoleic pathway such as: a lipase precursor, acyl-coA-binding protein ACBP/ankyrin, a dehydrogenase/reductase SDR, alcohol dehydrogenase, and S-adenosyl-L-methionine-dependent methyltransferases. There are also genes involved in insect defense such as ARF/SAR superfamily, pectin lyase fold/virulence factor, and Aldo/keto reductase. We found also a Jasmonic acid 2 gene, a DNA binding

protein involved in regulation of transcription that showed expression at 24h both in *C. arabica* and *C. liberica*, at 72h this transcript remains highly expressed in *C. liberica* while it got silenced in *C. arabica*

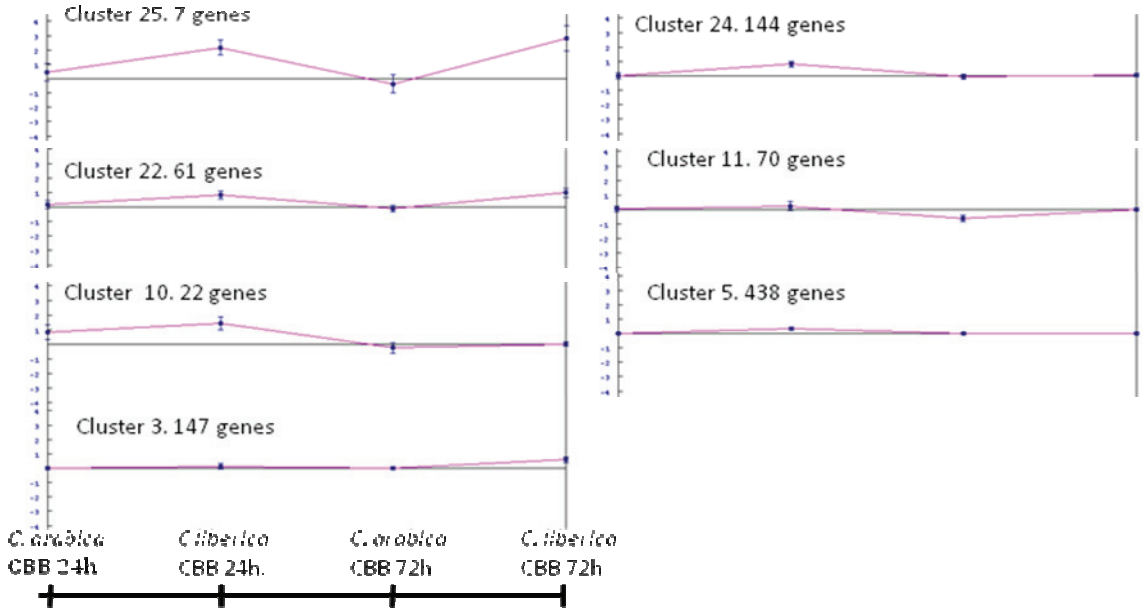


Figure 1. Clusters that showed differential transcriptional response between the *C. arabica* and *C liberica* species in response to the CBB attach.

Four of the clusters, 24, 10 11 and 5 corresponded to transcripts over-expressed at 24 h in *C. liberica* and with very low expression at 72 h and in *C. arabica.* In this group there are also genes involved in insect defense such as: proteinase inhibitor, plant protein trypsin-alpha amylase inhibitor, plant protein serine/threonine kinase-like, plant ascorbate peroxidase/Haem peroxidase, a putative class 5 chitinase as well as a S-adenosylmethionine decarboxylase/ Homocysteine S-methyltransferase.

Finally, there was a cluster containing transcripts that over expressed mostly at 72 h in *C. liberica*. Here there are many ubiquitin-associated/translation elongation, stress related proteins (heat shock proteins, UV excision repair protein), probably involved in the activation of other delayed responses.

In the case of *C. arabica* there are 10 clusters that showed over expression at 72 h, only 1 cluster with 116 genes showed some activity at 24 h. One cluster containing 40 transcripts displays the highest level of expression in all the experiment at 72 h. In this group: a cellulose synthase and a protein with unknown function are the ones with the highest levels of expression. Also a proteinase inhibitor -serpin, a proteinase inhibitor - Kunitz legume, and methyltransferase type 11 are defense proteins found here.

In conclusion both *C. arabica* and *C. liberica* respond in a different way to the CBB attack. *C. liberica* showed an early gene expression and a larger set of genes expressed at 24 h compared to *C. arabica*. At 72 h both genotypes showed expression but with a differential transcriptional responses. Those differences can be new sources of resistance that are absent in the known genetic pool of *C. arabica*.

REFERENCES

Bustillo. El papel del control biológico en el manejo integrado de la broca del café, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae. Rev. Acad. Colomb. Cienc. 2005. 29, 56-68.

Bustillo P.A.E. El manejo de los cafetales y su relación con el control de la broca en Colombia. Boletin Técnico N 24, Cenicafé , Chinchina. Colombia. 40 pp. 2002

Duarte N.M.T. Determinación de la atractividad de frutos de varios cultivares de café a la broca del fruto del café *Hypothenemus hampei* (Ferrari), utilizando el método de olfatometria a nivel de laboratorio. Guatemala, Universidad de San Carlos, Facultad de Agronomia , 55p (Tesis: ingeniero Agrónomo). 1992

Duque O.H., Chaves C.B., Hernández S.M. 1997. Costos del manejo de la broca *Hypothenemus hampei* (Ferrari) en lotes comerciales de café del departamento del Risaralda. Federación Nacional de Cafeteros de Colombia. Centro Nacional de Investigaciones de Café. Cenicafé. Chinchiná, Colombia. 37 pp. 2000

Koch V.J.M. Abondance de *Hypothenemus hampei* Ferr., scolyte des graines de café, en fonction de sa plante-hote et de son parasite *Cephalonomia stephanoderis* Betrem, en Cote d Ïvoire. Mededelingen Landbouwhogeschool Wageningen Nederland 73-16, 84 pp 1973.

Le Pelley R.H. Pest of Coffee. London; Longmans, Green and Co Ltda. 595 pp. 1968,

Reid J.C. Distribution of the coffee berry borer (*Hypothenemus hampei*) within Jamaica, following his discovery in 1978. Tropical Pest Management. 1983. 29, 224-230.

Romero V.C., Cortina G.H. Fecundidad y Ciclo de Vida de la broca *Hypothenemus hampei* F. (Coleoptera: Curculionidae: Scolytinae) en introducciones silvestres de café. Cenicafé. 2004. 55, 221-231.

Romero V.C., Cortina G.H. Tablas de vida de *Hypothenemus hampei* (Coleoptera: Curculionidae: Scolytinae) sobre tres introducciones de café. *Rev. Colomb. Entomol.* 2007. 33, 10-16.

Villagran G.W. Atractividad relativa y susceptibilidad de varias especies y cultivares de café *Coffea* spp. a la broca del fruto *Hypothenemus hampei* Ferr. en condiciones de laboratorio. Guatemala, Universidad de San Carlos de Guatemala. Facultad de Agronomía, 43 p. (Tesis: Ingeniero Agrónomo). 1991

High Density Genetic Mapping of Resistance Gene to Race II of *Hemileia vastatrix* in Hibrido de Timor Genotype

V. DIOLA[1], G.G.G. BRITO [1], A.P. GALLINA[2], E.T. CAIXETA[2,3], N.S. SAKYAMA[2], M.E. LOUREIRO[1,2]

[1]Lab. Fisiologia Molecular de Plantas, Dep. de Biologia Vegetal/UFV
[2]Lab. Biotecnologia do Café/BIOAGRO/UFV
[3]Embrapa-Café

SUMMARY

Coffee leaf rust caused by the fungus *Hemileia vastatrix* is the most important disease of *Coffea Arabica*, and can be responsible for reductions about 35 to 50% in coffee production in infected fields. In the Híbrido de Timor (HT) populations were detected resistance genes, that alone or in association, could confer resistance to several different physiologic races of *H. vastatrix*. The objective of this study was to generate a high density genetic map surrounding a single rust resistance gene. The mapping population (F_2 UFV 421-4) was composed by 224 plants, derived from F_1 self-fertilized individuals, which were derived from the cross between HT UFV 427-15 and the susceptible clone Catuai Amarelo IAC 30 (UFV 2143-236). Phenotypic characterization was performed by infection rate analysis of leaf disc inoculated with monospore cell culture rust race IIb spores. Phenotipic segregation in this population follow a rate of 3 resistant to 1 susceptible individual. Two BSAs (*Bulked Segregant Analysis*), one for resistant and other for susceptible plants, were composed by 5 genotypes each. About 770 primer combinations were used in AFLP analysis, and 15 markers were obtained. This marker delimited a chromosomal region of 90 cM. The nearest marker was positioned at 3.2 cM of the resistance gene, and it is inside a region of 6.6 cM between the markers limiting both gene sides. This make possible the obtaining of the physical map and to facility the studies of the signaling the defense answer in the interaction coffee x *H. vastarix*.

INTRODUCTION

The orange rust coffee, it is a disease caused by the biotrofic fungus *Hemileia vastatrix* Berk. et Br. More than 75% of the coffee cultivated in the world is susceptible to the majority of physiologic pathogenic races (Guzzo, 2004). Selection pressure against this fungi by the continuous use of fungicides and the wide cultivation of genotypes with lower genetic diversity, results in the constant arising of new fungi pathogenic races (Wagner e Bettencourt, 1965). Because coffee is a woody plant with a long juvenile phase, classical coffee plant breeding is slow and needs implementation of techniques that speed up the selection and phenotipic evaluation of elite genotypes for this resistance (Teixeira-Cabral et al., 2004). At the moment, resistance genes could be divided in nine classes, denominated as S_H1 to S_H9, that isolated or in combination, result in resistance to different leaf coffee rust disease. Pathogen virulence is codified by the genes *v1* to *v9* (Bettencourt & Rodrigues 1988). Between resistance genes, S_H1, S_H2, S_H4 e S_H5 could be found in *C. Arabica* genotype. The genes S_H6, S_H7, S_H8, S_H9 and others unknown, were introduced from *C. canephora*, and S_H3 from *C. liberica* (Wagner e Bettencourt 1965; Bettencourt e Rodrigues 1988; Prakash et al. 2004). At the present, are known 45 physiological races of *H. vastratrix* that are able to infect different genotypes (Várzea e Marques, 2005; Mahé et al., 2007). Nowadays, new molecular techniques allow us to quickly identify and characterize plant resistance genes,

making more feasible the pyramiding of several resistance genes. Actually is still very limited the number of molecular markers linked to coffee resistance. The locus S_H3 originated from introgression of *C. liberica* into *C. arabica*. Was fined mapped by Prakash et al. (2004), and characterized by Mahé et al. (2008). However, the physiological races of *H. vastratix* in Brazil have already overcome the S_H3 resistance gene, making the available markers not usefull to breeding purposes in Brazil. Previous work of your group (Brito ,2007), identified three markers linked to the single resistance gene present in Híbrido de Timor HT UFV 427-15.

The present work identified 15 AFLP markers linked to resistance locus to race II of *H. vastratrix*, and has a main to high genetic mapping of this resistance gene in order to clone this resistance gene.

MATERIALS AND METHODS

The mapping population was cultivated in the "Campo Experimental de Melhoramento do Cafeeiro da Universidade Federal de Viçosa -Viçosa-MG", and the molecular marker production was performed in the Plant Molecular Physiology Lab, in Plant Biology Department of UFV, from to 2005 to 2008. The mapping population of 224 F_2 individuals were originated from self-fertilization of F_1 plants originated from the cross between Híbrido de Timor UFV 427-15 (resistant parent) with cv. Catuaí amarelo UFV 2143-236 (susceptible parent).

Study of inheritance resistance for Coffee rust

Phenotipic evaluation of resistance was performed by inoculation of leaf discs with monosporic isolates of race IIb of *H. vastatrix* (Tamayo, 1988). Leaf disk were conditioned in magenta boxes with the button covered with 1 cm layer of foam saturated with water. In each Box were disposed 8 discs with diameter of one inch, remaining in darkness for 24 hours. After that, infection was done by inoculation of ten points of the under face with 5.0 µL uredospore suspension (2.0 $mg.mL^{-1}$), keeping the inoculated discs under photoperiod of 12 hours light at 22ºC (±2) and saturated air humidity. Phenotipic evaluation was performed at 18, 24, 36 and 48 days after inoculation as described by Eskes e Toma-Braghini (1982). The χ^2 test for the phenotypic segregation was calculated using the software "MapDisto Version 1.7 beta for Excel" (http://mapdisto.free.fr) (Lorieux, 2007).

Identification of molecular markers

DNA of mapping population was isolated after homogenization of leaf tissue under liquid nitrogen using the CTAB method (Murray e Thompson, 1980). The samples were quantified by spectrofotometry, and confirmed by analysis in 1% agarose gels.

Digestion of 600 ng of each genotype DNA was performed using 1.5 U of *EcoRI* and, or *MseI* restriction enzymes (*Promega*) (Sambrook et al., 1989), by 8 hours at 37 °C. The ligation reaction using *EcoRI* adaptor 5' CTAGTAGACTGCGTACC 3' or *MseI* adaptor 5' GACGATGAGTCCTGAGT 3' (2.5 mM each) was done using 400 ng digested DNA and 1 U of T4 DNA ligase (*Biosystem*), in 20 µL reaction volume by 12 h at 16 °C.

AFLP markers was obtained as described by Vos et al. (1995). Pre-amplification was performed with the primers *EcoRI* 5' GACTGCGTACCAATTC*N* 3' and *MseI* 5' GATGAGTCCTGAGTAA*N* 3' , using 24 cycles of amplification of 94ºC for 30", 58 °C for 60" and 72 °C for 60". The reaction was diluted 40 fold to further use. *Bulked segregant*

analysis (BSAs) was composed by 5 individuals, mixing the same amount of DNA of the diluted pré-amplification reaction.

Selective touch-down amplification was performed by 13 cycles of 94ºC for 30", 65ºC (-0,7ºC at each cycle) for 30" and 72ºC for 60"s, followed by 26 cycles of 94ºC for 30"s, 58ºC for 30"s and 72ºC for 60"s in a reaction volume of 20 μL, using 0,25 mM dNTP mixture (*Promega*), 50 ng each primer and 1.5 U Taq DNA polimerase (*Phoneutria*).

Denaturing eletrophoresis was performed in 6% gel at 2000v for 2.5 h, and DNA fragments detected by silver staining as described by Creste & Tulmann-Neto (2001) and modified by Britto (2007). Resistance linked polimorfisms were confirmed by individual analysis of each bulk member (Figure 1). Candidate markers were then further analyzed by segregation analysis in all 224 members of the segregating population.

LINKAGE ANALYSIS

The Mandelian segregation of the dominant markers in the F2 population was tested by χ^2 test (3:1), using a Excel sheet prepared to be used in the software "MapDisto Version 1.7 beta" (http://mapdisto.free.fr) (Lorieux, 2007). The minimal scores taken to consider the candidates as resistance markers was lower than 30% recombination (r max 0,3) and minimal LOD score of 5,0 (LODmin 5). The recombination frequency were converted to centiMorgans (cM) using the Haldane algorithm, using the SARF criterium (sum of adjacent recombination fractions) after LOD normalization.

RESULTS AND DISCUSSION

In the genitor and resistant genotypes, *H. vastatrix* didn't complete reproductive cycle in all assay evaluations and repetitions, while in the genitor and susceptible genotypes there were the successful infection and spore production. In the F_2 population UFV 421-4 were identified 166 resistant genotypes (74.1%) and 58 susceptible (25.9%). These results confirmed the pattern of segregation of 3:1, expected for a single dominant gene (χ^2=0,09524; *p* 75,76%) (Table 1). This results indicate that resistance in the HT genotype is conferred by a single resistance gene out closely cluster resistance genes. Similar results were found by Brito (2007) using the other population of the same parents.

Were analyzed 784 combinations of AFLP primers, resulting in the analysis of around 53 000 loci, detecting 1 221 polymorphisms, with approximate medium size polymorphic fragment of 480 bp. About 12.8% of polymorphic bands were associated with resistance, and all of them were used to individual analysis of each bulk member (Figure 1). This analysis resulted in the selection of 163 polimorfisms, from which only 15 were defined as molecular markets.

The 15 candidate markers were tested in the 224 F_2 genotypes and they confirmed the linkage to the coffee rust resistance locus, and this linkage group represents a chromosomal region of 90 cM. This reinforces the probability of being just a gene to determine the resistance to *H. vastatrix* IIb. We considered the interaction between the markers and the resistance loco and found values ~3.3 cM for each marker that flanks the gene including an region of ~6.6 cM (Figure 2).

These AFLP markers are in process of conversion to SCARS in order to facilitate its use in the Coffee Rust Resistance Breeding Program of UFV and EPAMIG. The availability of these primers will increase the efficiency and the speed of genetic selection, essential features of one strategy to pyramiding several resistance genes in order to produce a durable resistance to

the new *H. vastatrix*. Additionally, this SCARS will be used to construct the contig encompassing the resistance gene for further improvement of marker density and positional cloning of this gene. Better knowledge of the physiological mechanisms of this resistance could be achieved only by the characterization of the responsible gene, and it is essential to delineate successful strategies of durable resistance.

Table 1. Segregation analysis of AFLPs markers: primers combination, fragment polimorfic size, segregation and recombination for the R gene in the F_2 population UFV 424-1.

Locus tested	*Primer combination	Fragment size (bp)	Band -	Band +	** 3/1	** χ^2	*p* (%)	***RF (%)
R Gene	Fenotypes	-	58	166	2.86	0.095	75.76	0.89
M1	EGTA/ETGA	405	53	171	3.23	0.214	64.34	6.70
M2	ECTC/MTTT	280	58	166	2.86	0.095	75.76	6.25
M3	ECCT/MTTC	300	46	178	3.87	2.381	12.28	8.48
M4	ECGT/MTGT	280	48	176	3.67	1.524	21.70	14.29
M5	ECGT/MTGT	210	54	170	3.15	0.095	75.76	15.18
M6	ECAT/MTCT	150	57	167	2.93	0.024	87.74	20.54
M7	ECAT/MTCT	130	61	163	2.67	0.595	44.04	19.64
M8	ETGA/EGCA	310	46	178	3.87	2.381	12.22	13.39
M9	ETGA/EGCA	280	51	173	3.39	0.595	44.04	16.07
M10	ETAT/EGGC	480	57	167	2.93	0.024	87.74	12.95
M11	ETAT/EGGC	380	53	171	3.23	0.214	64.34	11.61
M12	ETAT/EGTT	385	60	164	2.73	0.381	53.71	16.96
M13	ETTC/EGAG	450	48	176	3.67	1.524	21.70	16.07
M14	ETTA/EGTA	650	47	177	3.77	1.929	16.49	14.73
M15	ETTA/EGTA	230	41	183	4.46	5.357	2.06	9.82

Note: - band absence, + band presence.

**Primer combination. E or M: restriction enzyme EcoRI or MseI, respectively and three additional bases; **Esperance proportion for monogenic inheritance in the population F_2 (3 resistance, R_, or band presence: 1 susceptible, r_, or band absence); ***Genetic recombination frequency between the gene and specific molecular markers.*

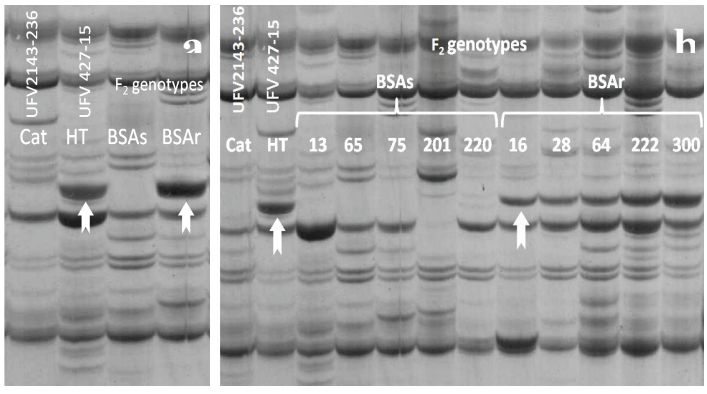


Figure 1. a)Polymorphism present the resistance BSA (BSAr) and absent in susceptible BSA (BSAs) . b) Validation of association of the polymorphism with rust resistance gene. (Cat and HT, susceptible and resistant progenitor). Numbers are designations for the F2 individuals.

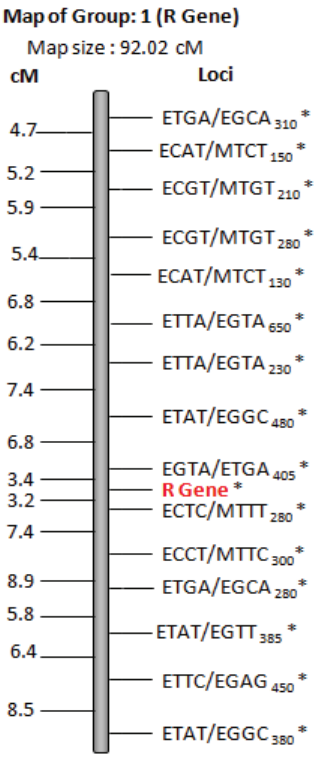


Figure 2. Genetic map of the markers for coffee rust resistance gene in the Hibrido de Timor UFV 427-15 markers and the resistance gene (MapDisto, 2007). E or M: restriction enzyme *EcoRI* or *MseI, respectively*; Numbers represent marker fragment size in base pairs. Asterisks denote χ2 significant segregation for a dominant gene.

REFERENCES

Bettencourt A.J., Rodrigues Jr.C.J., 1988. Principles and practice of coffee breeding for resistance to rust and other disease. In: Clarke RJ, Macrae R (Eds) Coffee. Agronomy, vol 4. Elsevier Applied Science, London, pp 199-234

Brito G.G., 2007. Mapeamento genético de marcadores AFLP ligados ao gene de resistência do Híbrido de Timor á *Hemileia vastatrix*. 57 p. Tese de doutorado. Universidade Federal de Viçosa – MG. 2007.

Creste S., Tulmann-Neto A., Figueira A., 2001. Detection of single sequence repeat polymorphisms in denaturing polyacrilamide sequencing gels by silver staining. Plant Mol Biol Repor 4:299-306

Eskes A.B., Toma-Braghini M., 1982. Assessment methods for resistance to coffee leaf rust (Hemileia vastatrix Berk. And Br.). Plant Prot Bull FAO (Rome) 29:56-66

Guzzo S.D., 2004 Aspectos bioquímicos e moleculares da resistência sistêmica adquirida em cafeeiro contra Hemileia vastatriN. Tese de doutorado, Piracicaba, Centro de Energia Nuclear na Agricultura 236p

Lorieux M., 2007. MapDisto Version 1.7 beta for Excel 2007. http://mapdisto.free.fr/. acessado em jun. 2008.

Prakash N.S., Marques D.V., Varzea V.M.P., Silva M.C., Combes M.C., Lashermes P., 2004. Introgression molecular analysis of a leaf rust resistance gene from *Coffea liberica* into *Coffea arabica* L. Theor Appl Genet 109:1311-1317

Mahé L., Combes M.C., Várzea V.M.P., Guilhaumon C., Lashermes P., 2008. Development of sequence characterized DNA markers linked to leaf rust (Hemileia vastatrix) resistance in coffee (Coffea arabica L.). Mol Breeding 21:105-113.

Murray M.G., Thompson W.F., 1980. Rapid isolation of high molecular weight plant DNA Nucleic Acids Reserch 8: 4321-4325

Sambrook J., Fritsch E.F., Maniatis T., 1989. Molecular cloning – a laboratory manual, 2nd edn. Cold Spring Habour Laboratory Press, New York

Tamayo P.J., 1988. Resistência de Progênies de Catimor a oito raças de *Hemileia vastatrix* Dissertação de Mestrado. Universidade Federal de Viçosa: UFV 64p

Teixeira-Cabral T.A., Sakiyama N.S., Zambolim L., Pereira A.A, Barros E.G., Silva D.G., 2004. Characterization of differential coffee tree hosts for *Hemileia vastatrix* Berk. et Br. with RAPD markers. Crop Breeding and Applied Biotechnology 4:68-73.

Várzea V.M.P., Marques D.V., 2005. Population variability of *Hemileia vastatrix* vs coffee durable resistance. In: Zambolim L, Zambolim E, Várzea VMP (Eds) Durable resistance to coffee leaf rust. Universidade Federal de Viçosa, Brasil. 53-74

Vos P., Hogers R., Bleeker M., Reijans M., Van de Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M., 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23: 4407-4414.

Wagner M., Bettencourt A.J., 1965. Inheritance of reaction to *Hemileia vastatrix* Berk & Br. in *Coffea arabica* L. Progress report 1960-1965, Coffee Rusts Research Center, Oeira, Portugal.

Study of Drought-Tolerance Mechanisms in Coffee Plants by an Integrated Analysis

P. MARRACCINI[1,2], H.J.O. RAMOS[3], L.G.E. VIEIRA[3], M.A.G. FERRÃO[4], F.R. DA SILVA[2], J.A. TAQUITA[2], C. BLOCH JR.[2], A.C. ANDRADE[2]

[1]CIRAD UMR DAP, Montpellier SupAgro, 2 place Viala - 34060 Montpellier, France
[2]EMBRAPA Recursos Genéticos e Biotecnologia, LGM-NTBio, Parque Estação Biológica, CP 02372, 70770-900 Brasília-DF, Brazil
[3]IAPAR, LBI-AMG, CP 481, 86001-970 Londrina-PR, Brazil
[4]INCAPER, Rua Afonso Sarlo 160, 29052-010 Vitória-ES, Brazil

SUMMARY

The principal aim of this study was to investigate the molecular mechanisms underlying the response to drought stress in coffee plants by different approaches. In order to indentify candidate genes involved in controlling drought tolerance in coffee plants, different strategies were followed in our laboratories. The first used the nucleic data generated by the Brazilian Coffee EST project to identify candidate genes (CG) by *in silico* analysis (electronic Northern-blot). Differential expression of these CG was verified in leaves and roots from drought-tolerant and susceptible clones of *C. canephora* var. Conilon by Northern-blot and quantitative PCR experiments. The second was based on the screening of macroarray membranes spotted with coffee ESTs which were hybridized separately with leaf cDNA probes of the same clones. Finally, 2D gel electrophoresis was also performed to selected proteins presenting differential accumulation in leaves of the same clones. These proteins were analyzed by MALDI-TOF-MS/MS leading to the identification of a new set of CG. Results concerning the identification of CG by these different approaches are presented and discussed.

INTRODUCTION

It is well known that drought periods affect coffee plant development and productivity (DaMatta and Ramalho, 2006). In case of severe drought, this could led to plant death and abortion of developing fruits but also affect flowering, bean development and consequently coffee bean quality, in case of moderate stress. As a consequence of elevation of temperature due to global warming, coffee growing geographical regions could suffer delocalization (Assad et al., 2004), leading environmental, economical and social problems. In such a context, the generation for drought-tolerant coffee varieties now turns one of the priorities of the coffee research mainly in Brazilian research institutions. The development of molecular tools generated by the recent advances in coffee genomic (Lin et al., 2005; Poncet et al., 2006 ; Vieira et al., 2006)] now opens the way to study the genetic determinism of drought-tolerance and the identification of molecular markers that could be used to speed up breeding programs (Lashermes et al., 2008).

We initiated the search of such markers by the *in silico* analysis of the Brazilian Coffee Genome project. The identification of CG was also performed using cDNA macroarrays and looking for CG underlying leaf expression profiles varying with water stress condition applied to coffee plants. Finally, protein profiles by 2-DE coupled with tryptic peptide identification by MALDI-TOF-MS/MS was also tested.

MATERIAL AND METHODS

Plant material

Clones of *C. canephora* var. Conillon tolerant (14, 73 and 120) and sensitive (22) to drought were selected by the INCAPER (Ferrão et al., 2000) and grown in greenhouse with (unstressed condition) or without (stress, Ψ_{H2O} leaves = -3.0 MPa) water. Some of them were already subjected to several physiological and biochemical analyses (DaMatta et al., 2003; Lima et al., 2002; Pinheiro et al., 2004; 2005; Praxedes et al., 2006). Leaves were collected, frozen in liquid nitrogen and further used for expression analyses.

In silico identification of candidate genes (CG)

cDNA libraries from leaves of drought-stressed plants of *C. arabica* cv. Rubi MG 1189 (drought sensitive) and *C. canephora* clone 14 (drought tolerant) of the coffee genome datadase (Vieira et al., 2006; Vinecky et al., 2005) were tested by a statistical test of Fisher (1922) to select ESTs over- or under-expressed in both libraries. Sequence homologies were made by screening the GenBank database using TBLASTX program (Altschul et al., 1997).

Northern-blot experiment

Total RNAs were extracted from collected tissues and tested by Northern-blot experiments as described before Geromel et al. (2006) using EST probes of CG labeled by random-priming with α-^{32}P-dCTP.

Quantitative PCR (qPCR)

Total RNAs were digested with DNaseI-RNase-free (Promega) and 1µg was reverse-transcribed with the ImPromII enzyme according to the recommendations of the furnisher (Promega). Synthesized single-strand cDNA were diluted (1/25 to 1/100) and tested by qPCR using CG primer pairs preliminary tested for their specificity and efficiency against cloned EST (data not shown). The qPCR was performed with 1µl of ss-cDNA in a final volume of 10µl with SYBR green fluorochrom (SYBRGreen qPCR Mix-UDG/ROX, Invitrogen) according to the manufacturer and using a Fast 7500 apparatus (Applied Biosystems). For each sample, GC expression levels were standardized to the expression of ubiquitine gene used as an internal control. Data were treated by SDS 1.3.1. program (Applied Biosystems). Expression levels were expressed in absolute quantification by comparison to standard curves of PCR realized with known concentrations of corresponding genes.

Screening of macroarrays

The inserts of 3,388 clones of an unigene set designed based on clustering ESTs from drought stressed cDNA libraries (SH1 and SH3) (Vieira et al., 2006) were PCR amplified with universal M13 reverse and forward primers. Concentration and quality of the PCR products were checked on agarose gels. Aliquots of 50 µl each (± 250 ng.µl^{-1}) were transferred to 384 plates. An equal volume of DMSO was added to each well. Spotting of the PCR products on nylon Hybond-N+ membranes (GE Healthcare) was performed using a Q-Bot (Genetix Inc.). Each PCR product was spotted in duplicate, in a 3x2 format, totalizing 6,912 spots including 68 controls. After spotting, nylon membranes were treated with denaturing and neutralizing solutions, according to the manufacturer. DNA was fixed onto membranes by UV-cross-linking. Membranes were further hybridized with probes corresponding to ss-cDNAs obtained

by RT of 30 μg of total RNA extracted from leaves of plants of *C. canephora* clones 22 and 14 grown with or without water stress conditions. Labeling was performed by random-priming with α-^{33}P-dCTP. After overnight hybridization, nylon membranes were washed and exposed for 3 days, before analysis on a Phosphoimager FLA3000 (Fuji). Differentially expressed genes were identified using the ArrayGauge software (Fuji).

Proteome Analysis by 2D gel electrophoresis

Proteins were extracted of leaves by a modified phenol/SDS method (Ramos et al., 2007) followed by two-dimensional electrophoresis. The first dimension (isoelectric focusing) was carried out using 13 cm IPG strips (pH 3-10 or pH 4-7) in an IPGphor system (GE Healthcare) and the samples (500-1000 micrograms of proteins) were loaded in during reswelling process at 20 °C for 12 h. The second dimension was in an SDS-PAGE (11%) using the Hoefer SE 600 Ruby system (GE Healthcare) under 15 mA/gel for 45 min and 30 mA/gel for 180 min at 12 °C. Gels were stained with Coomassie Blue G-250 and R-350, digitalized using an UMAX Image Scanner and analyzed with ImageMaster 2D Platinum 6.0. Protein spots differentially expressed were removed manually from gels and analyzed by mass spectrometry using a Maldi-Tof/Tof (Auto-flex, Bruker) mass spectrometer.

Protein identification by MS

Proteins were identified by PMF (Peptide Mass Fingerprinting) using PiumsGUI2.2 and MS/MS Ion Search using the X!Tandem software, against the translated HarvEST and Coffee Project Genone EST-based databases (Vieira et al., 2006). Trans-Proteomic Pipeline (TPP) and Scafold packages were used for analyzing, validating, and storing protein identification data. Additionally, the identification results were verified by visually inspection and by *de novo* sequencing using PepSeq software.

RESULTS

Selection of CG by electronic-northern

The *in silico* analysis ("electronic Northern") allowed us to identify several (n ≈ 20) CGs, some of them coding for putative proteins of known functions like the *rbcS* subunit (Marraccini et al., 2003) of the Rubisco [E-value 7e-^{84}], the cystatine [cystein protease inhibitor, E-value 7e-^{100}) and the mannose 6-P reductase [E-value 7e-^{120}]. However, some others (called "no hit") did not presented significant homologies after searches in public databases.

Expression of CG selected by electronic-northern

Firstly, CGs expression profiles were analyzed in leaves of *C. canephora* clones by Northern-blot and were classified in four types (Fig. 1). Independently of clones analyzed, some CG (i.e. *rbcS*) showed high expression under irrigation (I) but low expression under water limitation (NI) (Figure 1A). Inversely, other CG (i.e. mannose 6-P reductase) presented higher expression under water-stress independently of clones analyzed (Figure 1B). In that case, expression was higher in the clone 73 than in others. Interestingly, other CGs presented differential gene expression between the tolerant (14, 73 and 120) and sensible (22) clones of *C. canephora*. This was the case for CG10 that showed specific expression in the clone 22 that also increased under water-limitation (Figure 1C). Finally, CG with greater expression in tolerant than in sensible clones were also obtained like for the gene encoding cystatin-like protein (Figure 1D).

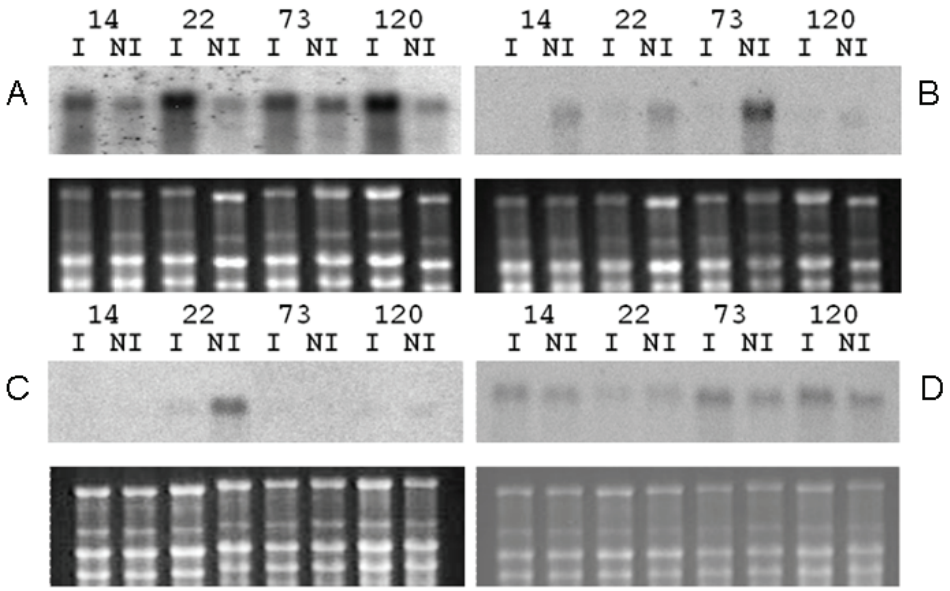


Figure 1. Total RNAs from leaves of *C. canephora* clones grown under irrigated conditions (I) and water deficit (NI) were separated on agarose gel, transferred to nylon membranes and hybridized with probes corresponding to CG labeled with $^{32}P\alpha$-dCTP (upper parts). Total RNAs colored with BET (lower parts) indicates equal loading of the samples.

Expression profiles of theses CGs were also checked by qPCR experiments that confirmed differential expressions detected by northern-blotting (Figure 2). In some cases, gene expression profiles obtained in roots were identical to those in leaves (data not shown).

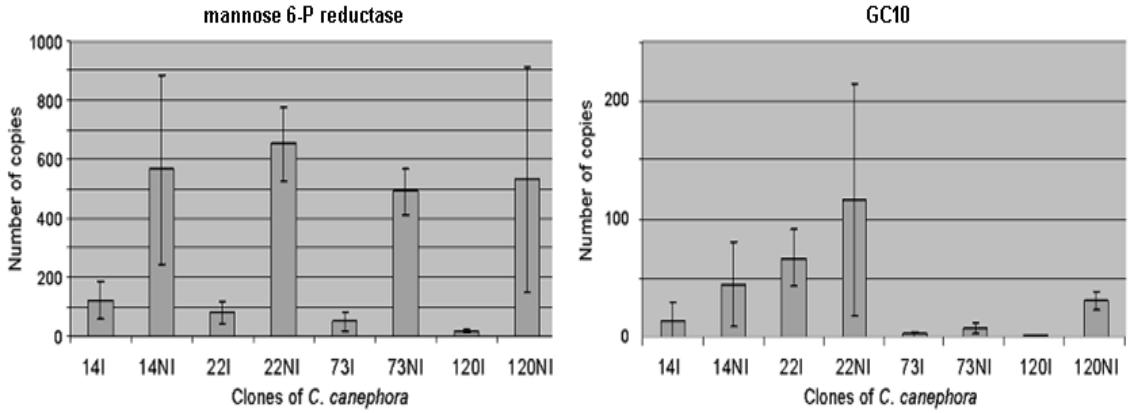


Figure 2. Expression of mannose 6-P reductase and CG10 genes in leaves of *C. canephora* clones grown under irrigated (1) and water stress (NI) conditions. Results are expressed in absolute quantification (Number of copies).

Identification of CG genes by of macroarray screening

After membrane hybridizations, several unigenes showing differential expression were observed. Hybridization profiles of 14I vs. 22I in the figure 3 are presented as an example. qPCR experiments are on-going to verify the differential expression of these unigenes.

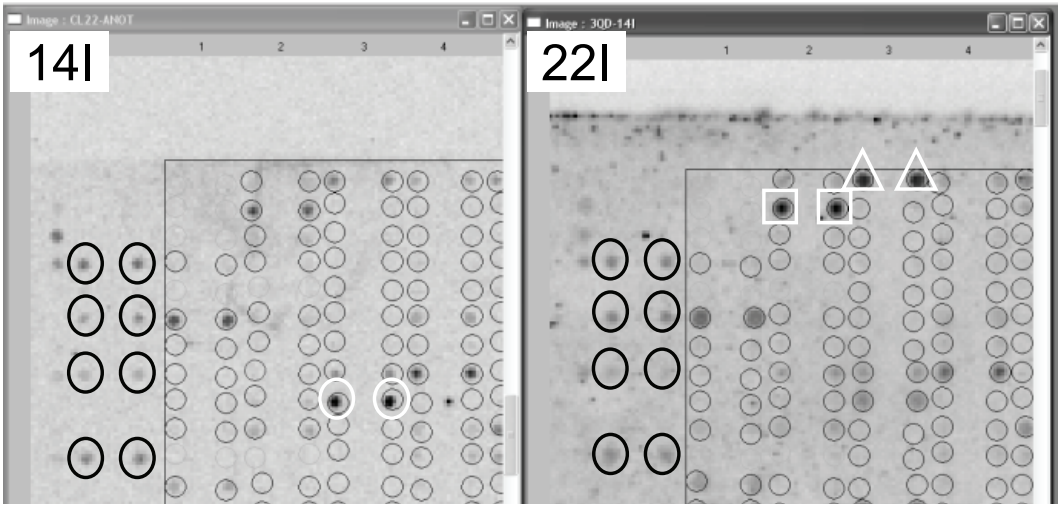


Figure 3. Nylon membranes with unigenes (spotted in horizontal duplicate) were hybridized with *a*-^{33}P-dCTP ss-cDNA probes coming from RNA extracted from leaves of the clones 14 (left) and 22 (right) of *C. canephora* var. Conillon grown under irrigation (I). Unigenes showing identical expression and used to standardize expression levels (control) in both membranes, are in black circles. Unigenes highly expressed in the clones 22I are in white triangles and boxes. A unigene highly expressed in the clone 14I is identified by a white circle.

Protein expression pattern in response to drought

In general, 700-1000 well resolved protein spots of each treatment were used comparative analyses and 40 more intense protein spots differentially expressed have been processed for protein identification by Maldi-Tof MS/MS.

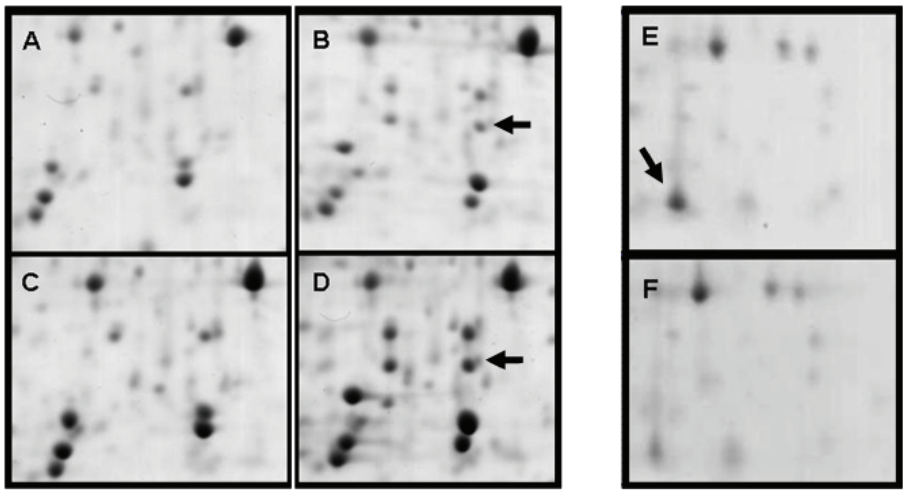


Figure 4. Regions of the 2D gels showing differential accumulation of the HSP26 protein (A to D) and of chloroplast carbonic anhydrase (E and F) are presented. In both cases, proteins are marked by black arrows. 2D gels correspond to leaf proteins of the clone 14 grown under irrigated conditions (A and E) and water deficit (C) and of the clone 22 grown under irrigated conditions (B and F) and water deficit (D).

The gel analysis permitted to identify several proteins that showed differential accumulation between the clones and with the water-stress conditions applied to the plants. This was the case of the coffee protein homologous to the *N. tabacum* HSP26 which was not detected in leaves of the drought-resistant clone 14 (Figure 4A and C) but was present in those of the drought-susceptible clone 22 (Figure 4B and D).

Another example concerns the chloroplast carbonic anhydrase which accumulate to higher amount in the clone 14 than in the clone 22 under unstressed conditions (Figure 4E-F). These 2D gel comparisons also permitted the identification of other proteins differentially expressed like those homologous to Oxygen-evolving enhancer (OEE) protein 2 that always accumulate to higher levels in the clone 22 (data not shown). In some cases, modifications of pI with water-stress conditions were also observed for several proteins particularly for the Rubisco small subunit (RbcS) (data not shown).

CONCLUSIONS AND DISCUSSIONS

This integrated analysis led us to identify several CG and proteins that showed differential expression and accumulation under water stress condition. Interestingly, a large number of them encode for proteins implicated in the photosynthesis suggesting that the drought-tolerance of coffee clones could be directly linked to this biological function. Work is now under way to validate expression of CG identified by macroarray screenings (see poster PB640) or coming from the comparisons of 2D gels, using drought-tolerant clones of *C. canephora* like those forming the clonal variety Conilon Vitória-Incaper 8142 (Fonseca et al., 2004) but also in field-grown plants of cultivars Rubi and Iapar59 of *C. arabica* submitted to different irrigation conditions (Embrapa Cerrados, Planaltina-DF). Expression of CG will be also checked in clones of *C. canephora* representing the genetic diversity of this species where a great variability for drought tolerance was yet reported (Montagnon and Leroy, 1993). In addition to the differences of CG expression profiles observed, it is also worth noting that post-translational modifications should occur under drought stress condition. In order to increase our knowledge about metabolic changes occurring in drought stressed coffee plants, the consequences of such modifications and their implications on cell metabolism are now under investigations. CGs showing differential expression profiles between drought-tolerant and susceptible clones of *C. canephora*, but also with water limitation, could now be used for the search of nucleic markers (i.e. SNP) and promoter regions (see poster PB620) that could be further used as genomic tools either to speed-up conventional breeding programs or for coffee genetic transformation.

FINANCIAL SUPPORT

This work was supported by CBP&D-Café, FINEP and Fundação Araucária (Brazil).

REFERENCES

Altschul S.F., Madden T.L., Schäffer A.A., Zhang J., Zhang Z., Miller W., Lipman D.J. (1997). “Gapped BLAST and PSI-BLAST: a newgeneration of protein database search programs”, Nucl. Acids Res. 25: 3389-3402.

Assad E.D., Pinto H.S., Zullo Jr J., Ávila A.M.H. (2004). Impacto das mudanças climáticas no zoneamento agroclimático do café no Brasil. Pesq. Agropec. Bras. 39: 1057-1064.

DaMatta F.M., Ramalho J.C. (2006). Impact of drought and temperature stress on coffee physiology and production: a review. Braz. J. Plant Physiol. 18: 55-81.

DaMatta F.M., Chaves A.R.M., Pinheiro H.A., Ducatti C., Loureiro M.E. (2003). Drought tolerance of two field-grown clones of *Coffea canephora*. Plant Sci. 164: 111-117.

Ferrão R.G., Fonseca A.F.A., Silveira J.S.M., Ferrão M.A.G., Bragança S.M. (2000). EMCAPA 8141 - Robustão Capixaba, variedade clonal de café conilon tolerante à seca, desenvolvida para o estado do Espírito Santo. Rev. Ceres 273: 555-560.

Fisher R.A. (1922). On the interpretation of χ^2 from contingency tables, and the calculation of P. J. Royal Stat. Soc. 85: 87-94.

Fonseca A.F.A., Ferrão M.A.G., Ferrão R.G., Verdin Filho A.C., Volpi P.S., Zucateli F. (2004). Conilon Vitória - Incaper 8142: improved Coffea canephora var. Kouillou clone cultivar for the State Espírito Santo. Crop Breed. Appl. Biotech. 4: 1-3.

Geromel C., Ferreira L.P., Cavalari A.A., Pereira L.F.P., Guerreiro S.M.C., Vieira L.G.E., Leroy T., Pot D., Mazzafera P., Marraccini P. (2006). Biochemical and genomic analysis of sucrose metabolism during coffee (*Coffea arabica*) fruit development. J. Exp. Bot. 57: 3243-3258.

Lashermes P., Andrade A.C., Etienne H. (2008). Genomics of coffee, one of the world's largest traded commodities. *In*: "Genomics of Tropical Crop Plants". H Moore & R Ming eds. Springer, pp. 203-226.

Lima A.L.S., DaMatta F.M., Pinheiro H.A., Totola M.R., Loureiro M.E. (2002). Photochemical responses and oxidative stress in two clones of *Coffea canephora* under water stress. Env. Exp. Bot. 47: 239-247.

Lin C., Mueller L.A., McCarthy J., Crouzillat D., Pétiard V., Tanksley S.D. (2005). Coffee and tomato share common gene repertoires as revealed by deep sequencing of seed and cherry transcripts. Theor. Appl. Genet. 112: 114-130.

Marraccini P., Courjault C., Caillet V., Lausanne F., Lepage B., Rogers W.J, Tessereau S., Deshayes A. (2003). Rubisco small subunit of *Coffea arabica*: cDNA sequence, gene cloning and promoter analysis in transgenic tobacco plants. Plant Physiol. Biochem. 41: 17-25.

Montagnon C., Leroy T. (1993). Réaction à la sécheresse de jeunes caféiers *Coffea canephora* de Côte-d'Ivoire appartenant à différents groupes génétiques. Café Cacao Thé 37: 179-190.

Pinheiro H.A., DaMatta F.M., Chaves A.R.M., Fontes E.P.B., Loureiro M.E. (2004). Drought tolerance in relation to protection against oxidative stress in clones of *Coffea canephora* subjected to long-term drought. Plant Sci. 167: 1307-1314.

Pinheiro H.A., DaMatta F.M., Chaves A.R.M., Loureiro M.E., Ducatti C. (2005). Drought tolerance is associated with rooting depth and stomatal control of water use in clones of *Coffea canephora*. Ann. Bot. 96: 1001-108.

Poncet V., Rondeau M., Tranchand C., Cayrel A., Hamon S., de Kochko A., Hamon P. (2006). SSR mining in coffee tree EST databases: potential use of EST-SSRs AS marker across *Coffea* genus. Mol. Gen. Genet. 276: 436-449.

Praxedes S.C., DaMatta F.M., Loureiro M.E., Ferrão M.A.G., Cordeiro A.T. (2006). Effects of long-term soil drought on photosynthesis and carbohydrate metabolism in mature robusta coffee (*Coffea canephora* Pierre var. *kouillou*) leaves. Environ. Exp. Bot. 56: 263-273.

Ramos H.J.O., Seixas D.F., Pereira L.F.P., Cruz L. M., Fungaro M. H., Hungria M., Osaku C., Petzl-Erler M.L., Ayub R.A., Peralta M., Marur C.J., Souza E.M., Vieira L.G.E.,

Pedrosa F.O. (2007). Análise proteômica do estresse hídrico em cafeeiro. V Simpósio de Pesquisa dos Cafés do Brasil, Águas de Lindóia, CD-rom.

Vieira L.G.E., Andrade A.C., Colombo C.A., Moraes A.A.H., Metha A., Oliveira A.C., Labate C.A., Marino C.L., Monteiro-Vitorello C.B., Monte D.C., Giglioti E., Kimura E.T., Romano E., Kuramae E.E., Lemos E.G.M., Almeida E.R.P., Jorge E.C., Barros E.V.S.A., da Silva F.R., Vinecky F., Sawazaki H.E., Dorry H.F.A., Carrer H., Abreu I.N., Batista J.A.N., Teixeira J.B., Kitajima J.P., Xavier K.G., Lima L.M., Camargo L.E.A., Pereira L.F.P., Coutinho L.L., Lemos M.V.F., Romano M.R., Machado M.A., Costa M.M.C., Grossi de Sá M.F., Goldman M.H.S., Ferro M.I.T., Tinoco M.L.P., Oliveira M.C., Sluys M.A.V., Shimizu M.S., Maluf M.P., Eira M.T.S., Guerreiro Filho O., Arruda P., Mazzafera P., Mariani P.D.S.C., Oliveira R.L., Harakava R., Balbao S.F., Tsai S.M., Mauro S.M.Z., Santos S.N., Siqueira W.J., Costa G.G.L., Formighieri E.F., Carazzolle M.F., Pereira G.A.G. (2006). Brazilian coffee genome project: an EST-based genomic resource. Braz. J. Plant Physiol. 18: 95-108.

Vinecky F, de Brito KM, Da Silva FR, Andrade AC (2005) Análise *in silico* de genes potencialmente envolvidos na resposta aos estresses abióticos, presentes na base de dados do projeto Genoma café. IV Simpósio de Pesquisa dos Cafés do Brasil, Londrina-PR, Embrapa Café, CD-rom

Construction of a Genetic Map Based on an Interspecific F_2 Population between *Coffea arabica* and *Coffea canephora* and its Usefulness for Quality Related Traits

R.H.G PRIOLLI[1], L.C.S. RAMOS[2], D. POT[3,4], M. MOLLER[2], P.B. GALLO[2], M.M. PASTINA[1], A.A.F. GARCIA[1], P.Y. YAMAMOTO[2], S.D. LANNES[3], L.P. FERREIRA[3], M.B.S. SCHOLZ[3], P. MAZZAFERA[5], L.F.P. PEREIRA[6], C.A. COLOMBO[2]

[1]ESALQ/USP, Departamento de Genética, Piracicaba (SP), Brazil
[2]IAC (Instituto Agronômico de Campinas), CPD de Recursos Genéticos Vegetais, Campinas (SP), Brazil
[3]IAPAR (Instituto Agronômico do Paraná), Londrina (PR) Brazil
[4]Cirad, UMR DAP, Montpellier, France
[5]UNICAMP, Instituto de Biologia, Campinas (SP), Brazil
[6] Embrapa Café, Brasília, DF, Brazil

SUMMARY

Genetic maps based on molecular markers have been developed in a large set of plants and this strategy has proven its efficiency towards the identification of tools for marker assisted selection. In the present study, AFLP and SSR markers were used to build a genetic map of an interspecific F_2 population between *Coffea arabica* and *Coffea canephora*. It was identified 349 AFLP markers and 50 SSR alleles segregating in 90 F_2 plants from forty four AFLP combinations and 19 SSR loci. For the map construction, only single dose markers segregating 3:1 in the F_2 were considered (248 AFLP markers and 27 SSR alleles, standing for to 68.9% of the polymorphic markers). The genetic map was build and one hundred and sixty nine markers were mapped, corresponding to 155 AFLP markers and 14 SSR loci. Thirty seven linkage groups corresponding to a total map length of 1011 cM were obtained, with an average distance between the markers of 5.98 cM and an average of 4.58 markers per linkage group. Forty-six marker trait associations were found; of which, nineteen were associated with sugar content, eight for caffeine, eight for CGA, one for caffeine and CGA and ten for total production per plant. Only four single markers associations were detected at both years of determinations. The single markers analysis for QTL detection allowed us to obtain previous information of putative QTL association for coffee quality and productivity. Additional markers are being added to this working linkage map for more complete coverage of the coffee genome.

INTRODUCTION

C. arabica L. the only self-fertile tetraploid species (2n = 4x = 44) of the *Coffea* genus, is characterized by low genetic diversity which has been attributed to its allotetraploid origin, its reproductive biology and its domestication history. In contrast, diploid species from the coffea genus (2n = 2X = 22) are alogamous and are highly diverse at the phenotypic and molecular levels. These species form valuable gene reservoirs for different breeding purposes (Carvalho 1988).

Transfer of desirable genes from *C. canephora* to *C. arabica* varieties through interspecific crosses is one of the breeding strategies used for coffee improvement. *C.arabica* x *C.canephora* hybrids, resulting from the hybridization between *C. arabica* and colchicine doubled *C. canephora* have been cited as reasonably fertile (Berthaud, 1978; Owuor and Van der Wossen, 1981). They are also particularly favorable to intergenomic recombination and gene introgressions (Lashermes et al., 2000; Herrera et al., 2002; Priolli et al., 2008).

Molecular markers are being used successfully in many crops to assist directed germplasm improvement. Marker-assisted selection allows screening of large numbers of trees for a gene of interest at early stage and reduces the number of backcrosses required to select elite genotypes (Lashermes et al., 1999).

In coffee, a saturated *Coffea canephora* genetic was built by Lashermes et al. (2001) and others partial genetic maps were obtained for interspecific crosses involving diploid species (*C. pseudozanguebarie x C. liberica* (Ky et al., 2000) e *C. canephora* x *C. heterocalyx* (Coulibaly et al.2003)). Identification of quantitative trait loci allowed the localization of two markers that flanked a fructification time genomic region (Akaffou et al. 2003) and three markers associated with pollen viability (Coulibaly et al., 2003), which could be used for early marker-assisted selection.

In *C. arabica*, according to its low polymorphism level associated with its tetraploid, the strategy consists on the construction of partial genetic maps (Pearl et al., 2004; Teixeira-Cabral et al., 2004) with posterior integration of the partial genetic maps.

In the present study, AFLP and SSR markers were used to build a genetic map of an F_2 interspecic population between *C. arabica* and *C.canephora.* In addition, association between segregating markers and quality related traits were analyzed.

MATERIAL AND METHODS

Plant material

The F_1 tetraploid hybrid between *Coffea arabica L*. var. Bourbon Vermelho and *Coffea canephora* var. Robusta 4x, an artificial tetraploid obtained by Mendes (1947), has, since 1996, been advanced to F_2 by selfing three F_1 clones from the same plant. The F_2 segregating population was grown in a field trial at a site near the municipality of Mococa (latitude 21º 28' S, longitude 47º 01' W and altitude 665 m) in São Paulo State Brazilian state received treatment with inorganic fertilizer, and weed and pest control and all other treatments recommended for growing coffee under Brazilian conditions (Thomaziello et al., 1996).

Sample preparation and field data

Leaves and fruits from each F2 were harvested two successive years (2004 and 2005). Leaves were collected from the third and fourth leaf pairs from different sides of the tree canopy. Fruits were harvested at mature stage for analysis of caffeine, chlorogenic acids and sugar contents.

Seeds were manually removed from the pericarp, dried at 70 °C for two weeks and then finely ground with a blade grinder or with a pestle and mortar. Caffeine and chlorogenic acids were extracted according to Priolli et al. (2008). Total and reducing sugars were extracted according to Rogers et al. (1999) and quantified using Somogyi and Nelson reagent. Sucrose

content was estimated by the substraction of reducing sugars content from total sugar contents. Production was also evaluated (Kg fruits / Plant).

Molecular marker assay

Total genomic DNA was extracted from freeze-dried leaves of the parental, F_1 hybrid and F_2 genotypes as described by Ky et al. (2000). Nineteen microsatellite loci, previously identified as polymorphic between *C. arabica* and *C. canephora*, were analyzed using PCR. Some of these microsatellite loci (Table 1) have been mapped in *C. canephora* (Lashermes et al, 2001) and other were obtained by Combes et al (2000). The specific primer pairs, amplification conditions, radioactive labelling and polyacrylamide gel electrophoresis were as reported elsewhere (Priolli et al., 2008). The amplified fragment length polymorphism (AFLP) procedure was performed as previously reported (Vos et al., 1995). Briefly, 500 ng of genomic DNA was digested with the restriction enzymes EcoRI and MseI. Restriction fragments were then ligated with double-strand EcoRI and MseI adapters. A selective pre-amplification was performed using the appropriate primers (named E and M, respectively) without selective nucleotide at the 3′ end (ie E+0/M+0). The reaction mixture was diluted 1/10 and 3 µL was used for the final amplification with two primers, each containing three selective nucleotides (Table 1).

Data analysis

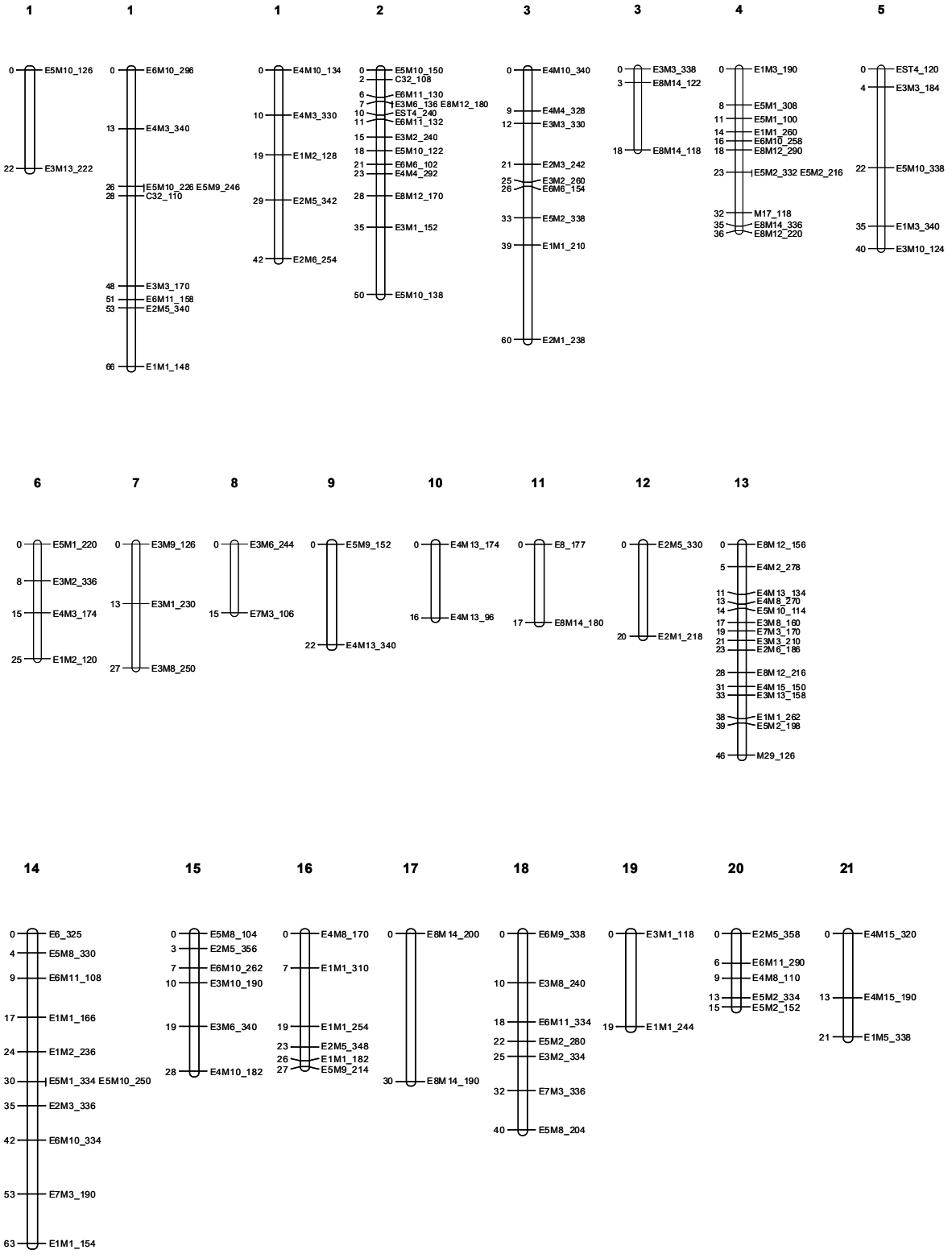
Only markers polymorphic between the parents and present in the F_1 were considered. This strategy was applied whatever the marker used. Consequently, both SSRs and AFLPs were considered here as dominant markers. Segregation distortion from the 3:1 expected ratio for single dose markers was analyzed by the chi-square test. Bonferoni correction was applied to control type I error for multiple tests. Map construction was carried out using Joinmap version 3.0 (Stam 1993). Linkage groups were established using two-point analysis with LOD threshold values of 4 and recombination fraction of 0.50. The Kosambi function was used for converting recombination fractions into map distances. Associations between markers and the analyzed traits (total sugar, reducing sugar, sucrose, caffeine, chlorogenic acids contents and production) were analyzed by one-way ANOVA. Significant association were considered for *P* value lower than 0.001 and suggestive associations were considered for *P* value between 0.001 and 0.005.

RESULTS AND DISCUSSION

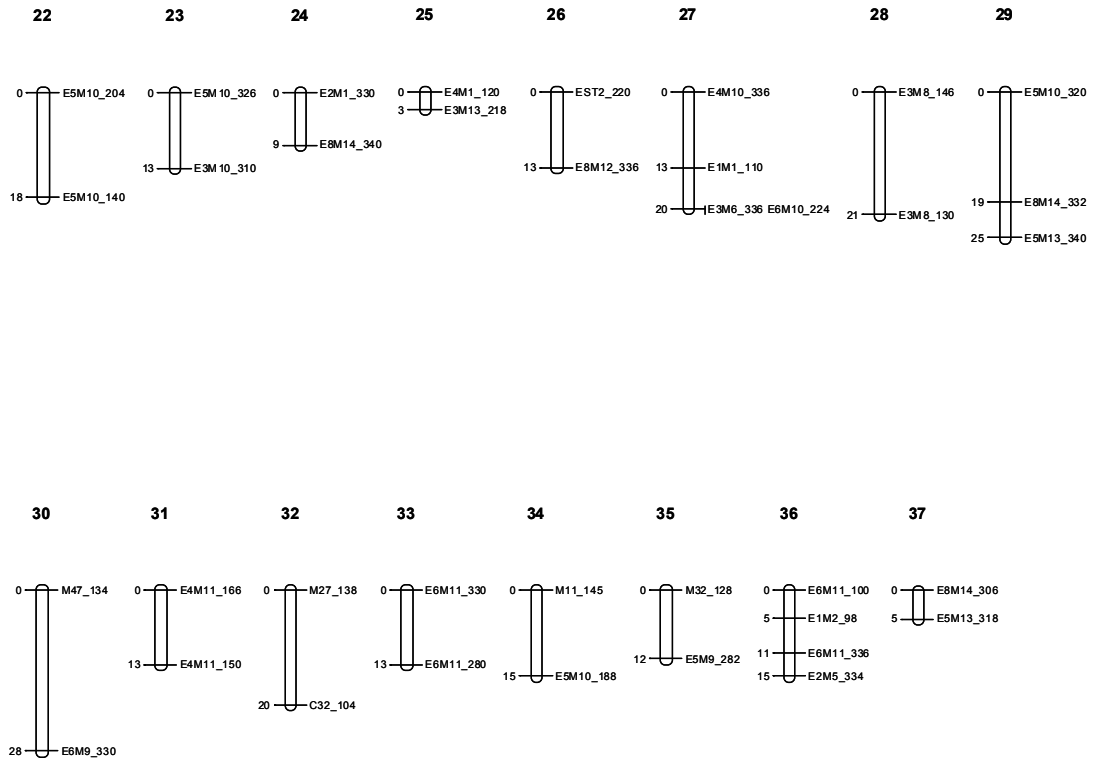
Linkage map

Fifty alleles of SSR markers and 349 AFLP markers were polymorphic in F_2 populations. Chi-square analysis revealed 275 markers (69%) fitted a 3:1 ratio. The remaining 99 (33%) showed segregation distortion within the population. One hundred and sixty nine markers were mapped, corresponding to 155 AFLP markers and 14 SSR alleles. 66% of the mapped markers came from the *Coffea arabica* parent, 30% from the *Coffea canephora* 4x and 4% from both parent. These results suggest that the divergence between the two ancestral genomes of *Coffea arabica* (i.e *C. canephora* and *Coffea eugenioides*) is two times larger than the one between the two haplotypes of the *C. canephora* genotype used as a parent to create the F_1 hybrid. Similar results were observed by Pearl et al (2004) who found in a pseudo F_2 population derived from a cross between the cultivars of *C. arabica* , 68% of AFLP markers were from cv. Catimor, 30% from cv. Mokka hybrid, and 2% were codominant.

Thirty seven linkage groups corresponding to a total map length of 1011 cM were obtained, with an average distance between the markers of 5.98 cM and 4.58 markers per linkage group (Figure 1). Twenty linkage groups include only 2 markers.



(A)



(B)

Figure 1.Genetic linkage map constructed from *C.arabica* x *C.canephora* containing 155 AFLP markers and 14 SSR markers. Mapping distances are represented in centiMorgans (cM) and markers codes are provided on the right side of each linkage group.

Segregation analyses of restriction fragment length polymorphism (RFLP) loci-markers have indicated tetrasomic inheritance resulting from the pairing of homologous chromosomes in meiosis of first generation *C. arabica* x *C. canephora* 4x hybrids (Lashermes et al., 2000). In two BC_1F_1 populations, (*C. arabica* × *C. canephora* 4x) × *C. arabica*, segregations and co-segregation of RFLP and microsatellite loci-markers conformed to the expected ratio assuming random chromosome segregation and the absence of selection (Herrera et al., 2002). In a study using SSR loci, the hybrid F_1 showed that the ratios of the gametes genotype did not differ significantly from those expected assuming random associations and tetrasomic inheritance (Priolli et al., 2008).

In *C. arabica*, according to its low polymorphism level associated with its tetraploid, the strategy consists on the construction of partial genetic maps with posterior integration of the partial genetic maps. A linkage map of arabica coffee was constructed from 288 AFLP primer combinations resulting in 16 major linkage groups containing 4-21 markers, and 15 small linkage groups consisting of 2-3 linked markers. The total length of the map was 1,802.8 cM, with an average distance of 10.2 cM between adjacent markers (Pearl et al., 2004). In a backcross population of the *C. arabica*, a partial genetic map was constructed with 82 RAPD loci and covered the estimated length of 540.6cM, average distance of 7.3 cM between adjacent markers in a total of eight linkage groups (Teixeira-Cabral et al 2004). In *C. canephora*, a genetic map of 1402 cM with 15 linkage groups was reported using 47 RFLP and 100 RAPD markers (Paillard et al., 1996). Eleven linkage groups that putatively correspond to the 11 gametic chromosomes of *C. canephora* were identified from 162 loci (97 AFLP, 11 RAPD, 18 microsatellite, and 36 RFLP) in a total map length of 1041 cM and

average distance of 6.5 cM. (Lashermes et al., 2001). Genetic maps for interspecific diploid crosses were also obtained for *C. pseudozanguebarie x C. liberica* (Ky et al., 2000) and *C. canephora* x *C. heterocalyx* (Coulibaly et al., 2003) leading to the identification of 14 linkage groups covering 1,144 cM and 15 linkage groups with 1,360 cM respectively.

Table 1. List of SSR loci and selective AFLP primers (EcoRI+3 and MseI+3) with their codes presents in *C.arabica* X *C.canephora* genetic map.

Loci SSR	Code	Primer AFLP	Code	Primer AFLP	Code
17-2CTG	M17	ACC	E1	CAA	M7
32-2CTG	C32	ACT	E2	CAG	M8
C2-2CATC	C2	AAC	E3	CGA	M9
E6-3CTG	E6	ACA	E4	CGT	M10
E8-3CTG	E8	AAG	E5	CGG	M11
EST1	EST1	AGC	E6	CCC	M12
EST2	EST2	AGG	E7	CGC	M13
EST4	EST4	ACG	E8	CCG	M14
M11	M11	CAC	M1	CCA	M15
M25	M25	CTG	M2		
M27	M27	CTT	M3		
M29	M29	CTC	M4		
M32	M32	CAT	M5		
M47	M47	CTA	M6		

Single marker analysis

Single marker analysis to detect associations between phenotypic traits including total sugar, reducing sugar, sucrose, caffeine, chlorogenic acids contents and production and molecular markers were performed. Two threshold levels corresponding to significant association (*P* value < 0.001) and suggestive level (0.001 < P value < 0.005) were considered (Table 2). Overall, 46 marker trait associations were found corresponding to seven from SSR makers and 39 from AFLP markers. The percentage of the phenotypic variance explained by each marker ranged from 10.62 (E3M1_118 marker) to 20.69% (E3M3_170 marker). In relation to biochemical contents, ten marker-trait associations were found for total sugar, seven to reducing sugar, eleven to sucrose, nine to caffeine and nine to CGA. For field production data, ten markers presenting suggestive and significant effect were detected. Nine markers were associated to total sugar and sucrose but only two of these markers presented significant or suggestive effects in the two years analysed (E3M8_250 and E5M1_308). Marker E4M3_330 also presented association with caffeine content in the two successive years, the same effect stability was observed for E3M8_146 in relation with the production level. These markers can be viewed as consistent markers, with potential to be applied in a further marker assisted selection.

In a few cases the same markers presented association with two distinct traits. Such results were observed for marker E2M5_342 for caffeine and GCA contents. The same observation was done for markers that presented associations with total sugars and sucrose contents. However such association was expected as sucrose content was derived from the total sugar and reducing sugar contents.

Some QTLs in Coffea were detected using segregating population, derived from interspecific crosses. Three significant QTLs (LOD > 3 and p < 0.001 by ANOVA) were detected for

pollen viability in a backcrossed progenies originating from a cross between *Coffea canephora* and *Coffea heterocalyx* (Coulibaly et al., 2003).

Table 2. Single marker-test for total and reducing sugar (TS and RS), sucrose, caffeine, chlorogenic acids (CGA), and production at years 2004 and 2005, with their effects in % of the phenotypic variability.

Markers	TS		RS		Sucrose		Caffeine		CGA		Production	
	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
E3M8_250*	**11.23**	**12.36**			**11.16**	**12.24**						
E5M1_308**	**16.76**	**16.97**			**16.39**	**16.79**						
E5M2_216**	15.21				15.53							
E5M2_332*	12.00				11.93							
E5M8_330*	10.96				11.07							
E3M1_118*		10.62				11.01						
E3M1_230*		12.33				12.31						
E5M1_100**		14.16				13.80						
EST1_110*		13.96				14.38						
E8_179*		11.11										
E5M9_290**			14.55									
E6M6_272**			12.92									
E7M3_126*			12.79									
M47_151**			16.57									
E4M4_292**				13.01								
E6M9_108*				10.64								
M11_45**				14.96								
E3M6_244*						11.16						
E6_325*						11.01						
E4M3_330*							**11.36**	**11.69**				
E6M8_120**							16.74					
E8M12_156*							13.44					
E8M14_180**							13.78					
E3M10_120**								16.31				
E3M1_260*								11.96				
E5M1_270*								12.04				
E8M14_118*								12.17				
E2M5_342**							17.77		11.28			
E1M1_166*									10.84			
E7M3_120**									16.99			
E2M5_350**										14.17		
E3M3_338*										11.29		
E4M4_328**										16.36		
E5M1_326**										14.13		
E8M14_118**										14.46		
E8M14_122**										16.86		
E2M3_336*											12.20	
E2M5_340**											16.51	
E3M3_170**											20.69	
E3M8_146**											**13.99**	**13.34**
E3M8_320**											18.35	
E6M11_158**											15.36	
E6M9_336*											11.29	
EST4_240**											18.23	
C32_110**											12.95	
E3M6_244*												11.34

**Significance at P < 0.005*

***Significance at P < 0.001. Bold letters: significance at 2004 and 2005.*

Red letters indicate one mark associated with two traits.

Only one QTL was identified for fructification time using a one-way ANOVA with a significance level of $P < 0.001$ in a cross between *Coffea pseudozanguebariae* X *C. liberica* var. Dewevrei . The QTL was located on linkage group E defined by Ky et al. (2000), between the AFLP marker ACCCTT1 and the RFLP marker G13 The ACCCTT1 marker explained 64% of the fructification variance (Akaffou et al., 2003).

The single markers analysis allowed us to obtain preliminary information of putative QTL for biochemical components involved in the quality of coffee beverage an their relation with production. However, others QTL detection approaches such as interval mapping (Lander and Botstein, 1989) and composite interval mapping (Zeng, 1994), both, having more power to detect single QTL marker association are planned to be applied to our data set in a short-term.

REFERENCES

Akaffou D.S., Ky C.L., Barre P., Hamon S., Louarn J., Noirot M. (2003) Identification and mapping of a major gene (Ft1) involved in fructification time in the interspecific cross *Coffea pseudozanguebariae* X *C. liberica* var. Dewevrei: impact on caffeine content and seed weight Theor Appl Genet (2003) 106:1486-1490

Berthaud J. (1978) L'hybridation interspécifique entre *Coffea arabica* L. et *Coffea canephora* Pierre. Obtention et comparaison des hybrides triploïdes arabusta et hexaploïdes. Café Cacao Thé 22: 87-109

Carvalho A. (1988) Principles and practices of coffee plant breeding for productivity and quality factors: *Coffea arabica*. In Coffee. Vol. 4. Agronomy. Edited by R.J. Clarke and R. Macrae. Elsevier Applied Science, London. pp. 129-165.

Combes M.C., Andrzejewski S., Anthony F., Bertrand B., Rovelli P., Graziosi G. and Lashermes P. (2000) Characterization of microsatellite loci in *Coffea arabica* and related coffee species. Mol Ecol 9: 1178-1180.

Coulibaly I., Louarn J., Lorieux M., Charrier A., Hamon S., Noirot M. (2003) Pollen viability restoration in a *Coffea canephora* P. and *C. heterocalyx* Stoffelen backcross. QTL identification for marker-assisted selection Theor Appl Genet 106: 311-316

Coulibaly I., Revol B., Noirot M., Poncet V., Lorieux M., Carasco-Lacombe C., Minier J., Dufour M., Hamon P. (2003) AFLP and SSR polymorphism in a *Coffea* interspecific backcross progeny [(*C. heterocalyx* X *C. canephora*) X *C. canephora*] Theor Appl Genet 107: 1148-1155

Herrera J.C., Combes M.C., Anthony F., Charrier A. and Lashermes P. (2002) Introgression into the allotetraploid coffee (*Coffea arabica* L.) segregation and recombination of the *C. canephora* genome in the tetraploid interspecific hybrid (*C. arabica* X *C. canephora*). Theor Appl Genet 104: 661-668.

Ky C.L., Barre P., Lorieux M., Trouslot P., Akaffou S., Louarn J., Charrier A., Hamon S. and Noirot M. (2000) Interspecific genetic linkage map segregation distortion and genetic conversion in coffee (*Coffea* sp.). Theor Appl Genet 101: 669-676.

Lander E.S., Botstein D. (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps Genetics. 121: 185-199.

Lashermes P., Combes M.C., Robert J., Trouslot P., D'hont A.A.F. and Charrier A. (1999) Molecular characterization and origin of the *Coffea arabica* L. genome. Mol. Gen. Genet. 261: 259–266.

Lashermes P., Paczek V., Trouslot P., Combes M.C., Couturon E. and Charrier A. (2000) Single-locus inheritance in the allotetraploid *Coffea arabica* L. and interspecific hybrid *C. arabica* X *C. canephora*. J Heredity 91: 81-85

Lashermes P., Combes M.C., Prakash N.S., Trouslot P., Lorieux M. and Charrier A. (2001) Genetic linkage map of *Coffea canephora*: effect of segregation distortion and analysis of recombination rate in male and female meioses. Genome 44: 589-596

Mendes A.J.T. (1947) Observações citológicas em Coffea: Métodos de tratamento pela colchicina. Bragantia 7: 221-230.

Owuor J.B.O. and Van der Vossen H.A.M. (1981) Interspecific hybridization between *Coffea arabica* L. and tetraploid *Coffea canephora* Pierre: I: Fertility in F1 hybrids and backcrosses to *C. arabica*. Euphytica 30: 861-866.

Paillard M., Lashermes P. and Pétiard V. (1996) Construction of a molecular linkage map in coffee. Theor Appl Genet 93: 41-47.

Pearl H.M., Nagai C., Moore P.H., Steiger D.L., Osgood R.V. and Ming R. (2004) Construction of a genetic map for arabica coffee. Theor Appl Genet 108: 829-835.

Priolli R.H.G., Mazzafera P., Siqueira W.J.G., Möller M., Zucchi M.I., Ramos L.C., Gallo P.G., Colombo C.A. (2008) Caffeine inheritance in interspecific hybrids of *Coffea arabica* X *Coffea canephora* (Gentianales, Rubiaceae). Genetics and Molecular Biology 31: 498-504

Stam P. (1993) Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J. 3: 739-744.

Teixeira-Cabral T.A., Sakiyama N.S., Zambolim L., Pereira A.A. and Schuster I. (2004) Single-locus inheritance and partial linkage map of *Coffea arabica* L. Crop Breeding and Applied Biotechnology 4: 416-421.

Thomaziello R.A., Oliveira E.G., Toledo Filho J.A. and Costa T.E. (1996) Cultura do café, Fundação Cargill, Campinas, 69 pp.

Vos P., Hogers R., Bleeker M., Reijans M., Van de Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M., Zabeau M. (1995) AFLP: a new technique for DNA fingerprinting. Nuclei Acid Res 23: 4407-4414.

Zeng Z.B. (1994) Precision mapping of quantitative trait loci. Genetics 136: 1457-1466.

Chloroplast Membrane Lipids from *Coffea* sp. under Low Positive Temperatures

F.L. PARTELLI[1], H.D. VIEIRA[2], I.P. PAIS[3], V.L. QUARTIN[3,4], P.S. CAMPOS[3], A. FORTUNATO[4], P. EICHLER[4], A.P. VIANA[2], A. RIBEIRO[4], J.C. RAMALHO[4]

[1]Universidade Federal de Goiás - UFG, Escola de Agronomia e Engenharia de Alimentos. Campus II Samambaia. Caixa Postal 131, 74001-970, Goiânia - GO, Brazil
[2]Universidade Estadual do Norte Fluminense Darcy Ribeiro - UENF, Centro de Ciências e Tecnologias Agropecuárias. Av. Alberto Lamego 2000, Parque Califórnia, 28013-602, Campos dos Goytacazes - RJ, Brazil
[3]L-Instituto Nacional de Investigação Agrária / INRB, Av. República, Quinta do Marquês 2784-505 Oeiras, Portugal
[4]Centro de Ecofisiologia, Bioquímica e Biotecnologia Vegetal / Inst. Inv. Científica Tropical, Av. República, Quinta do Marquês, 2784-505 Oeiras, Portugal

SUMMARY

Plants have acclimation mechanisms conferring them tolerance to environmental limiting conditions and better recovering ability after the stress ceases. The objective of this work was to evaluate the influence of low positive temperatures (*chilling*) on the photosynthetic structures of *Coffea* sp. genotypes, through the analysis of the chloroplast membrane lipid composition and leaf shedding. For that, two genotypes of *Coffea canephora* (clone 02 and 153) and one of *C. arabica* (Catucaí IPR 102) were used. Plants were placed in growth chambers under environmental controlled conditions with temperature 25/20 °C (day/night), irradiance 700-900 μmol m^{-2} s^{-1}, external CO_2 concentration *ca*. 380 μL L^{-1}, relative humidity 70% and a 12 h photoperiod. Plants were successively submitted to a gradual temperature decrease from 25/20 °C down to 13/8 °C (0.5 °C day^{-1}), 3 days at 13/4 °C and 14 days at temperature of 25/20 °C, allowing the plants to recover. Conilon clone 02 was the most affected genotype, showing stronger leaf shedding, followed by clone 153, while Catucaí was less affected. The latter genotype usually showed a higher degree of lipid unsaturation, as well a higher proportion of linolenic acid and a lower of palmitic acid in phosphatidyglycerol, suggesting greater membrane fluidity. Quantitative and qualitative differences in chloroplast fatty acids and lipid classes, together with leaf shedding evaluation, may contribute to the management and adequate genotype selection for low temperature occurrence areas, thus constituting valuable tools in *Coffea* sp. breeding programs.

INTRODUCTION

The genus *Coffea* comprehends at least 103 species, but only *C. arabica* and *C. canephora* have worldwide commercial relevance (Davis et al., 2006). When cultivated in latitudes higher than 15 °C, coffee shows a clear decrease in growing rates in winter season (Libardi et al., 1998; Silva et al., 2004). However, coffee plants have acclimation mechanisms, which includes quantitative and qualitative membrane lipid modifications and increased ability of energy excess dissipation, conferring higher tolerance to low temperatures and recovering capacity after the stress finishes (Campos et al., 2003; Ramalho et al., 2003).

In fact, when submitted to low temperatures some *Coffea* sp. genotypes may undergo an unsaturated fatty acid (FA) increase, such as in linolenic acid (C18:3), specially at the

beginning of acclimation (Campos et al., 2003). This modification helps membrane fluidity, since the *cis* double bonds on these FA chains, stimulate the formation of curvatures that increase chain flexibility, thus ensuring an adequate photosynthetic activity (Xin, and Browse, 2000). However unsaturated FA (abundant in galactolipids) are also the preferential substrate for peroxidases, hydrolytic enzymes and reactive oxygen species (Campos et al., 2003), the latter with a common occurrence when plants are submitted to cold.

Cultivation of Conilon coffee in higher altitude areas (lower temperatures) has recently increased. In spite of its high susceptibility to low temperatures when compared to *C. arabica* (Ramalho et al., 2003), growers and researchers believe that cultivation of *C. canephora* cv. Conilon may be an alternative for these areas, once this cvs. shows higher tolerance to biotic and abiotic stresses.

The objective of this work was to evaluate the influence of low positive temperatures over the photosynthetic structures through the analysis of the lipid composition of membrane chloroplasts and leaf shedding (by leaf senescence and/or necrosis) in 3 *Coffea* sp. genotypes.

MATERIALS AND METHODS

One year old plants of *Coffea canephora* cv. Conilon, clones 02 and 153 and *C. arabica* cv. Catucaí IPR 102 were used for the experiments. Plants were placed in a growing chamber with an irradiance of 700-900 μmol m^{-2} s^{-1}, 380 μL CO_2 L^{-1}, 70% relative humidity, 12 h photoperiod and 25/20 °C (day/night) for about 15 days, in order to promote plant acclimation to this environmental conditions, set as control. Coffee plants were then submitted to a gradual temperature reduction (0.5 °C day^{-1}), from 25/20ºC down to 13/8 °C (during 24 days), followed by 3 consecutive cycles of 13/4 °C (3 x 13/4 °C). After this chilling treatment the temperature was raised to 20/15 °C during the first day and to 25/20 °C up to 14 days. Analysis were performed on recent mature leaves at 25/20 °C (control), 18/13 °C, 13/8 °C (end of the acclimation period), 3x13/4 °C (after chilling treatment) and after 7 and 14 days of recovery.

Evaluation of leaf number (with less than 50% affected tissue) was performed in 8-10 plants per genotype, being analysed young and mature leaves separately.

Determination of lipid classes and fatty acid quantification was performed as described in Campos et al. (2003), using 3 g of leaf material, from 6 to 8 plants per genotype, collected after 2-2:30 h of illumination. Double bond index [DBI =(% monoenes + 2 x % dienes + 3 x % trienes) / (saturated fatty acids)] was calculated as in Campos et al. (2003).

Data was subject to an ANOVA on a factorial design (genotype *vs*. temperature, including the recovery period) ($P = 0.05$). Treatment means were compared by the Tukey test of significant difference at $P < 0.05$ probability.

RESULTS AND DISCUSSION

Until temperatures of 13/8 °C no significant effect was observed concerning leaf shedding. However, immediately after the exposure to 4 °C, leaf shedding was observed, stronger among mature leaves when compared with the younger ones, independently of the genotype (Figure 1 A, B), what agrees with Strauss et al. (2007).

Immediately after the chilling cycles, mature leaves in Catucaí IPR 102 and Conilon clone 153 showed significant leaf shedding. However, the post-stress effects were more pronounced in Conilon clone 02 from the 7th day of recovery onwards, preserving only 8.7% of these leaves by the end of the experiments.

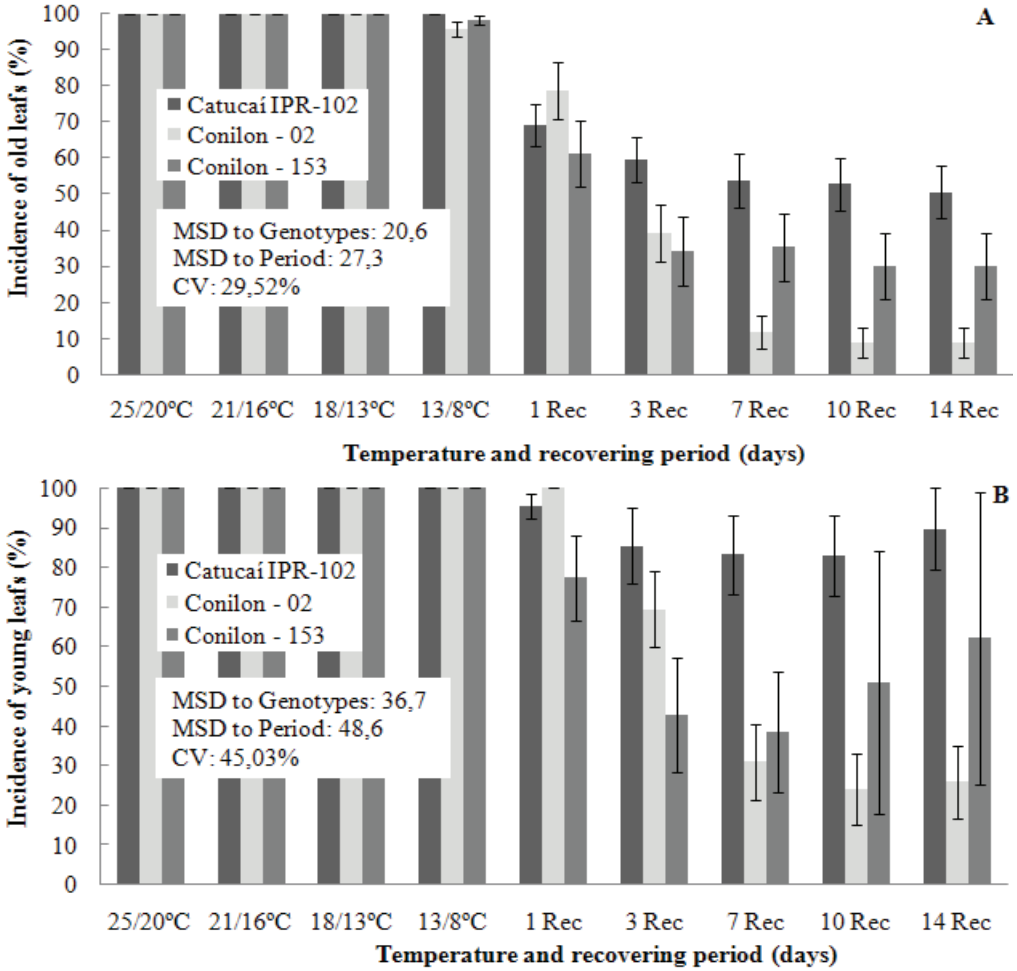


Figure 1. Evaluation of mature (A) and young (B) leaves proportion in *Coffea* sp. genotypes under gradual temperature reduction (25/20 °C to 13/8 °C) during 24 days, 3 cycles at 13/4 °C (3x13/4 °C) and 14 days of a recovery period (Rec) at 25/20 °C. Each mean value was originated from 7 to 14 replicates. Bars correspond to the mean standard error. *Note: MSD = Minimum significant difference. CV = Coefficient of variation.*

Concerning the young leaves, Catucaí showed a non-significant leaf shedding after the chilling exposure, showing a maximal leaf loss of 17%, as compared to its control at the beginning of the experiment. Furthermore, a tendency to increase was observed at the 14th day of the recovery period. On the other hand, clone 153 showed a strong impact of the low temperature treatment, with a clear shedding of young leaves until the 7th the recovery period. However, after that this genotype showed some ability to develop new leaves, recovering from 38.6% to 62.2% between the 7th and the 14th day. As for the older leaves, clone 02 showed the highest young leaf shedding, retaining about 25% of these leaves by the end of the experiments.

Changes in the lipid constituents of chloroplast membranes could represent an important part of the acclimation process (Campos et al., 2003). Among the studied genotypes, clone 153

presented the highest values of total fatty acids (TFA) at 13/8ºC and at the 7th day of recovery, when it showed increases of 84 % and 96 % as compared to its control, thus reflecting *de novo* lipid synthesis. This response may constitute an advantage of this clone when submitted to low temperatures, since it may allow plant to repair damaged structures and, eventually, to promote qualitative modifications at the membrane lipid constitution level (Campos et al., 2003), thus preserving its function. In fact, after the reduction in TFA provoked by the chilling cycles at 13/4 °C new TFA increment was observed, suggesting an effective ability to repair cold damaged membranes. This genotype had the lowest values in control treatment, with an inversion at the end of the recovery (14th day).

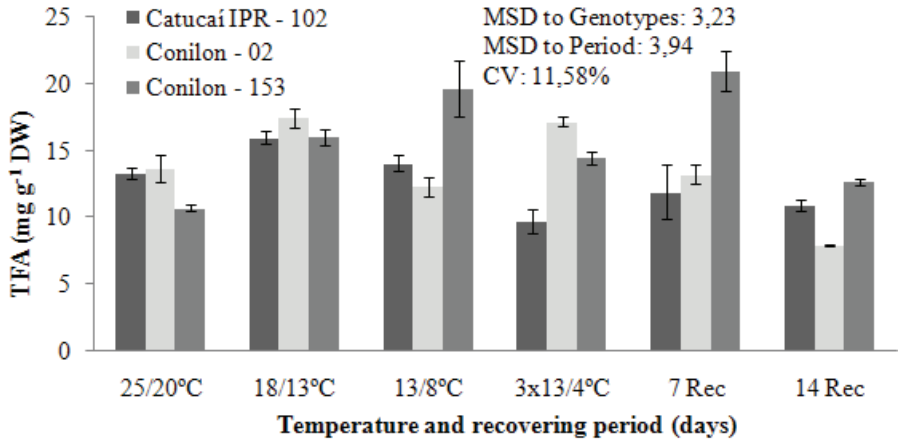


Figure 2. Evaluation of the percentage variation of total fatty acids (TFA) in chloroplast membranes from *Coffea* sp. genotypes leaves, under a gradual temperature reduction (25/20 °C to 13/8 °C), during 24 days, 3 cycles at 13/4ºC (3x13/4 °C) and 14 days of a recovery period (Rec) at 25/20 °C. Each mean value was originated from 3 replicates. Bars correspond to the mean standard error. *Note: MSD = Minimum significant difference. CV = Coefficient of variation.*

Conilon clone 02 showed also TFA increments around 25% at 18/13 °C and after chilling exposure (3x13/4 °C), what could indicate some ability to maintain lipid metabolism at low temperatures. However, the TFA clear decreased after that, representing only 57.6% of the control by the end of the recovery period.

Catucaí IPR 102 showed no significant variations of TFA values along the experiment. Considering the quick recovery of the photosynthetic rate observed in this genotype after the end of stress (data not showed), the TFA stability suggests a lower chilling impact, thus not requiring an increase on membrane lipid synthesis.

Concerning the changes of saturation degree of the FA during the gradual temperature decrease imposition, Catucaí IPR 102 showed an increased unsaturation degree (DBI) at 18/13 °C, both compared to its control and to the other genotypes (Table 1). That results from a simultaneous increase in linolenic acid (C18:3) and a decrease of palmitic acid (C16:0) at this temperature, as also observed in *Nicotiana tabacum* submitted to cold (Kodama et al., 1995). That could increase membrane fluidity (Campos et al., 2003), preserving the photosynthetic processes at low temperatures (Silva et al., 2004; Kodama et al., 1995). These changes are more common when plants are submitted to gradual reductions of temperature, favoring the acclimation to low temperature conditions (Zhang et al., 2006). However, despite some FA variations, for the rest of temperatures Catucaí presented DBI values that did not significantly differ from the control, indicating stability of the unsaturation degree (Table 1), as it happens also for the TFA.

Table 1. Evaluation of the percentage variation of the fatty acids composition and unsaturated lipids degree (DBI) from the chloroplast membrane in leaves of *Coffea* sp. genotypes, under gradual temperature reduction (25/20 °C to 13/8 °C), during 24 days, 3 cycles at 13/4 °C (3x13/4 °C) and 14 days of a recovery period (Rec) at 25/20 °C. Each mean value was originated from 3 replicates.

-	Genotype	Temperatures and days of recovering period					
-	-	25/20ºC	18/13ºC	13/8ºC	3x13/4ºC	7 Rec	14 Rec
C16:0 (%)	Catucaí	16,90Aab	12.3Ab	22.49Bab	22.12Aab	25.93Aa	23.60Aa
CV: 18.86	Clone 02	17.39Aa	18.58Aa	23.93ABa	23.04Aa	26.03Aa	27.54Aa
-	Clone 153	16.82Abc	14.95Ac	31.52Aa	25.72Aab	23.09Aabc	24.77Aabc
C16:1*t* (%)	Catucaí	11.26Aa	9.44Aa	10.43Aa	11.35Aa	12.45Aa	12.08Aa
CV: 20.94	Clone 02	8.33Aa	10.47Aa	7.94Aa	11.39Aa	13.08Aa	10.17Aa
-	Clone 153	12.81Aa	13.48Aa	11.46Aa	9.51Aa	11.82Aa	11.21Aa
C18:1 (%)	Catucaí	1.38Bab	1.32Bab	0.81Bb	1.87Aa	1.90Aa	1.43Bab
CV: 16.52	Clone 02	1.66Ba	1.62ABa	2.13Aa	1.81Aa	0.64Bb	0.72Cb
-	Clone 153	2.44Aa	2.03Aa	1.24Bb	2.16Aa	2.43Aa	2.44Aa
C18:2 (%)	Catucaí	13.23Ba	11.82Aab	12.03Aab	6.52Bc	5.15Bc	9.66Bb
CV: 11.12	Clone 02	15.97Aa	12.46Ab	10.39Abc	8.29ABc	10.65Abc	13.19Aab
-	Clone 153	10.68Ca	9.38Bab	7.63Bb	9.44Bab	10.54Aa	10.67Ba
C18:3 (%)	Catucaí	57.22Aab	65.12Aa	54.24Aab	58.14Aab	54.56Aab	53.22Ab
CV: 8.31	Clone 02	56.65Aa	56.87Aa	55.62Aa	55.48Aa	49.60Aa	48.38Aa
-	Clone 153	57.25Aab	60.16Aa	48.15Ab	53.18Aab	52.12Aab	50.91Aab
DBI (%)	Catucaí	12.74Ab	18.88Aa	9.10Ab	9.37Ab	9.05Ab	8.36Ab
CV: 22.14	Clone 02	12.42Aa	11.49Bab	8.27Aab	8.52Aab	7.06Aab	6.64Ab
-	Clone 153	13.24Aab	14.44Ba	5.47Ac	7.40Ac	8.30Abc	7.58Ac

Values within columns followed by the same capital letter (contrast between genotypes), and followed by the same lower-case within lines (contrast between temperatures and days of recovering period), are not statistically different based upon Tukey's HSD means separation test at $P < 0.05$. CV = Coefficient of variation. Note: C16:0 = palmitic acid; C16:1t = trans-hexadecenoic acid; C18:1 = oleic acid; C18:2 = linoleic acid and C18:3 = linolenic acid.

On the other hand, the two Conilon genotypes showed stronger DBI decreases along the experiment, mostly due to increases in C16:0, accompanied by reductions in C18:2 and C18:3. These lipid qualitative modifications (Table 1) would turn the chloroplast membranes less fluid under cold conditions, what could have negative impact in its functionality. Moreover, with the gradual cold imposition, the leaves in clone 02 (but not in clone 153) became progressively yellowish, due to strong decreases in chlorophylls and carotenoids (data not showed), what could reflect 'photobleaching' due to the action of ROS. This massive loss of pigments and the probable lower fluidity of membranes may have contributed to the slow and incomplete recovery of the photosynthetic rate in this genotype, despite that the TFA content was lower than the control only at the 14th day of recovering period.

It seems noteworthy that palmitic acid (C16:0), which is the highest detected saturated FA, showed the strongest variations among FA, tending to increase with cold exposure and afterwards in the 3 studied genotypes, thus strongly contributing to DBI reduction.

Despite its small contribution among the several lipid classes phosphatidylglycerol (PG), is considered of upmost importance, since it presence contributes to the stabilization of PS I complex and influences the molecular organization of proteins and pigments (Yang et al., 2005). In fact, despite its relatively small contribution to the whole membrane lipid content, a decrease in PG quantities leads to macro-organizational changes of the photosynthetic complexes by the modification of the electrical charge of membrane surface. That affects molecular organization and functionality of PS I and PS II, reducing photochemical efficiency (Apostolova et al., 2008).

In PG significant rises of C16:0 were observed during cold imposition, while C18:3 followed an opposite pattern, which was maintained under recovery conditions. Those changes were stronger in Catucaí and lead to a strong unsaturation decrease of PG, contrary to what was observed in genotypes that presented a lower cold sensitivity (Xin and Browse, 2000). However, Catucaí consistently presented a lower proportion of C16:0 and a higher of C18:3 and C18:2 when compared to the Conilon genotypes, thus maintaining a higher unsaturation degree and, probably, a higher fluidity of this important chloroplast lipid at low positive temperatures. In fact, increases of C18:3 and C16:1*t* was observed in Xu and Siegenthaler (1997) studying *Cucurbita moschata* under decreasing temperatures, suggesting that these effects would be associated with adaptation to adverse environmental conditions.

On the other hand, the values of the trans-hexadecenoic acid (C16:1*t*), which is specific from PG, were very similar in all genotypes when analyzing TFA distribution (Table 1). Although, when analyzed the distribution of FA in PG, the value of C16:1*t* seems to be somewhat reinforced in some low temperatures in the Conilon genotypes, being maintained quite stable in Catucaí (Table 2). The C16:1*t* is involved in the oligomerization of the light harvesting complexes (Dubertret et al., 2002), contributing to its stabilization and to the preservation of the photochemical efficiency of the photosynthesis (Gray et al., 2005).

In conclusion, our results point out Conilon clone 02 as the most sensitive among the studied genotypes, showing higher leaf shedding and a worse recovery, followed by clone 153. Catucaí IPR 102 showed to be less affected by cold what might be related to a higher unsaturation level, namely in PG. Qualitative changes in fatty acids and in lipid classes, together with the evaluation of leaf shedding and photosynthetic parameters could be helpful tools for an adequate selection/breeding of coffee genotypes for areas prone to low temperature occurrence.

Table 2. Composition of the fatty acids (mol %) phosphatidilglycerol (PG) in the chloroplastid membranes from leaves of *Coffea* sp. genotypes, under gradual temperature reduction (25/20 °C to 13/8 °C), during 24 days, 3 cycles at 13/4 °C (3x13/4 °C) and a recovering period (Rec) and 14 days of a recovery period (Rec) at 25/20 °C. Each mean value was originated from 3 replicates.

-	Genotype	Temperatures and days of recovering period					
-	-	**25/20ºC**	**18/13ºC**	**13/8ºC**	**3x13/4ºC**	**7 Rec**	**14 Rec**
PG	Catucaí	6.57Cd	15.77Bc	34.38Aab	35.11Aab	26.96Bb	38.49Aa
C16:0	Clone 02	29.87Ab	30.34Ab	41.75Aa	41.00Aa	43.91Aa	43.91Aa
CV: 11.20	Clone 153	21.86Bd	31.91Abc	38.70Aab	41.65Aa	44.65Aa	27.66Bcd
PG	Catucaí	47.55Aa	44.40Aa	45.12Aa	44.31Aa	43.89Aa	41.11Aa
C16:1*t*	Clone 02	33.80Bb	42.17Aab	49.72Aa	41.97Aab	44.92Aab	45.34Aab
CV: 11.17	Clone 153	38.30ABb	39.59Ab	45.00Aab	43.80Aab	51.71Aa	38.37Ab
PG	Catucaí	15.20Bb	18.73Aa	9.71Ade	11.99Acd	13.40Abc	8.55Ae
C18:2	Clone 02	16.48Ba	12.65Bb	4.74Be	9.91Abc	8.00Bcd	5.02Bde
CV: 11.78	Clone 153	19.64Aa	14.18Bb	7.13Bc	7.28Bc	1.92Cd	11.12Ab
PG	Catucaí	25.73Aa	21.09Ab	10.79Acd	8.60Ad	12.48Ac	11.85Bc
C18:3	Clone 02	8.77Ca	9.78Ca	3.80Cc	7.12Aab	3.17Bc	5.73Cbc
CV: 10.59	Clone 153	11.93Bb	14.32Bb	6.22Bc	7.27Ac	1.73Bd	22.85Aa

Values within columns followed by the same capital letter (contrast between genotypes), and followed by the same lower-case within lines (contrast between temperatures and days of recovering period), are not statistically different based upon Tukey's HSD means separation test at $P < 0.05$. CV = Coefficient of variation. Note: C16:0 = palmitic acid; C16:1t = trans-hexadecenoic acid; C18:2 = linoleic acid and C18:3 = linolenic acid.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. T. Sera (IAPAR, Brazil) for the Catucaí IPR 102 and G. Burgarelli (Brazil) for the Conilon plant materials. Thanks are also due to Tech. Eng. C. Santiago, Lab. Tech. Ana P. Ramos (L-INIA/INRB) and Lab. Tech. P. Alves (IICT) for technical support. This work was supported by Fundação para a Ciência e a Tecnologia, through the projects POCTI/AGG/43101/2001 and PTDC/AGR-AAM/64078/2006 (co-financed by the European Fund FEDER) and by the grants SFRH/BPD/32006/2006 (V.L. Quartin), SFRH/BPD/21884/2005 (A. Fortunato), SFRH/18361/2004 (P. Eichler), co-financed by the Portuguese PIDDAC program and European Social Fund under the 3rd framework program, and PDEE (F. Partelli) financed by CAPES, Brazil.

REFERENCES

Apostolova E.L., Domonkos I., Dobrikova A.G., Sallai A., Bogos B., Wada H., Gombos Z., Taneva S.G. (2008) Effect of phosphatidylglycerol depletion on the surface electric properties and the fluorescence emission of thylakoid membranes. J. Photochem. Photobiol. B: Biology, 91: 51-57.

Campos P.S., Quartin V., Ramalho J.C., Nunes M.A. (2003) Electrolyte leakage and lipid degradation account for cold sensitivity in leaves of *Coffea* sp. Plants. J. Plant Physiol., 160: 283-292.

Davis A.P., Govaerts R., Bridson D.M., Stoffelen P. (2006) An annotated taxonomic conspectus of the genus *Coffea* (*Rubiaceae*). Bot. J. Lin. Soc., 152: 465-512.

Dubertret G., Gerard-Hirne C., Trémolières A. (2002) Importance of *trans*-Δ3-hexadecenoic acid containing phosphatidylglycerol in the formation of the trimeric light-harvesting complex in *Chlamydomonas*. Plant Physiol. Biochem., 40: 829-836.

Gray G.R., Ivanov A.G., Krol M., Williams J.P., Kahn M.U., Myscich E.G., Huner N.P.A. (2005). Temperature and light modulate the *trans*-Δ^3-hexadecenoic acid content of phosphatidylglycerol: light-harvesting complex II organization and non-photochemical quenching. Plant Cell Physiol., 46: 1272-1282.

Kodama H., Horiguchi G., Nishiuchi T., Nishimura M., Iba K. (1995) Fatty acid desaturation during chilling acclimation is one of the factors involved in conferring low-temperature tolerance to young tobacco leaves. Plant Physiol., 107: 1177-1185.

Libardi V.C.M., Amaral J.A.T., Amaral J.F.T. (1998) Crescimento vegetativo sazonal do cafeeiro (*Coffea canephora* Pierre var. Conilon) no sul do Estado do Espírito Santo. *Rev. Bras.* Agrometeor., 6: 23-28.

Ramalho J.C., Quartin V.L., Leitão E., Campos P.S., Carelli M.L.C., Fahl, J. I., Nunes, M. A. (2003) Cold acclimation ability and photosynthesis among species of the tropical *Coffea* genus. Plant Biol., 5: 631-641.

Silva E.A., DaMatta F.M., Ducatti C., Regazzi A.J., Barros R.S. (2004) Seasonal changes in vegetative growth and photosynthesis of Arabica coffee trees. Field Crops Res., 89: 349-357.

Strauss A.J., Krüger G.H.J., Strasser R.J., van Heerden P.D.R. (2007) The role of low soil temperature in the inhibition of growth and PSII function during dark chilling in soybean genotypes of contrasting tolerance. Physiol. Plant., 131: 89-105.

Xin Z., Browse J. (2000) Cold comfort farm: the acclimation of plants to freezing temperatures. Plant Cell Environ., 23: 893-902.

Xu Y., Siegenthaler P.A. (1997) Low temperature treatments induce an increase in the relative content of both linolenic and Δ^3-*trans*-Hexadecenoic acids in thylakoid membrane phosphatidyglycerol of squash cotyledons. Plant Cell Physiol., 38: 611-618.

Yang Z., Su X., Wu F., Gong Y., Kuang T. (2005) Effect of phosphatidylglycerol on molecular organization of photosystem I. Biophys. Chem., 115: 19- 27.

Zhang J-H., Liu Y-P., Pan Q-H., Zhan J-C., Wang X-Q., Huang W-D. (2006) Changes in membrane-associated H^+-ATPase activities and amounts in young grape plants during the cross adaptation to temperature stresses. Plant Sci., 170: 768-777.

Variability in the Caffeine and Trigonelline Levels in Clones of Robusta Coffee*

C.A. BRAGHINI[1], L.C. FAZUOLI[1], T.J.G. SALVA[1], M.T. BRAGHINI[1], M.B. SILVAROLLA[1], J.C. MISTRO[1], P. B. GALLO[2]

[1]Coffee Center 'Alcides Carvalho', Agronomic Institute, Campinas (IAC/APTA), SP, Brazil.
Email: café@iac.sp.gov.br
[2]Regional Center of Northeast Paulista, Mococa (APTA Regional), SP, Brazil.
*Supported by the CBP&D and the CNPq

SUMMARY

As part of the genetic improvement program for *Coffea canephora* Pierre ex A. Froehner developed by the Agronomic Institute, at Campinas, State of São Paulo, Brazil, more than 300 coffee plants were selected, all of which were highly productive, rust-resistant, rustic and produced large beans of high medium-sieve values (16 to 19), in addition to presenting different fruit ripening patterns. The levels of caffeine and trigonelline of 125 robusta coffee stock plants and several F1 hybrids were determined with the objective to evaluate the variability of these chemical components. Caffeine levels varied from 1.58% to 3.09% and trigonelline levels ranged from 0,56% to 1,35% in the 125 coffee plants analyzed. The results clearly show great variability in the concentrations of caffeine and trigonelline in selected robusta stock plants and make it possible to identify clones with high or low levels of caffeine, which is of great interest to both consumers and coffee roasters that use robusta coffee in blends of roasted ground coffee or in the manufacture of soluble coffee.

INTRODUCTION

The *Coffea canephora* species, usually known as robusta coffee, has considerable importance in the worldwide market, representing about 36% of the commercialized coffee all over the world. The Agronomic Institute, at Campinas, State of São Paulo, Brazil, has developed several superior robusta coffee genotypes, selected from field trials and from the germplasm bank collection. Besides the desirable agronomic characteristics such as high productivity and resistance to pests and diseases, there is a high interest in identifying coffee germplasm attributes that benefit the beverage quality.

It is well known that alkaloids such as caffeine and trigonelline present in green coffee fruits, are substances related to the beverage quality and with physiological action. Caffeine contributes for the characteristic bitter flavor of the coffee beverage (Trugo, 1984) and is responsible, for example, by the known stimulating effect on the central nervous system (Nehlig, 1999).Trigonelline, by the way, is the precursor of aromatic compounds and acid nicotinic, which is important for the sensorial quality as well as for the nutritional beverage quality (Viani and Horman, 1974; Taguchi et al., 1985). The present work aimed at selecting elite matrix plants of robusta coffee, characters of high productivity and grains with low contents of caffeine and high contents of trigonelline.

MATERIALS AND METHODS

Seed samples of *C. canephora* and derivates

Seeds of 125 selected coffee plants were used from the germplasm bank of the Agency of Agricultural Research and Technology – APTA, located at Mococa county, State of São Paulo, Brazil, and coordinated by the Coffee Center 'Alcides Carvalho', Agronomic Institute, at Campinas, State of São Paulo, Brazil. The treatments are described as follows: 29 coffee plants from the EP 329 trial, corresponding to F1 hybrids of *C. canephora*; 69 coffee plants from the 'Lote Chácara', corresponding to several selections of *C. canephora*; and 27 coffee plants from 'Lote 78', corresponding to 21 plants of cv. Apoatã IAC 2258 of *C. canephora* and six plants from the Bangelan population, natural hybrids between *C. congensis* and *C. canephora*. The coffee fruits were collected at the berry stage and the pulps and mucilage were removed. Then, beans were transferred to trays and sun-dried during one day, and afterwards, they were left to dry under shade until reaching 11% humidity. Green peeled coffee seeds were ground to pass a 0.5 mm sieve and sent to chemical analysis.

Caffeine and trigonelline quantifying

Compound extraction procedure: 100 mg of ground green coffee seeds were transferred to 5 mL of 70% methanol solution, pure for HPLC, at 60 °C, during one hour. This mixture was centrifuged and the supernatant was filtered through a 0.22 μm thick membrane. The caffeine and trigonelline compounds were quantified by high-performance liquid chromatography (HPLC), according to procedure adapted from Casal et al. (2000). A constant-composition mobile phase (isocratic elution) was used, constituted of methanol: acetic acid: water (50:0.5:49.5, v:v:v), at a rate of 1 mL/min, at room temperature. The compound concentrations were determined through daily obtained standard curves using standard solutions of caffeine and trigonelline (Sigma). Analysis were run in duplicates.

RESULTS AND DISCUSSION

The data evidenced that the largest concentration range of caffeine (1.65-3.09%) and trigonelline (0.56-1.23%) occurred in the F1 hybrid plants from the EP 329 trial (Table 1). And the lowest concentration range was observed in the Bangelan population from 'Lote 78' (caffeine = 1.92 to 2.61% and trigonelline = 0.93 to 1.13%). The mean values obtained for robusta coffee and for hybrids from *C. canephora* x *C. congensis* were similar.

Table 1. Variation range and means for the caffeine and trigonelline concentrations in the coffee beans of 125 elite matrix plants selected from *C. canephora* of 'EP 329', 'Lote Chácara', cv. Apoatã IAC 2258 and Bangelan population of 'Lote 78'.

Elite matrix plants selected in the trials	Total # of analyzed plants	Variation range of chemical components concentrations		Means of chemical components concentrations	
		Caffeine (%dm)	Trigonelline (%dm)	Caffeine (%dm)	Trigonelline (%dm)
EP 329	29	1.65 – 3.09	0.56 – 1.23	2.24	1.03
Lote Chácara	69	1.58 – 2.76	0.68 – 1.17	2.24	0.98
Lote 78-cv. Apoatã IAC 2258	21	1.84 – 2.94	0.87 – 1.35	2.36	1.06
Lote 78-Bangelan pop.	6	1.92 – 2.61	0.93 – 1.13	2.24	1.03
TOTAL	125				

Plants from 'Lote Chácara' did not show significant caffeine variability (Table 1), but plant 21 from this trial showed up against them, due to its least caffeine concentration (1.58%), showed in the table 2. Ten plants, among the studied robusta coffee plants, were identified with low caffeine levels (Table 2). Plant 700 is also considered of interest for genetic breeding due to its low caffeine concentration (1.84%) associated to relatively high trigonelline content (1.23%). The ten plants showing lower caffeine concentrations (1.58 to 1.87%) are of interest for the *C. canephora* breeding improvement, because this species usually presents high caffeine levels).

Table 2. The ten elite matrix coffee plants showing lower caffeine concentrations selected from 125 plants, and their correspondent trigonelline concentration values.

Selected coffee plants	Chemical component concentrations	
	Caffeine (%dm)	Trigonelline (%dm)
Plant 21 – Lote Chácara	1.58	0.98
Plant 74 – Lote Chácara	1.62	1.05
Plant 10 – EP 329	1.65	0.56
Plant 58 – Lote Chácara	1.73	0.91
Plant 656 – Lote Chácara	1.78	0.93
Plant 700 – L.78- Apoatã cv.	1.84	1.23
Plant 58 – EP 329	1.84	1.06
Plant 60 – EP 329	1.85	0.92
Plant 385 – L.78- Apoatã cv.	1.86	1.08
Plant 43 – EP 329	1.87	1.06

Following are presented the ten coffee plants with higher caffeine concentration values, varying from 2.62 (plant 43) to 3.09% (plant 53) from EP 329.

Table 3. The ten elite matrix coffee plants showing higher caffeine concentrations selected from 125 plants, and their correspondent trigonelline concentration values.

Selected coffee plants	Chemical component concentrations	
	Caffeine (%dm)	Trigonelline (%dm)
Plant 53 – EP 329	3.09	1.10
Plant 323 –L.78- Apoatã cv.	2.94	1.02
Plant 438 – L.78- Apoatã cv.	2.78	1.20
Plant 276 – Lote Chácara	2.76	0.94
Plant 614 – Lote Chácara	2.76	0.90
Plant 530 – Lote Chácara	2.76	0.68
Plant 1003 – Lote Chácara	2.76	0.98
Plant 35 – Lote Chácara	2.74	0.85
Plant 681 – L.78- Apoatã cv.	2.69	1.01
Plant 43 – EP 329	2.62	1.35

CONCLUSIONS

1. The selected coffee plants presented significant variation on coffee bean caffeine and trigonelline concentrations, allowing plant selection with lower caffeine and associated to higher trigonelline levels.
2. Genotypes showing higher caffeine levels might also be selected for industrial purposes of alkaloid extraction and use.

3. The population results did not suggest association between caffeine and trigonelline concentrations.

REFERENCES

Casal S., Oliveira B., Ferreira M.A. HPLC/diode-array applied to the thermal degradation of trigonelline, nicotinic acid and caffeine in coffee. Food Chemistry, London, 68(4); 481-485, 2000.

Trugo L.C. PhD Thesis, University of Reading, England, 1984.

Nehlig A. Chemtech, 29, 30p, 1999.

Viani R., Horman I. Thermal behavior of trigonelline. J. Food Sci., 39, 1216-1217, 1974.

Taguchi H., Sakaguchi M., Shimabayashi Y. Trigonelline content in coffee beans and the thermal conversion of trigonelline into nicotinic acid during the roasting of coffee beans. Agric. Biol. Chem. 49; 3467-3471, 1985.

Genetic Variability of Agronomic Characteristics in 'Tupi Iac 1669-33' Progenies*

M.R. PETEK[1], J.C. MISTRO[1], A.C.B. DE OLIVEIRA[2], L.C. FAZUOLI[1,], P.B. GALLO[3], M.T. BRAGHINI[1]**

[1]Instituto Agronômico de Campinas, São Paulo, Brasil
[2]EPAMIG, Minas Gerais, Brasil
[3]APTA/Pólo Nordeste Paulista, São Paulo, Brasil.
*Partly funded by the Consórcio Brasileiro de Pesquisas Cafeeiras (CBP&D/Café)
**Scholarship granted by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) as part of the scientific produtivity program.

SUMMARY

The main characteristic of cultivar Tupi IAC 1669-33 – a variety with high nutritional demands and indicated for dense spacing – is its high resistance to coffee rust. However, when planted at wide spacings (i.e. plants more than 0,7 m apart), this cultivar were found to exhibit reduced vegetative vigor, affecting its longevity. In addition, Tupi IAC 1669-33 produces higher percentages of elephant beans compared to cultivars such as 'Catuaí Vermelho IAC-144', which degrades the quality of the product. The high ratio of elephant beans may be more related to adverse weather conditions, such as drought, than to factors inherent to the genetic constitution of the cultivar. The objective of this study was to determine the genetic variability within the Tupi IAC 1669-33 cultivar, in addition to identifying the genetic lines that should be eliminated from the genetic seed production fields. The experiment was conducted in an experimental area of APTA Regional, in Mococa, São Paulo (BR), using a random block design with three replications and 10 plants/plot. The treatments were 31 'Tupi IAC 1669-33' progenies and three control cultivars: 'Ouro Verde IAC H5010-5', 'Obatã IAC 1669-20' and 'Catuaí Vermelho IAC-144'. Throughout an experimental period of 3 years, genetic parameters were estimated for the following characteristics: productivity, vegetative vigor, fruit size, fruit ripening, reaction to rust, plant height and percentage of elephant beans. Heritability varied from 56,29% to 84,79%. Thus, genetic variability was detected for all the characteristics analyzed, however, based on the index of variation (b=CVg/CVe), only vegetative vigor, fruit ripening and plant height exhibited favorable potential to achieve an efficient selection. It was concluded that the variability which exists within the 'Tupi IAC 1669-33' cultivar should be eliminated in order to improve its vegetative vigor and, consequently, its longevity and adaptability. Fruit ripening of most progenies was similar to that exhibited by 'Ouro Verde IAC H5010-5' and differed from that of 'Obatã IAC 1669-20' and 'Catuaí Vermelho IAC-144' which are later-ripening cultivars. Fourteen progenies presented higher percentages of elephant beans compared to the control 'Obatã IAC 1669-20'. Consequently, these fourteen progenies should be eliminated from the formation of cultivar Tupi IAC 1669-33. Since most of the progenies investigated exhibited higher productivity than the control plants, the Tupi IAC 1669-33 cultivar constitutes an excellent option for coffee growers, provided all recommended planting and managing procedures are followed.

RESUMO

A cultivar Tupi IAC 1669-33, indicada para plantios adensados e exigente em nutrição, tem como característica principal a resistência à ferrugem do cafeeiro. Porém, quando submetida a espaçamentos largos, acima de 0,7 m entre plantas, verifica-se que há redução em seu vigor vegetativo, afetando assim a sua longevidade. Verificam-se também nesta cultivar porcentagens de grãos do tipo concha, superiores as encontradas em cultivares como 'Catuaí Vermelho IAC-144', o que provoca depreciação a qualidade do produto. Os grãos concha podem estar mais relacionados a condições climáticas adversas, como seca, do que inerente à constituição genética da cultivar. O objetivo deste trabalho é determinar a existência de variabilidade genética na cultivar Tupi IAC 1669-33 além de indicar quais linhagens devem ser eliminadas do campo de produção de sementes genética. O experimento está instalado no Pólo Nordeste Paulista (APTA Rgional), em Mococa (SP), em blocos casualizados com três repetições e 10 plantas/parcela. Os tratamentos utilizados foram 31 progênies da 'Tupi IAC 1669-33' e as cultivares controle 'Ouro Verde IAC H5010-5', 'Obatã IAC 1669-20' e 'Catuaí Vermelho IAC-144'. Foram avaliadas durante 3 anos e estimados parâmetros genéticos para as seguintes características: produtividade, vigor vegetativo, tamanho de grãos, maturação dos frutos, reação à ferrugem, altura das plantas e porcentagem de grãos concha. As herdabilidades variaram de 56,29% a 84,79%. Portanto, detectou-se variabilidade genética para todas as características analisadas, porém, de acordo com o índice de variação (b=CVg/CVe), apenas o vigor vegetativo, maturação dos frutos e altura das plantas apresentam condições favoráveis para que a seleção seja eficiente. Conclui-se que existe variabilidade dentro da cultivar 'Tupi IAC 1669-33' a qual deve ser eliminada, a fim de melhorar seu vigor vegetativo e consequentemente a sua longevidade e adaptabilidade. A maioria das progênies apresentaram maturação dos frutos semelhantes a 'Ouro Verde IAC H5010-5' e diferiram de 'Obatã IAC 1669-20' e 'Catuaí Vermelho IAC-144' que são mais tardias. Quatorze progênies apresentaram porcentagem de grãos concha com valores significativamente superiores à 'Obatã IAC 1669-20', as quais deverão ser eliminadas da formação da 'Tupi IAC 1669-33'. Grande parte das progênies apresentaram produtividades superiores às testemunhas, portanto, desde que esta cultivar seja plantada e manejada de acordo com as recomendações é uma excelente opção para o produtor.

INTRODUCTION

The Brazilian coffee sector has undergone several impacting changes in attempts to develop a more self-sustainable growing system. Important advances have been made, such as the development of the dense planting system and organic coffee growing techniques, which mainly aim at increasing the profitability of the coffee grower and reducing, at the same time, contamination of the environment. The use of disease and pest resistant cultivars, fully adapted to each regional growing system, is the most efficient and lowest-cost technology strategy for achieving self-sustainability, capable not only of providing greater profitability and social return, but also of reducing the use of pesticides and other toxic farm chemicals.

The main diseases affecting coffee production in Brazil are coffee leaf rust, caused by *Hemileia vastatrix* (Várzea et al., 2002), halo blight, caused by *Pseudomonas syringae* pv. *garcae* (Sera, 2001; Sera et al., 2002), cercospora leaf spot, caused by *Cercospora coffeicola* (Sera, 2001; Sera et al., 2002) and, more recently, anthracnose, caused by *Colletotrichum spp.* (Paradela Filho et al., 2001).

Throughout the world, leaf rust is the most widespread and most damaging disease of coffee plants. There are already several Brazilian cultivars resistant to coffee rust, such as TUPI IAC 1669-33, Obatã IAC 1669-20, IAPAR 59, OEIRAS MG6851, Paraíso, Catucaí and others.

Although the mechanisms and factors of resistance are well understood and have been the object of intense study, continued research efforts aimed at improving *H. vastatrix*-resistant cultivars with larger numbers of qualitative and quantitative genes is recommended (Varzea et al., 2002). The continued emergence of new physiological strains of this pathogen has resulted in breakdown of resistance of cultivars that were earlier considered resistant. For that reason, duration of resistance of current cultivars is difficult to predict (Varzea et al., 2002).

The main characteristic of cultivar Tupi IAC 1669-33 – a variety with high nutritional demands and indicated for dense spacing – is its high resistance to coffee rust (Fazuoli, et al. 2002) and the reason for its importance to coffee production. However, when planted at wide spacings (i.e. plants more than 0,7 m apart), specimens of this cultivar were found to exhibit reduced vegetative vigor, affecting its longevity. In addition, Tupi IAC 1669-33 produces higher percentages of elephant beans compared to cultivars such as 'Catuaí Vermelho IAC-144', which degrades the quality of the product. The high ratio of elephant beans may be more related to adverse weather conditions, such as drought, than to factors inherent to the genetic constitution of the cultivar.

The objective of this study was to determine the genetic variability within the Tupi IAC 1669-33 cultivar, in addition to identifying the genetic lineages that should be eliminated from the genetic seed production fields.

MATERIAL AND METHODS

The present study is being conducted in an experimental area located on the premises of the Northeast Regional Development Zone of APTA – the São Paulo State Agency for Agribusiness Technology - (IAC/APTA), in the town of Mococa (SP), Brazil. The experiment was planted on 04/07/2003 at a spacing of 3,5 x 0,5 m, using a random block design with three replications, 34 treatments and 10 plants per plot. The treatments investigated were 31 'Tupi 1669-33' progenies and 3 control cultivars: 'Obatã IAC 1669-20', 'Ouro Verde IAC H5010-5' and 'Catuaí Vermelho IAC 144'.

The following plant characters were evaluated: productivity (Pr), measured by transforming the weight of freshly harvested coffee berries into number of sacks processed green coffee beans per ha., based on the processing of a sample; vegetative vigor (VV), evaluated by assigning a vigor rating on a 1 to 10 scale, with 1 indicating a very weak, non-growing plant and 10 indicating a highly vigorous plant; fruit ripening (MF) with plants being classified on a 1 to 5 scale (1 = early-ripening, 2 =, semi-early-ripening, 3 = mid-ripening, 4 = semi-late-ripening, and 5 = late ripening0; bean size (TF) was evaluated by assigning ratings from 1 to 5 using commercial cultivars that produce fruits of known size, where: 1 = (very small) similar to 'Mokka', 2 = (small) similar to 'Icatú Precoce IAC 3282', 3 = (medium) similar to 'Catuaí Vermelho IAC 81', 4 = (large) similar to 'Acaiá IAC 474-7' and 5 = (very large) similar to 'Maragogipe'; reaction to rust (Ferr), evaluated by assigning one of the following ratings: 1 = immune, 2 = resistant, 3 = moderately resistant, 4 = moderately susceptible, 5 = susceptible, and 6 = highly susceptible; plant height was measured in centimeters from the soil level to the tip of the orthotropic branch; the percentage of elephant beans was estimated by separating defective beans from a sample. Next, the defective and non-defective beans were weighed and the respective weights transformed into percentiles.

The Genes (CRUZ, 2001; CRUZ, 2006) computer program was used for the genetic-statistical analyses. All analyses were performed based on the mean values of the plants of each plot. Analysis of variance was performed using the statistical model $Yij = \mu + Ti + Bj + \varepsilon ij$, where Yij is the value of the i-th treatment of the j-th block; the symbol μ represents the overall

average of the experiment; Ti is the fixed effect of the i-th treatment; Bj is the effect of the j-th block; and εij is the experimental error. Analysis of the results of the effects of treatments on genotypes and control cultivars was also performed, in addition to the significance test between genotypes and control cultivars. The genetic parameters were estimated from the expected mean squares, also with the Genes software program.

RESULTS AND DISCUSSION

The one-way analysis of variance F-test detected significant differences for all plant characters evaluated, indicating that there was sufficient genetic variability between and within the progenies of the Tupi IAC 1669-33 cultivar to allow selection with chances of success (Table 1). With the exception of the trait "percentage of elephant beans", the experimental coefficient of variation of all characters was relatively low, indicating reduced sensitivity to non-controllable variations in the experimental conditions (Table 1). The experimental coefficient of variation of 51,52% of the character "percentage of elephant beans" clearly demonstrates the need for high research rigor when conducting scientific experiments involving this plant character.

Table 1. Summary of analysis of variance for the results of the effects of treatments on the characters productivity (Pr), vegetative vigor (VV), fruit size (TF), fruit ripening (MF), reaction to rust (RF), plant height (AP) and percentage of elephant beans (GC).

Sources of variation	GL	Mean squares						
		Pr	VV	TF	MF	RF	AP	GC
Blocks	2	319.044	5.214	0.258	0.820	0.916	15.291	0.071
Treatments	33	168.620	1.739	0.237	0.449	1.341	288.284	7.568
Gen. (Tupi)	30	167.842 **	1.419 **	0.244 **	0.469 **	0.314 **	305.880 **	8.040 **
Control	2	104.817 ns	2.087 **	0.090 ns	0.004 ns	6.637 **	50.684 ns	4.264 ns
Gen. vs Test.	1	319.574 *	10.619 **	0.328 ns	0.737 **	21.551 **	235.592 *	0.008 ns
Residue	66	67.218	0.292	0.106	0.071	0.125	53.635	2.830
Overall average		43.20	7.42	2.40	3.19	1.68	112.80	3.26
Gen. average (Tupi)		43.75	7.52	2.42	3.16	1.54	112.33	3.27
Average control cultivars		37.51	6.38	2.22	3.46	3.16	117.69	3.24
CVe(%)		18.98	7.28	13.58	8.37	21.02	6.49	51.52

*ns not significant; * Significant at the 5% probability level by the F test; ** Significant at the 1% probability level by the F test.*

According to the results of analyses of variance, the progenies of 'Tupi IAC 1669-33' were significantly different from the control cultivars regarding the characters productivity (Pr), vegetative vigor (VV), fruit ripening (MF), reaction to rust (RF) and plant height (AP). These results were expected since rust had developed on control cultivars 'Ouro Verde IAC H5010-5' and 'Catuaí Vermelho IAC 144', causing reduction in productivity and vegetative vigor of the control plants (Table 1). As for plant height, the same results also confirmed the smaller stature of 'Tupi IAC 1669-33' as compared to the control cultivars. The results regarding the

percentage of elephant beans are relevant since they demonstrate that, on average, the 'Tupi IAC 1669-33' progenies are similar to the control cultivars concerning this defect.

The heritability coefficient values calculated based on the means of the plots varied from 56,29 % to 84,79% (Table 2), indicating a high level of variability between and within the 'Tupi IAC 1669-33' progenies. The high variation index values (b = CVg/CVe) – all greater than the unit -, along with the high heritability coefficient estimates for the characters vegetative vigor (VV), fruit ripening (MF) and plant height (AP) are clearly indicative of the primacy of genetic components over environmental factors which, in turn, make these traits good potential targets for genetic selection and improvement programs. On the other hand, although the variation index values (b = CVg/CVe) for the characters productivity (Pr), fruit size (TF), reaction to rust (RF) and percentage of elephant beans (GC) are smaller than the unit, the relatively high heritability coefficient estimates also indicate considerable chances of success for genetic selection efforts (Table 2).

Table 2. Estimates of genetic parameters for productivity (Pr), vegetative vigor (VV), fruit size (TF), fruit ripening (MF), reaction to rust (RF), plant height (AP) and percentage of elephant beans (GC) of 31 'Tupi IAC 1669-33' progenies.

Parameters[1]	Pr	VV	TF	MF	RF	AP	GC
σ_p^2	55.947	0.473	0.081	0.156	0.105	101.960	2.680
σ_g^2	33.541	0.376	0.045	0.132	0.063	84.082	1.736
σ_e^2	22.406	0.097	0.035	0.024	0.042	17.878	0.943
h_m^2	59.95	79.45	56.29	84.79	60.24	82.46	64.79
b	0.706	1.135	0.655	1.363	0.711	1.252	0.783

[1] *σ_g^2 = genetic variance among the progenies; σ_p^2 = the phenotypic variance; σ_e^2 = residual variance; h_m^2 = coefficient of heritability; b = variation index (CVg/CVe).*

Thus, especially because of its resistance to rust – the main disease affecting Brazilian coffee production - the Tupi IAC 1669-33 cultivar constitutes an excellent option for dense planting on small family farms. It is of utmost importance that the variability between and within the progenies of Tupi IAC 1669-33 described in this study be used as point of departure to further improve the cultivar and develop a coffee variety with improved and more stable agronomic characteristics and subsequently make it available to small coffee farmers.

REFERENCES

Cruz C.D. Programa GENES – versão Windows. Viçosa: UFV, 2001. 642p.

Cruz C.D. Programa GENES: Estatística experimental e matrizes. Viçosa: UFV, 2006. 285p.

Paradela-Filho O., Paradela A.L., Thomaziello R.A., Ribeiro I.J.A., Sugimori M.H., Fazuoli L.C. O complexo *Colletotrichum* do cafeeiro. *Série Tecnológica APTA,* Boletim técnico IAC, 191. Campinas, 2001, 11p.

Sera T. Coffee genetic breeding at IAPAR. Crop Breeding and Applied Biotechnology, v.1, n.2, p. 179-190, 2001.

Sera T., Alteia M.Z., Petek M.R. Melhoramento do Cafeeiro: Variedades Melhoradas no Instituto Agronômico do Paraná (IAPAR). In: Zambolim L (Ed.). O estado da arte de tecnologias na produção de café. UFV, 2002. p.217-251.

Várzea V.M.P., Rodrigues Jr. C.J., Silva M.C.M.L., Gouveia M., Marques D.V., Guerra-Guimarães L., Ribeiro A. In: Zambolim L (Ed.). O estado da arte de tecnologias na produção de café**.** UFV, 2002. p.297-320.

Zambolim L., Vale F.X.R., Costa H., Pereira A.A. And Chaves Gm (2002) Epidemiologia e controle integrado da ferrugem-o-cafeeiro. In: Zambolim L (ed.) O estado da arte de tecnologias na produção de café. Viçosa, Departamento de Fitopatologia, UFV, p.369-450.

Initial Characterization of Durandé Hybrid F_3 Progenies

L. PADILHA[1*], J.B. MATIELLO[2], S.R. ALMEIDA[2], C.H.S. CARVALHO[1], R.A. FERREIRA[2], S.M. MENDONÇA[2], S.L. FILHO[2], L.B. JAPIASSU[2], A.C.R. SANTOS[2], R.N. PAIVA[2]

[1]Embrapa Café, Brasília, DF. Brazil. [*]E-mail: lilian.padilha@embrapa.br
[2]Fundação Procafé, Varginha, MG

SUMMARY

The leaf rust (*Hemileia vastatrix*) is a disease responsible to cause a lot of losses in coffee culture. An alternative to the chemical control is the use of resistant cultivar. The aim of this work was to evaluate and to select F_3 progenies with good agronomic traits such as high yield, uniform maturation, leaf rust resistance and good grain quality. Seeds from 22 progenies with high vigor and high initial productivity were used to obtain 531 F_3 progenies that were planted at Varginha, MG, Brazil, in 2005. In 2007 a total of 25 plants F_3 with leaf rust resistance and high vigor were selected. From these, in 2008 only 19 keep the desirable characteristics. Plants 1-21, 7-27 and 21-18 produced 4,1L, 6,7 L and 4,3 L/plant each, with 54%, 55% and 77% of flat beans, respectively. These three highlighted plants have produced 38%, 39% and 13% of peaberry beans, with 2,0%, 8,0% and 1,0% of coreless fruits. In the F_3 generation there was a great evolution in the flat grains percentage when compared with F_2 progenies. These results have showed interesting traits in several F_3 generation such as short plants, grains with the same traits as a commercial arabica cultivars and leaf rust resistance. The selected genotypes will be evaluated during more three production years when the traits will better established. Progress of generations will be established with the best plants.

INTRODUCTION

The leaf rust (*Hemileia vastatrix*) is the one of the most important disease in the coffee culture. This disease can be handled with cultural practices, chemical control and genetic resistance. The chemical is the usually practice employed to *H. vastatrix* control and it is an important factor to increase the production costs and environmental pollution. The genetic resistance is a desirable trait and the objective of this work was to evaluate and to select F_3 progenies with good agronomic traits such as high yield, uniform maturation, leaf rust resistance and good grain quality.

MATERIAL AND METHODS

In the year of 2000, a vigorous hybrid coffee plant was found in a robusta plantation at Heringer's farm. It was a result of the natural cross between *Coffea arabica* and *C. canephora* and was named Durandé hybrid. F_2 seedlings were grown in a nursery and evaluated for nematode resistance. The F_2 plants that were resistant to nematode were planted in field, at CEPEC, Martins Soares, MG, Brazil. After 18 months, plants were evaluated and it was found that 35% F_2 plants were resistant to the leaf rust (*Hemileia vastatrix*).

Seeds of 22 progenies with high vigor and high initial productivity were used to obtain 531 F_3 progenies that were planted in Varginha, MG, Brazil, in 2005. Plants were evaluated in 2007

and 2008 years for the agronomic traits, yield, uniform maturation, leaf rust resistance and grain quality (Table 1).

RESULTS AND DISCUSSION

In 2007, a total of 25 plants F_3 with leaf rust resistance and high vigor were selected and from these, 19 remained in 2008. An average of 3,3 L/plant was observed in 2007/08 (Table 1). Plants 1-21, 7-27 and 21-18 produced 4,1L, 6,7 L and 4,3 L/plant each, with 54%, 55% and 77% of flat beans, respectively. The elephant beans were produced in a range of 2 to 11%. The three highlighted plants have produced 38%, 39% and 13% of peaberry beans, with 2,0%, 8,0% and 1,0% of coreless fruits. In the F_3 generation there was a great evolution in the flat grains percentage when compared with F_2 progenies. These results have showed interesting traits in several F_3 generation such as short plants, grains with the same traits as a commercial arabica cultivars and leaf rust resistance

Table 1. Coffee bean and production F3 progenies of Durandé hybrid. Varginha, 2008.

Genotype	Ripening	Color fruit	Production (L/Plant)		Average Prod. (L/plant)	Peaberry	Elephant bean	Corelesss fruit
			2007	2008	(07/08)			(%)
1-21	early	Red	8,0	0,1	4,1	38	8	2,0
1-28	normal-late	Red	2,5	1,4	2,0	68	3	14,0
3-14	early	Red	5,0	2,2	3,6	70	3	24,0
5-3	late	Yellow	3,0	1,5	2,2	15	6	1,0
7-26	normal	Red	6,5	1,3	3,9	73	3	22,0
7-27	normal-late	Red	9,5	3,9	6,7	39	6	8,0
7-29	normal-late	Yellow	4,0	2,0	3,0	18	4	4,0
7-39	late	Yellow	2,5	3,5	3,0	15	6	14,0
11-15	normal	Red	4,5	1,8	3,2	75	2	26,0
11-21	normal	Red	5,5	2,0	3,8	76	2	41,0
11-30	normal-early	Red	3,5	1,3	2,4	35	4	10,0
14-2	late	Yellow	2,5	2,7	2,6	35	5	5,0
14-4	normal	Red	3,5	0,9	2,2	47	5	71,0
14-11	normal	Red	3,5	1,3	2,4	55	3	25,0
12-21	normal	Red	6,5	1,2	3,8	51	2	13,0
21-18	normal-early	Red	8,0	0,6	4,3	13	10	1,0
22-2	normal-early	Red	6,5	0,2	3,1	10	11	7,0
22-5	early	Red	4,5	1,9	3,2	10	4	5,0
22-16	normal-early	Red	5,0	0,6	2,8	14	6	2,0
		Average	5,0	1,6	3,3	40	5	18

CONCLUSIONS

The selected genotypes will be evaluated during more three production years when the traits will better established. Progress of generations will be established with the best plants.

Changes in Physiological Quality of *Coffea arabica* and *Coffea canephora* Seeds Submitted to Different Drying Rates

A.C.R. FRAIZ[1], O.A.O. TONETTI[1], J. ANDERSON CLEITON[1], E.A. AMARAL DA SILVA[1], M.T. SOUZA EIRA[2]

[1]Universidade Federal de Lavras (UFLA), Lavras, MG, Brasil
[2]Embrapa Café/Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brasil

SUMMARY

Long-term conservation of coffee is hampered by the desiccation sensitivity of its seeds. This poses a very serious problem for *ex situ* conservation of coffee germplasm. One important factor to be considered during conservation and storage are the seed water content and the drying rate used to dry the seeds. Thus, the objective of this work was to study the influence of different drying rates on the physiological quality of *Coffea arabica* cv. Rubi and *Coffea canephora* cv. Apoatã seeds. Therefore, fruits were harvested at maturity (cherry state) and were depulped mechanically. Part of the seeds were hermetically dried using activated silica gel (fast drying), and part were hermetically dried over saturated salt solutions (slow drying) to different water contents. The results showed that both species showed higher germination when the seeds were submitted to slow drying. The speed of germination for *Coffea arabica* seeds was higher after slow drying.

INTRODUCTION

Conservation of coffee seeds is hampered by the desiccation sensitivity of its seeds. Ellis et al. (1990) observed that Coffea arabica seeds when dried and stored, they presented an intermediary behavior, tolerating loss of water to certain point (11-13% of moisture) and they do not tolerate storage at low temperatures for long periods of time. However, the capacity to tolerate drying and storage can vary from recalcitrant to intermediate among the coffee species.

Seeds from a high number of species when dried slowly acquire tolerance to desiccation. However, for coffee seeds any type of drying method influences the viability negatively, which demonstrates the sensitivity to dry of the coffee seeds, although the intensity of that influence is less drastic than for seeds with behaviour typically recalcitrant (Guimarães et al., 2002).

Therefore, the occurrence of desiccation sensitivity in coffee seeds hinders or makes impossible to storage the seeds in germplasm banks for long periods of time. Thus, studies that attempt to elucidate the mechanisms of tolerance/sensitivity to desiccation of coffee seeds may provide necessary information for the correct storage of the coffee species. An important factor to be considered for seed storage and conservation is the water content of the seeds. However, water content of the seeds adjusted to conservation of coffee seeds is still not properly defined. Another point to be considered is the drying rate of the seeds, which may allow the repair mechanism to be activated during drying in order to protect the seeds against damages caused by water removal.

Thus, the objective of this work was to study the influence of different drying rates on the physiological quality of *Coffea arabica* cv. Rubi and *Coffea canephora* cv. Apoatã seeds.

MATERIALS AND METHODS

Seed material

Fruits from *Coffea arabica* cv. Rubi e *C. canephora* cv. Apoatã were harvested at the cherry state and depulped mechanically.

Determination of seed water content

The water content of the seeds was determined in an oven regulated at the temperature of 103 °C for 17 hours. The results were expressed in percentage of water on a fresh weight basis, according to the International Seed Testing Association (2004).

Slow and fast drying

Part of the seeds were hermetically dried using activated silica gel (fast drying), and part were hermetically dried over saturated salt solutions (slow drying) to different water contents. The saturated salt solutions with its respective relative humidity (RH) were: $MgSO_4.7H_2O$ (89%RH), NaCl (75%RH), $Mg(NO_3)_2$ (53% RH) and LiCl at the concentration of 5g/100ml of water (95%RH). For *Coffea arabica* the final water contents were 20, 15, 10 and 5%, and for *Coffea canephora* were 30, 15, 10 and 5% (on fresh weight basis).

Seed germination

Immediately, after drying the seeds had the seed coat removed by hand. Following, the seeds were pre-humidified in a chamber with 100% RH for 48 hours and 30 °C. Seeds were surface sterilized in 1% of sodium hypochlorite for 2 minutes and subsequently rinsed in tap water and placed in Petri dishes on filter paper with 10 ml of water. During imbibition seeds were kept at 30 ± 1 °C in the dark (da Silva et al., 2004). The total germination percentage as well as the germination speed index was calculated. The data were subjected to ANOVA and the averages were compared with the Tukey test at the level of 5% of probability.

RESULTS AND DISCUSSION

For *C. arabica* the drying rate influenced the total germination percentage. The seeds that were slowly dried to water content of 5% and 10%, showed higher germination percentage when compared with seeds that were fast dried at the same water contents. For the seeds with 15% and 20% of water content there was no difference regarding to the dry method used.

For *C. canephora* the only treatment that showed differences was the treatment of 15% of water content. In this case, there was higher germination percentage for the seeds slowly dried. Seeds of *C. arabica* with a water content of 5% (slow dry), showed relatively higher germination (71%), when compared with seeds of *C. canephora*, which showed only 6% of germination. This confirms the difference in sensitivity to desiccation among *Coffea* species, corroborating the results shown by others authors (Hong e Ellis, 1995, Eira et. al, 1999; Brandão, 2000).

Table 1. Germination of *C. arabica* and *C. canephora* seeds with different water content obtained through slow and fast drying.

Species	Drying rate	Water content			
		5%	**10%**	**15%**	**20%**
C. arabica	**Slow**	71 a B	92 a A	79 a B	93 a A
	Fast	23 b B	77 b A	85 a A	85 a A
		5%	**10%**	**15%**	**30%**
C. canephora	**Slow**	6 a C	39 a B	44 a B	65 a A
	Fast	7 a B	25 a B	11 b B	69 a A

Averages followed by the same lower case letter in the columns and capital letter in rows do not differ by the Tukey test at 5% level of probability.

Table 2. Speed of germination index of *C. arabica* and *C. canephora* dried at different water contents, through slow drying and fast drying.

Species	Drying rate	Water content			
		5%	**10%**	**15%**	**20%**
C. arabica	**Slow**	1,54 a B	2,5 a A	2,1 a B	2,26 a A
	Fast	0,7 b B	1,8 b A	2,09 a A	2,02 a A
		5%	**10%**	**15%**	**30%**
C. canephora	**Slow**	0,24 a B	0,99 a A	0,97 a A	1,37 a A
	Fast	0,44 a C	0,93 a B	0,16 b C	1,80 a A

Averages followed by the same lower case letter in the columns and capital letter in rows do not differ by the Tukey test at 5% level of probability.

Table 2 shows the speed of germination index of seeds of *C. arabica* e *C. canephora* seeds submitted to slow and fast drying. The speed of germination for *C. arabica* varied significantly according to the drying method used, where the seeds submitted to fast drying had smaller values of speed of germination index. Therefore, this shows that slow drying is more effective and the differences between the drying methods are observed mainly at low seed water contents.

CONCLUSION

Coffea arabica and *Coffea canephora* seeds showed higher germination when the seeds were submitted to slow drying. The speed of germination for *Coffea arabica* seeds was higher after slow drying

REFERENCES

Brandão Júnior D.S. Marcadores da tolerância à dessecação de sementes de cafeeiro. 2000. 144 p. Tese (Doutorado em Fitotecnia) – Universidade Feral de Lavras, Lavras.

Eira M.T.S., Walters C., Caldas L.S., Fazuoli J.B.S., Dias M.C.L.L. Tolerance of *Coffea* spp. seeds to desiccation and low temperature. Revista Brasileira de Fisiologia Vegetal, v. 11, n.2, p. 97-105, 1999.

Ellis R.H., Hong T.D., Roberts E.H. An Intermediate Category of seed storage behavior? I. Coffee. Journal of Experimental Botany, v. 41, n. 230, p. 1167-1174, set. 1990.

Guimarães R.M., Vieira M.G.G.C., Fraga A.C., Pinho E.V.R. von., Ferraz V.P. Tolerância à dessecação em sementes de cafeeiro. Ciência e Agrotecnologia, Lavras, v. 26, n. 1, p. 128-139, jan/fev. 2002.

Hong T.D., Ellis R.H. Optimum air-dry seed storage environments for arabica coffee. Seed Science and Technology, v. 20, n. 3, p. 547-560. 1995.

International Seed Testing Association. 2004. International rules for seed testing. Amendments 2004. Bassersdorf, Switzerland. ISTA

Factors Demanding the Conservation of the Coffee Gene-Pool in Montane Rainforests of Ethiopia

H. HINDORF

INRES University of Bonn/Germany

SUMMARY

The existence of the wild coffee populations of Arabica coffee (*Coffea arabica* L.) in the montane rainforests of Ethiopia is highly threatened by diseases, pests, settlements and land-use pressure. This development is alarming, as, on the one hand, coffee production and consumption is of considerable economic and social importance to Ethiopia. On the other hand, the destruction of the montane rainforests leads to the loss of the natural genetic diversity of *C. arabica*. The genetic erosion of its gene pool is irreversible, leading to high consequential costs also for international coffee breeding and production. As the research project "Conservation and use of wild populations of *Coffea arabica* in the montane rainforest of Ethiopia (CoCE)" aims at the conservation and use of *C. arabica* in its natural habitat and also in the traditional forest coffee systems, the objectives of the concepts include:

1. Genetic studies supporting conservation efforts of wild coffee populations in montane rainforests of Ethiopia
2. Phytomedical studies supporting conservation efforts in wild coffee populations of Ethiopia
3. Ecophysiological and quality studies supporting conservation efforts in wild coffee populations of Ethiopia
4. Identification of coffee forest areas and their documentation by means of a coffee forest atlas for Ethiopia
5. Incentive and financing mechanisms for coffee forest conservation and use in Ethiopia - impacts, prospects and challenges of certification
6. Coffee forest conservation education, communication and deliberation: Innovation systems and implementation support

The investigation on wild coffee populations in their comprehensive biodiversity context calls for an approach that considers natural scienctists, economics and social sciences as well as biological and ecosystem cause-effect chains. Only a transdisciplinary research approach allows the development of conservation and use concepts that are ecologically sustainable, economically efficient and at the same time socially acceptable.

INTRODUCTION

Coffee is by far Ethiopia's most important export crop (32% are exported to Germany) and, with 40-60%, contributes decisively to the country's foreign currency income. 96% of the Ethiopian coffee is produced in traditional forest and garden coffee production systems and is characterised by high quality.

Coffea arabica originates from Southwest Ethiopia, where its wild populations naturally occur in the understory of the montane rainforests at altitudes between 1 000 and 2 100 m.

Wild Arabica coffee is not only consumed by local people, but it is also a cash crop for the local as well as the international specialty market. Above all, it is a unique gene pool for national and international coffee breeding, due to its high natural genetic diversity. The aims of the project "Conservation and use of wild populations of *C. arabica* in the montane rainforest of Ethiopia (CoCE)" are to assess the diversity and the economic value of the montane rainforests and the wild coffee gene pool, and to develop concepts for conservation and use of the wild coffee populations in its center of diversity (Feyera et al., 2007). These concepts include:

1. the conservation of the genetic diversity of the wild Arabica coffee populations,
2. the conservation of the species diversity of the montane rainforests and
3. the sustainable use of wild coffee by the local population.

These objectives require an interdisciplinary approach, integrating natural sciences, economics and social sciences. Accordingly, the CoCE project carried out vegetation studies, forest mapping, molecular genetic analyses, phytopathological and ecophysiological surveys, quality screening, a valuation of the forest and the coffee gene pool as well as institutional analyses. The results of these activities are the basis for achieving CoCE's implementation-oriented goals, such as:

1. establishing a protected area for wild coffee and its forest habitat,
2. developing a coffee forest information system,
3. establishing in-situ gene banks for the conservation of wild coffee genetic resources,
4. developing a certification scheme for wild coffee and
5. working out concepts for environmental and conservation education and public awareness raising.

(Information: **Manfred Denich** m.denich@uni-bonn.de **Franz Gatzweiler** f.gatz@uni-bonn.de and **Woldemariam Tadesse Gole** twgole@ethionet.et).

RESULTS

Genetic studies supporting conservation efforts of wild coffee populations in montane rainforests of Ethiopia

The genetic diversity of wild *C. arabica* from eight regions in the montane rainforest area were studied using different molecular markers to reveal the extent and patterns of the diversity of the coffee populations. Based on chloroplast genome sequence data, *C. arabica* appears as a species that arose in rather recent times through a single allopolyploidization event involving the ancestor of *C. eugenioides* as a mother (Kassahun et al., 2007). Even though large portion of chloroplast genome were sequenced, no deviating chloroplast haplotypes were encountered in *C. arabica*. The interregional analysis of wild *C. arabica* shows a complex pattern of genotype distribution over the different regions; whereby coffee samples collected from some regions are observed to be very diverse, whereas the coffee genotypes from others tend to be more homogeneous and form their own groups. Moreover, the analyses show that wild *C. arabica* populations generally form a group distinct from landraces. The in-depth genetic diversity analyses of three regions demonstrate a clear differentiation and fine scale spatial patterning within region. The over all genetic diversity results suggest the need for a multi-site *in situ* conservation approach. Only this type of conservation approach allows capturing the entire genetic diversity found in different wild coffee regions. The outcomes from the genetic diversity assessment are used as the bases for designing *in situ* gene banks. The ongoing effort of the *in situ* gene bank establishment allows

the conservation of wild coffee-genetic diversity together with the diversity of the associated forest tree species at the peripheral sites of the forest within the same environment (Information: **Kassahun Tesfaye** kassahuntesfaye@yahoo.com)**.**

Phytomedical studies supporting conservation efforts in wild coffee populations of Ethiopia

The three major diseases of coffee, namely coffee berry disease (CBD), coffee wilt disease (CWD) and coffee leaf rust (CLR) were found in association with forest coffee in Harenna, Bonga, Berhane-Kontir and Yayu montane rainforests of Ethiopia (Zeru et al., 2008).

The frequency and intensity of CBD varied among and within forest coffee areas depending on environmental conditions like altitude, rainfall, temperature etc. and genetic diversity of the forest coffee. Survey results indicated that the disease frequency ranged from 0-50%, 20-60%, 0-20% and 0-50% and the intensity from 0-1%, 12.5-22.5%, 0-6.5% and 0-7.8% in forest coffee areas of Harenna, Bonga, Berhane-Kontir and Yayu, respectively. The mean frequency ranged between 6% at Berhane-Kontir and 40 % at Bonga, respectively. High frequencies of CBD may be explained by the particularly high rainfall found in relatively high altitudes of Bonga and to some extent in Yayu. There occurred no CBD at low lands of Harenna (around Majete) and Berhane-Kontir (around Gizmeret) forest coffee areas. These results are the first informations in both areas for the existence of CBD infestations in limited pocket parts of Harenna (around Mekabaldo) and Berhane-Kontir (around Wesheka) forest and semi-forest coffee areas.

The coffee wilt disease (CWD) is caused by the ascomycete *Gibberella xylarioides* forming conidia of *Fusarium xylarioides* in the imperfect life cycle. The soil borne fungus attacks the host by penetrating the stem base with ascospores and invading the vascular system. After blockage of the water transport in the host the tree starts showing wilting symptoms on one site of the host first, before dying completely during the following three months. Lateron, black to violet perithecia occur on the bark of the stem base and more often underneath of the bark. When removing the bark a brown discolouration of the vascular system appears. The dark perithecia contain numerous asci with always 8 two celled ascopsores. The imperfect stage, *F. xylarioides*, produces 2-4 celled macroconidia in sporodochia and slimy microconidia in droplets. In a comparative study of Adugna (2004, 2005) with different *G. xylarioides* isolates the pathogenicity to Arabica and Robusta varieties could be tested, morphological and genetic characteristics in DNA-analyses obtained and similarities were summarized in a dendrogramme. After comparing strains from Arabica and Robusta varieties it could be proved that the fungal population exists of two different *formae speciales*: *G. xylarioides* f. sp. *abyssiniae* hosting Arabica coffee only and *G. xylarioides* f. sp. *canephorae* invading Robusta coffee. Since 1997 *G. xylarioides* attacks *C. arabica* (Arabica coffee) in Ethiopia more seriously and is now found in all coffee growing areas. The pathogen even occurs in very remoted sites like the montane rainforests with an indigenous coffee population. The damage after invasion of the pathogen is a total loss of the coffee tree within 3-5 months. CWD was found in all assessed forest coffee areas suffering considerably in coffee tree losses. Its frequency varied from 0-16%, 0-10%, 0-6% and 0-30% in forest coffee areas of Harenna, Bonga, Berhane-Kontir and Yayu, respectively. The mean frequency varied between 2.4% at Berhane-Kontir and 16.9% at Yayu. The disease seems to expand and damage coffee trees particularly in Yayu and Harenna. Coffee farmers in Yayu, Bonga and Harenna are accustomed to work in groups and use cutlasses (bushman knives) to slash weeds around coffee trees once per year from July to mid September and thereby occasionally coffee trees are wounded becoming then susceptible for an attack of the pathogen.

In all investigated forest coffee areas coffee leaf rust (CLR) was prevalent to a high extend. Results of the assessments, counting exactly the number of infected trees for the frequency and scoring the intensity on single infected leaves, showed more or less high frequencies of CLR in all forest coffee areas varying from 32.2 % at Berhane-Kontir to 96 % at Harenna forest coffee. The highest CLR frequency occurred in 2005 at Harenna followed by Yayu. Young seedlings under the forest coffee at Harenna were covered completely with rust sori influencing the survival and further growth. We scored intensities of CLR during the complete observation period from 2003 – 2007. A tendency of an increase of the disease in all autochthonous coffee areas of Ethiopia could be observed (Information: **Holger Hindorf** h.hindorf@uni-bonn.de and **Girma Adugna** girma.adugna@yahoo.com).

Ecophysiological and quality studies supporting conservation efforts in wild coffee populations of Ethiopia

Investigations on ecophysiology and quality of wild Arabica coffee were conducted in the montane rainforests of Ethiopia. The objectives were to investigate ecophysiological diversity of wild Arabica coffee across their natural environmental gradients (*in situ*) and under controlled identical environment (*ex situ*); and to assess variability in cup quality (aroma, acidity, flavour, body) and biochemical contents (caffeine, chlorogenic acid, trigonelline) of wild Arabica coffee and their interactions with environmental factors. Results indicated that there existed a high variability in water use strategies among the wild coffee populations and they were well-adapted to their local environments. Under drought stress conditions, different accessions exhibited different strategies; those from the southeastern area exhibited an opportunistic way of water use, whereas those from the southwestern areas exhibited the conservative way. High variability in caffeine and trigonelline contents of wild Arabica coffee was observed. Caffeine content was negatively correlated with the soil total N. Trigonelline was negatively correlated with most of soil nutrients. Chlorogenic acid was not influenced by soil nutrients. Shade tree species influenced coffee cup quality; *Acacia* was the best followed by *Cordia* (Information: **Jürgen Burkhardt** j.burkhardt@uni-bonn.de and **Abebe Yadessa** abebeyadessa@hormail.com).

2.4 Identification of coffee forest areas and their documentation by means of a coffee forest atlas for Ethiopia

The Ethiopian montane rainforests are home to hundreds of plant species, many of them rare or endemic. In an economic as well as a cultural sense, the most important one is the coffee plant, *C. arabica*. Popular around the world and widely planted in the tropics, its wild populations are highly endangered by deforestation, threatening its centre of origin and genetic diversity.The development of conservation and use concepts for wild coffee and its forest habitat requires information on the extent of the forest cover and areas with wild coffee populations. To this end, forest areas are mapped based on current satellite imagery. As a reference for the estimation of long-term deforestation rates, satellite images from the 1960s are used. By means of an ecological niche modelling, considering site factors and environmental gradients, suitable habitat areas for the occurrence of wild coffee populations are determined. This analysis provides guidance to locate wild coffee stands, particularly in less-known regions.

All spatial information is compiled in a Coffee Forest Atlas and in an online Coffee Forest Information System. Besides the coffee forest maps, the atlas includes further ecological and socio-economic information, e.g. population density, relevant to the location and design of coffee forest conservation areas. Thus it facilitates the planning of a protected area for wild coffee in its forest habitat. (Information: **Georg Lieth** lieth@uni-bonn.de).

Incentive and financing mechanisms for coffee forest conservation and use in Ethiopia - impacts, prospects and challenges of certification

The economic value of forest coffee can contribute to the development of incentives for the sustainable use and conservation of the Ethiopian coffee forests. Certification is a marketing tool to add value to a product. Certification addresses a growing worldwide demand for more healthy, socially and environmentally-friendly produced products, based on the consumers' motivation to pay price premia for products meeting defined and assured standards.

The research topic provides empirical findings on forest coffee cooperatives currently certified under generic certification standards (Wiersum et al., 2008). It illustrates that certification provides incentives to destroy the forest rather than to promote its conservation. Certification price benefits motivate producers to intensify production by slashing the forests' undergrowth and cutting trees providing the coffee with more sunlight. This destroys the forest ecosystem and biodiversity. Additionally, most producers do not know whether their cooperative is certified and what the respective standards say.

In conclusion, there is need for an Ethiopian forest coffee standard that uses the added value of forest coffee to stimulate its producers to sustainable use and conserve the forest and biodiversity. This could be part of a national initiative of a forest certification system such as the Forest Stewardship Council (Information: **Till Stellmacher** t.stellmacher@uni-bonn.de and **Jörg Volkmann** volkmann@evolve-sustainable-development.de).

Coffee forest conservation education, communication and deliberation: Innovation systems and implementation support

A research project, which aims to contribute towards conservation and sustainable use of biodiversity, requires not only the integration of different disciplines but also of various stakeholders. In its second phase CoCE therefore evolved from a mainly multidisciplinary into a more inter- and transdisciplinary project. With the support of two external consultants the project team uses implementation targets as organizing elements for this integration process. In a series of workshops the team members identified their contributions to the implementation targets. Thus the various interfaces between the involved disciplines and possible stakeholders became clear. For each implementation target the results of the ongoing research are integrated not just at the end but throughout the whole project. (Information: **Peter Moll** moll@science-development.de and **Ute Zander** zander@lernprozesse.com).

CONCLUSION

CoCE as a research project under the umbrella of the BMBF-funded program "BioTeam - Biosphere Research – Integrative and Application-Oriented Model Projects" focusing on the conservation and sustainable use of wild coffee in southwestern Ethiopia. Since 2002, research and implementation-oriented activities are jointly carried out by German and Ethiopian institutions, amongst others, University of Bonn, Institute for Biodiversity Conservation (IBC, Addis Ababa), Ethiopian Institute of Agricultural Research (EIAR) and Addis Ababa University.

In the first project phase (2002-2006), the activities of the project included

1. studies on the floristic diversity of montane rainforests with occurrences of wild coffee populations,
2. molecular analyses as a basis for managing the genetic diversity of the wild coffee,

3. studies on water stress adaptation of wild coffee populations along a climatic gradient,
4. a survey of fungal pathogens in wild coffee populations and the selection of disease-tolerant coffee types,
5. an assessment of the economic value of the gene pool of wild coffee and its forest habitat, and
6. analyses of institutional factors influencing the conservation and use of wild coffee.

Based on the findings of the first project phase, the activities of the still ongoing second phase (2006-2009) direct to the implementation of the results into a sustainable conservation of the coffee potential in the montane rainforests.The coffee forests of Ethiopia are important for both biodiversity conservation and the livelihood of people in the area. For conservation and sustainable use of the forests, the biosphere reserve (BR) approach is the most suitable option, as BRs are established to promote and demonstrate a balanced relationship between nature conservation and human development. However, the BR concept is new to Ethiopian. The Ethiopian Coffee Forest Forum (ECFF) – an NGO established by CoCE – is carrying out the implementation oriented activities of the CoCE project, particularly on conservation education, advocacy, lobbying and networking at different levels. In collaboration with its partners, ECFF has prepared a road map and a task force for the BR establishment in SW Ethiopia. ECFF also organizes workshops and trainings for policy makers and experts on BR and landscape planning, the proceedings of which are published and distributed to the public. Trainings on forest resources assessment, inventory, coffee disease management and monitoring were also given to selected farmers (para-ecologists). ECFF also uses radio, TV, newspapers and documentary film to disseminate information and has established an *in situ* coffee gene-bank at Yayu, where a diverse pool of seedlings from wild coffee areas are cultivated and prepared for dissemination.

REFERENCES

Adugna G. and Mengistu H.: Cultural characteristics and pathogenicity of *Gibberella xylarioides* isolates on coffee. Pest Management J. Ethiopia 4,11-18,2000.

Adugna G.: Diversity in pathogenicity and genetics of *Gibberella xylarioides* (*Fusarium xylarioides*) population and resistance of coffee spp. in Ethiopia. PhD thesis, Bonn/Germany, pp. 81,2004.

Adugna G., Hindorf H., Steiner U., Nirenberg H.I., Dehne H.-W. and Schellander K.: Genetic diversity in the coffee wilt pathogen (*Gibberella xylarioides*) populations: Differentiation by host specialization and RAPD analysis. J. Pl. Dis. Prot. 112,134-145,2005.

Feyera S., Denich M., Böhmer H.J., Tadesse W., Teketay D. and Demissew Sebsebe: Wild *Coffea arabica* L. in the afromontane rainforests of Ethiopia: Distribution, ecology and conservation. SINET: Eth. J. Science 30,13.24,2007.

Kassahun T., Borsch T., Govers K. and Bekele Endeshaw: Characterization of *Coffea* chloroplast microsatellites and evidence for the recent divergence of *C. arabica* and *C. eugenioides* chloroplast genomes. Genome 50,1112-1129,2007.

Wiersum K.F., Tadesse W., Gatzweiler F., Volkmann J., Bognetteau E. and Wirtu Olani: Certification of wild coffee in Ethiopia: Experiences and challenges. Forsts, Trees and Livelihoods 18,9-21,2008.

Zeru A., Assefa F., Adugna G. and Hindorf H.: Occurrence of fungal diseases of *Coffea arabica* L. in montane rainforests of Ethiopia. J. appl.Botany, 2008 (in press).

Genetic Divergence in Clones of *Coffea canephora* Variety Conilon by Different Methods of Multivariate Analysis

R.G. FERRÃO[1], M.A.G. FERRÃO[2], A.F.A. DA FONSECA[2], C.D. CRUZ[3], P.R.CECON[3], P.C.S. CRESCENCIO[3]

[1]Capixaba Institute of Resarche (Incaper), Espírito Santo, Brazil
[2]Embrapa Caffee/Incaper, Espírito Santo, Brazil
[3]Federal University of Viçosa, Minas Gerais, Brazil

SUMMARY

The objective of this work was to study by different methods of multivariate analysis the genetic divergence of forty genotypes of conilon coffee of the Coffee Genetic Improvement Program of the Capixaba Institute of Research - Incaper. In the analysis of dissimilarity, evaluated by the generalized distance of Mahalanobis, the genotypes most dissimilar were ES 318 and ES 01 in Sooretama, and ES 309 and ES 315 in Marilândia, Those most similar were ES 330 and ES 336 in Sooretama and ES 324 and ES 333 in Marilândia. In the study of clustering by the technique of Tocher, the genotypes were distributed in ten groups in Sooretama and five in Marilândia. In both locations there was good agreement in the disposition of the genotypes,in amount of genetic divergence, when the hierarchical nearest neighbor method and the method of Tocher were used. We verified good genetic divergence among the clones for the differents biometric methodologies studies. The results obtained, associated to the main agronomic characteristics evaluated, were important for the strategies addressing genetic improvement through sexual and asexual reproduction and propagation. The clones ES 01, ES 308, ES 309, ES 311, ES 321, ES 327, ES 328, ES 329, ES 330 and ES 337 are promising clones to be maintained and used in the Program of Improvement.

INTRODUCTION

The conilon of the *Coffea canephora* species has great economic and social importance in the State of Espirito Santo. This State, in 2007, produced 73% of the conilon coffee of Brazil (7,5 million prosessed sacks). The Capixaba Institute of Research (Incaper) has been conducting a research program in genetic improvement with this species since 1985. As applied results of this program, newly developed seeds and seedlings of six conilon varieties were made available to the producers from the state of Espírito Santo (Ferrão et al., 2007).

The study of genetic divergence is of great importance in a genetic improvement program, because, biometric techniques with the use of multivariate analyses, dialelics analyses and, or, molecular, are decisive tools in the identification of promising parents and with complementarity of hybridization, in the quantification of genetic variability of the genetic material studied, in the grouping of genotypes more similar aiming at the formation of synthetic varieties in the identification of the importance of each characteristic in the process of selection and also in the availability of information about the available genetic resources in the program and in the exchange of genetic material (Ferrão et al., 2007).

The objective of this work was to study by different methods of multivariate analysis the genetic divergence of forty genotypes of conilon coffee of the Coffee Genetic Improvement Program of the Capixaba Institute of Research - Incaper.

MATERIALS AND METHODS

The studies were carried out during the period of 1996 to 2002, in Sooretama and Marilândia, in Espírito Santo, Brazil, for fourteen characteristics. The experiments were installed in random blocks with four repetitions and spacing of 3.0 x 1.5 m. The statistical and biometric analyses utilized were: dissimilarity avaliation, distance of Mahalanobis, Tocher group and graphic representation of the genotypes for the technique of variable canonical. For those statistical and biometric analyses were utilized the Genes program (Cruz, 2001).

RESULTS AND DISCUSSION

In the analysis of dissimilarity, evaluated by the generalized distance of Mahalanobis, the genotypes most dissimilar were ES 318 and ES 01 in Sooretama, and ES 309 and ES 315 in Marilândia, Those most similar were ES 330 and ES 336 in Sooretama and ES 324 and ES 333 in Marilândia.

Table 1. Grouping, by the Tocher method, of 40 genotypes conilon coffee based or the dissimilarity expressed by the generalized distance of Mahalanobis estimated from 14 characteristics, at Sooretama and Marilândia, ES.

Groups	Genotypes in Sooretama	Genotypes in Marilândia
I	7(ES 312) 19(ES 324) 20(ES 325) 23(ES 328) 9(ES 314) 14(ES 319) 21(ES 326) 11(ES 316) 15(ES 320) 2(ES 307) 27(ES 332) 40(VSM-T_5) 28(ES 333) 18(ES 323)	19(ES 324) 28(ES 333) 29(ES 334) 40(VSM-T_5) 39(VCP-T_4) 25(ES 330) 20(ES 325) 36(ES 36-T_1) 14(ES 319) 9(ES 314) 8(ES 313) 21(ES 326) 2(ES 307) 7(ES 312) 15(ES 320) 18(ES 323) 11(ES 316) 31(ES 336) 30(ES 335) 17(ES 322) 35(ES 340) 23(ES 328) 5(ES 310) 26(ES 331) 27(ES 332) 24(ES 329) 1(ES 306) 34(ES 339)
II	25(ES 330) 31(ES 336) 38(ES 23-T_3) 29(ES 334) 8(ES 313) 24(ES 329) 39(VCP-T_4) 16(ES 321) 17(ES 322) 35(ES 340)	6(ES 311) 32(ES 337) 37(ES 01-T_2) 4(ES 309) 16(ES 321)
III	22(ES 327) 26(ES 331) 5(ES 310) 12(ES 317)	3(ES 308) 33(ES 338) 13(ES 318) 12(ES 317) 22(ES 327)
IV	10(ES 315) 33(ES 338)	38(ES 23-T_3)
V	30(ES 335) 34(ES 339)	10(ES 315)
VI	4(ES 309) 32(ES 337)	
VII	6(ES 311) 37(ES 01-T_2)	
VIII	3(ES 308) 13(ES 318)	
IX	1(ES 306)	
X	36(ES 36-T_1)	

In the study of clustering by the technique of Tocher, the genotypes were distributed in ten groups in Sooretama and five in Marilândia. In both locations there was good agreement in the disposition of the genotypes, in amount of genetic divergence, when the hierarchical nearest neighbor method and the method of Tocher were used (Table 1).

By graphical dispersion with the technique of canonical variables, the clones most divergent in Sooretama were ES 318, ES 311, ES 308 and ES 01, and in Marilândia, ES 315, ES 318,

ES 338, ES 317, ES 309, ES 337 and ES 321. In Sooretama, by canonical analysis, 80.59% of the total variance was explained by the first three discriminate canonical functions with variances of 39.42%, 29.94% and 11.23%, respectively, while in Marilândia the first three canonical functions accounted for 77.70% of the total variance, with variances of 50.51%, 16.35% e 10.81%, respectively (Figure 1 and 2).

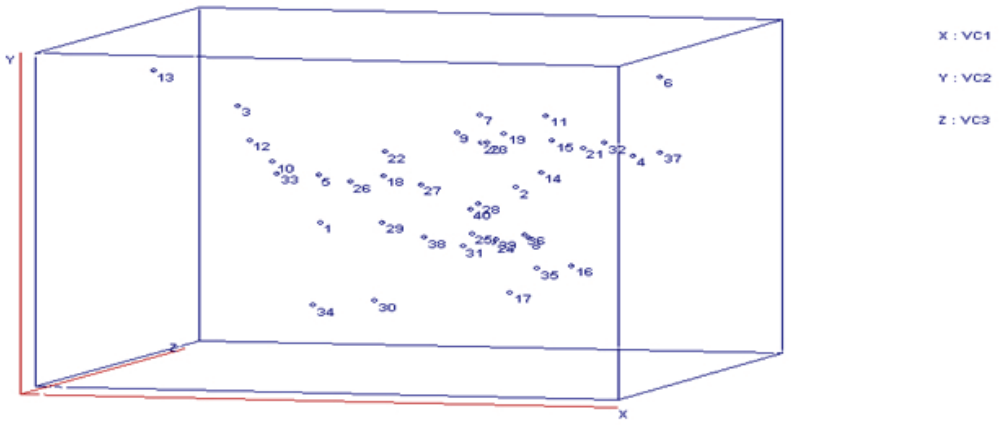


Figure 1. Three-dimensional graph of the dispersion of 40 genotypes of conilon coffee in relation to the canonical variables VC_1, VC_2 and VC_3, in Sooretama, ES.

.

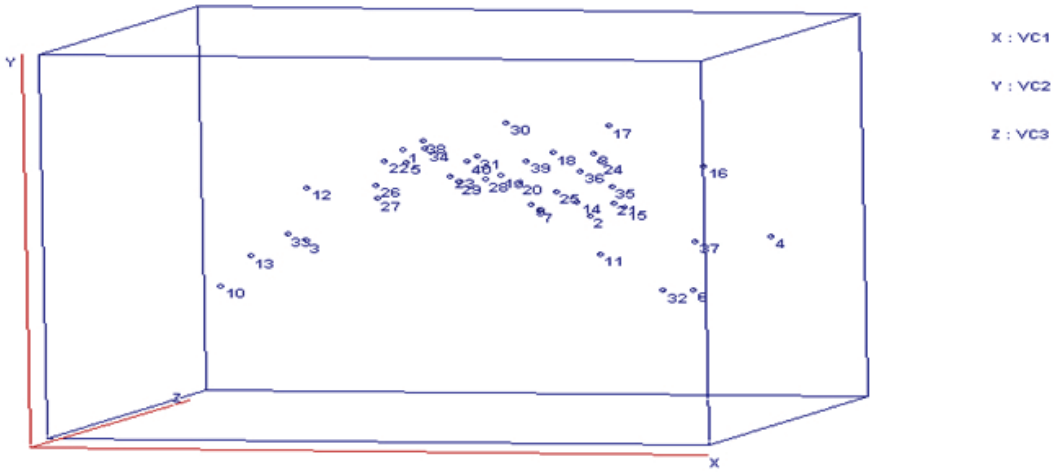


Figure 2. Three-dimensional graph of the dispersion of 40 genotypes of conilon coffee in relation to the canonical variables VC_1, VC_2 and VC_3, in Marilândia, ES.

CONCLUSIONS

We verified good genetic divergence among the clones by the different biometric methods studied. The results obtained, associated to the main agronomic characteristics evaluated, were important for directing strategies of genetic improvement through sexual and asexual reproduction and propagation.

The clones ES 01, ES 308, ES 309, ES 311, ES 321, ES 327, ES 328, ES 329, ES 330 and ES 337 are promising clones to be maintained and used in the Program of Improvement.

ACKNOWLEDGMENT

We thank Mark P. Culik and Fernando Colnago Gonçalves, Incaper, for review and translation into English of this work.

REFERENCES

Cruz C.D. Programa Genes: versão windows, aplicativo ao computacional em genética e estatística. Viçosa: UFV, 2001. 1 CD.

Ferrão R.G., Ferrão M.A.G., Fonseca A.F.A. Da., Pacova B.E.V. Melhoramento genético de *Coffea canephora*. In; Ferrão et al., (Eds.). Café Conilon. Vitória, ES: Incaper, 2007. p. 121-173. Cap. 5.

Microsatellite Markers from the Brazilian Coffee Genome Project

E.T. CAIXETA[1,2], G.F. PENA[2], E. MACIEL-ZAMBOLIM[2,3], R.F. MISSIO[2], L. ZAMBOLIM[2,3], N.S. SAKIYAMA[2,4]

[1]Embrapa Café. E-mail: eveline.caixeta@embrapa.br
[2]Universidade Federal de Viçosa, Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO), 36570-000, Viçosa, MG, Brazil; [3]Dept. Fitopatologia; [4]Dept. Fitotecnia

SUMMARY

A total of 24,031 microsatellites present in the *Coffea arabica* genome were identified in the expressed sequence tags database from the Brazilian Coffee Genome Project. All perfect di-, tri-, and tretranucleotide microsatellites having a minimum of 12 bp were mined. Tetranucleotides repeats were the most frequent, among which the most abundant class was (AGGG)n. The (AG)n and (AGG)n motifs were the most abundant among dinucleotides and trinucleotides, respectively. Some of the microsatellite-containing ESTs were randomly selected for primer design to develop EST-SSR molecular markers and 146 primer pairs were synthesized and analyzed in six coffee genotypes. Well-defined bands patterns were obtained with 109 primers, which were then tested in 22 coffee tree genotypes. The number of alleles per primer ranged from 1 to 15. Only nine monomorphic markers were found. The level of polymorphism was 75% among *C. canephora*, 39% among Híbrido de Timor, and 33% among *C. arabica* individuals. Also, 82% and 74% of markers, which were obtained from *C. arabica* sequences, amplified DNA fragment in *C. canephora* and *C. racemosa*, respectively. All markers were functional in interspecific hybrids. Seventeen of the EST-SSRs markers were used in genetic diversity studies of 17 accessions from the germplasm collection and of six *C. arabica* commercial varieties. Coffee trees were divided into groups, as follows: (1) *C. arabica* genotypes; (2) *C. canephora*; (3) Híbrido de Timor (*C. arabica* x *C. canephora*); (4) Triploids (*C. arabica* x *C. racemosa)*; and (5) commercial varieties. All of the primers were able to detect genetic differences between *C. arabica* and *C. canephora* populations, and between *C. canephora* and all other populations. About 29% of the markers differentiated *C. arabica* from Híbrido de Timor. Analysis of the genotypes grouped according to population revealed a 64% genetic diversity among populations and a 36% within populations. Six primers distinguished four commercial varieties from the six analyzed. These results highlight the potential of these markers in the crossings orientations in breeding programs, in diversity studies, in genetic mapping and in variety fingerprinting.

INTRODUCTION

Molecular marker-assisted selection has been used with increased frequency in plant genetic breeding to attain higher genetic factor transfer efficiency. Microsatellite sequences have been widely used in different species as molecular markers. Microsatellites are multiallelic, highly variable genetic loci that contain a significant amount of information. This information capability, combined with the specificity and speed of PCR technology, make these markers efficient tools for breeding and for studying eukaryotic genes. The major limitation of microsatellites is that they must be individually isolated and developed for each species. A standard method for the SSR marker development involves the construction of a genomic library, sequencing of the clones and designing the primers. The availability of thousands of sequences generated by Expressed Sequence Tag (EST) sequencing projects has opened an

opportunity for using electronic analysis to develop these markers more easily and at a relatively low cost. These EST-SSRs have been used successfully in genetic diversity studies (Aggarwal et al., 2007; Poncet et al. 2006), genetic mapping (Feingold et al., 2005; Han et al., 2006) and map integration (Song et al., 2004).

There is a large coffee EST database developed by the Brazilian Coffee Genome Project (Vieira et al., 2006). This study used this database to develop and validate EST-SSR markers based on expressed sequences of *Coffea arabica*.

MATERIAL AND METHODS

C. arabica ESTs from the Brazilian Coffee Genome Project were accessed and analyzed using the bioinformatics platform of the Laboratório de Genomica e Expressão (http://www.lge.ibi.unicamp.br/cafe/) to identify microsatellites. Data mining was done by using all of the microsatellite-forming di-, tri- and tetranucleotide combinations, totaling 46 classes (4 di-, 10 tri- and 32 tetranucleotides). The mining criterion was ESTs that contained perfect microsatellites larger than 12 bp. Some ESTs were selected randomly for designing the primers. Microsatellites were located using the *Gramene* software (www.gramene.org); the *Primer3* (http://frodo.wi.mit.edu/) was used for designing primer pairs flanking the repetitions, and the *PrimerSelect* (DNASTAR, Madison, USA) was used for primer stability test. The primers were analyzed in six genotypes, two *C. arabica* genotypes, two *C. canephora*, and two Híbrido de Timor (HT). PCR and amplification were done according to Rufino et al. (2005). Marker efficiency and polymorphisms were tested in 22 coffee genotypes (Table 1). Genetic diversity was analyzed using 23 genotypes (Table 1), which were divided into groups: (1) *C. arabica*; (2) *C. canephora*; (3) HT (*C. arabica* x *C. canephora*); (4) Triploids (*C. arabica* x *C. racemosa)*; and (5) rust-resistant commercial varieties. Analysis of molecular variance was used to investigate genetic diversity among and within populations, using the *Genes* software (Cruz, 2006).

Table 1. Coffee genotypes used for EST-SSR markers polymorphism test and for genetic diversity study.

UFV/EPAMIG germplasm accessions	
UFV 2144 (Catuaí Vermelho IAC 44) – *C. arabica*	Guarini UFV 514 (Robusta) – *C. canephora*
Típica UFV 2945 – *C. arabica*	Apoatã IAC 2258 (Robusta) – *C. canephora*
Arábica UFV 10832 – *C. arabica*	HT CIFC 832/2 – *C. arabica* x *C. canephora*
Bourbon UFV 2952 – *C. arabica*	HT CIFC 4106 – *C. arabica* x *C. canephora*
Bourbon Amarelo UFV 535-1 – *C. arabica*	HT CIFC 1343/269 – *C. arabica* x *C. canephora*
Bourbon Amarelo UFV 10745 – *C. arabica*	UFV 557-2 – *C. arabica* x *C. racemosa*
T 3751 (Robusta) – *C. canephora*	UFV 557-3 – *C. arabica* x *C. racemosa* *
T 3580 (Robusta) – *C. canephora*	UFV 557-4 – *C. arabica* x *C. racemosa* *
Conillon UFV 513 (Conillon) – *C. canephora*	Racemosa – *C. racemosa* **
Rust-resistant commercial varieties	
Catiguá MG2 (*C. arabica* x HT)	Sacramento MG1 (*C. arabica* x HT)
IAPAR 59 (*C. arabica* x HT)	Catucai Amarelo 2SL (*C. arabica* x Icatu vermelho)
Oeiras MG6851 (*C. arabica* x HT)	Obatã Amarelo IAC 4932 (*C. arabica* x HT)

** Used only for genetic diversity study; ** Used only for polymorphism test of the EST-SSR markers*

RESULTS AND DISCUSSION

There were 24,031 microsatellites identified in the *C. arabica* genome. Of these, 4,380 were dinucleotides (18.2%), 8,811 were trinucleotides (36.7%) and 10,840 were tetranucleotides (45.1%). These numbers demonstrate that tetranucleotides were the most frequent in the coffee genome transcribed region. Among the 46 microsatellites classes, the most abundant was (AGGG)n (in this class 3,603 EST-SSRs were found), followed by (AGG)n, (AG)n, (AAG)n and (AAAG)n, respectively with 2,713, 2,500, 1,891 and 1,847 EST-SSRs. From the dinucleotides, 57.1% belonged to class (AG)n, 18.1% to (AT)n, 16.2% to (AC)n and 8.6% to (CG)n. For trinucleotides, 30.8% were (AGG)n, 21.5% were (AAG)n and 9.4% were (ACC)n; other had a much lower frequency. The most frequent tetranucleotides were (AGGG)n (33.2%), (AAAG)n (17.0%) and (AAGG)n (8.4%).

Some microsatellite-containing ESTs were used for primer design. After stability testing, 146 primer pairs were synthesized and analyzed in six coffee trees. The result was that 109 primers (75.0%) amplified well-defined bands. These markers were analyzed in 22 coffee trees (Table 1); only nine were monomorphic. The number of alleles per primer ranged from 1 to 15; the mean was 5.2 alleles per primer. Thirty-three percent of the primers amplified polymorphic bands in *C. arabica* genotypes. For *C. canephora* and Híbrido de Timor (HT) accessions, 75.0% and 39.0% respectively of primers were polymorphic. A lower polymorphism found in HT, compared to *C. canephora* genotypes, may be explained by their origin. The designation Híbrido de Timor is given to the descent of a coffee tree originated most probably from natural crossing between *C. arabica* and *C. canephora*. Its descendants, such as the accessions evaluated in this paper, come from natural backcrossing with *C. arabica* coffee trees. Thus, the genotypes that were assessed had a higher proportion of the *C. arabica* genome, and carried the narrow genetic basis of this species.

Microsatellites were mined only in *C. arabica* ESTs, but were tested in different species and interspecific hybrids that are important for breeding. The result was that 82.0% and 74.0% respectively of markers amplified DNA fragments in *C. canephora* and *C. racemosa*. All markers were functional in interspecific hybrids. The use of heterologous primers, together with the single locus nature of these markers, assures their value as connectors for a future consolidation of genetic and physical maps; it also makes possible to associate these maps with phenotypes of interest. In particular, it provides the opportunity of using microsatellite markers for investigating a wide range of genetic diversity, as may be seen in related wild species that are relevant for coffee breeding.

The potential of some of these markers has also been confirmed in genetic diversity studies within and among five coffee populations (Table 1). Seventeen markers that amplified 87 alleles were analyzed; the mean was 5.1 alleles per primer (Table 2) in 23 genotypes. All primers were polymorphic among the genotypes that were evaluated, and were able to detect differences between *C. arabica* and *C. canephora* populations and between *C. canephora* and all other populations. About 29.4% of primers differentiated *C. arabica* from HT. This result is relevant for breeding programs that use HT as the main source of coffee rust resistance. A further observation was that 35.3% and 47.0% of markers were polymorphic between commercial varieties and *C. arabica* and HT accessions respectively. Six primers distinguished four commercial varieties from the six analyzed. These results demonstrate the potential of this class of markers in variety identification studies and in genetic fingerprinting. An analysis of genotypes groups by population revealed 64.0% genetic diversity among populations and in 36.0% within populations; the differentiation rate was 0.637.

This work demonstrated the high abundance of microsatellite repeats in coffee transcriptome, which may be used as efficient molecular markers. These markers can assist conventional breeding by transferring genetic information in a more precise and controlled manner.

Table 2. EST-SSR primers used for the genetic diversity study.

Marker	Primer Sequence		Alleles number
EST-SSR005	F: GGTCCCTGATACCAAACCTC	R: CAAGATCACAGGAAGGCACA	3
EST-SSR007	F: AGTGGCTGGGAACAAAGAGA	R: TTCTCCTCCCGCAAACAGAG	6
EST-SSR010	F: CTTCTTCATCCAACAACACG	R: TGCCATTCCACTGTGTCACT	6
EST-SSR012	F: CGCGTCTGCAACAAAGGTA	R: GGGTGCTAGTCAGAGCCATTT	5
EST-SSR013	F: GCCTTGCTCATATCTGCTGTCT	R: GATCCTTCAACTGAGCCAAA	7
EST-SSR023	F: GCCATTTCACAATCTCACCTC	R: AGACCCAGCAGACAACAACA	4
EST-SSR025	F: AGATACCCACCGCCTAATCCT	R: GCAACAACTTCTGCTCATCC	3
EST-SSR027	F: ATGGAAGTGTCCTTGTCGTG	R: ATGTCGGTGGGTCGGTCAAA	4
EST-SSR029	F: TTAACCTCCTGCCACACA	R: GCCCAAATAAATCCCTCCA	4
EST-SSR047	F: GCGTCAATTAAGCCTCATCATC	R: CAGCCGCTTGCAAAGTAATC	10
EST-SSR048	F: TCCTCCTCGTGCTTCTCAAC	R: GGCAGCATTCTCCTGATCCT	4
EST-SSR054	F: GTTAGCCGTTGGTGATGGAA	R: TTGGTCGAGGGAGGAAGAAC	4
EST-SSR055	F: TACCACCAGCATCCAGACCA	R: TGGGAGGAAATCAAGAGCAA	11
EST-SSR057	F: TTGTGTTCTTTCTCCACCTC	R: CAGGAGTCGTATAACGCTGAA	4
EST-SSR058	F: CACACTTGATTCCGCTCACA	R: GGATGCTTGCTGCTGCTATT	3
EST-SSR069	F: TGAGCTAACCAAGACCAGTTCC	R: CAACAGGAAATCACCGCCTA	3
EST-SSR073	F: GAGGTCTTCCCACCACAACA	R: GGATACGAGAGTCCCTTCCA	6
Mean			5,12

REFERENCES

Aggarwal R.K., Hendre P.S., Varshney R.K., Bhat P.R., Krishnakumar V., Singh L. (2007) Identification, characterization and utilization of EST-derived genic microsatellite markers for genome analyses of coffee and related species. Theor Appl Genet 114:359-372.

Cruz C.D. (2006) Genes versão 2006.4.1: Programa GENES versão Windows. Editora UFV, Viçosa, MG.

Feingold S., Lloyd J., Norero N., Bonierbale M., Lorenzen J. (2005) Mapping and characterization of new EST-derived microsatellites for potato (*Solanum tuberosum* L.). Theor Appl Genet 111:456-466.

Han Z., Wang C., Song X., Guo W., Gou J., Li C., Chen X., Zhang T. (2006) Characteristics, development and mapping of *Gossypium hirsutum* derived EST-SSRs in allotetraploid cotton. Theor Appl Genet 112 :430-439.

Poncet V., Rondeau M., Tranchant C., Cayrel A., Hamon S., De Kochko A., Hamon P. (2006) SSR mining in coffee tree EST databases: potential use of EST-SSRs as markers for the Coffea genus. Mol Gen Genomics 276:436-449.

Rufino R.J.N., Caixeta E.T., Maciel-Zambolim E., Pena G.F., Almeida R.F., Alvarenga S.M., Zambolim L, Sakiyama NS (2005) Marcadores microssatélites para o cafeeiro. In: IV Simpósio de Pesquisa dos Cafés do Brasil, 2005, Londrina.

Song Q.J., Marek L.F., Shoemaker R.C., Lark K.G., Concibido V.C., Delannay X., Specht J.E., Cregan P.B. (2004) A new integrated genetic linkage map of the soybean. Theor Appl Genet 109:122-128.

Vieira L.G.E. et al. (2006) Brazilian coffee genome project: an EST-based genomic resource. Braz J Plant Physiol 18:95-108.

Híbrido de Timor: a Valuable Source of Genetic Variability for Arabica Coffee

N.S. SAKIYAMA[1,4], E.T. CAIXETA[2], T.A. SETOTAW[1], E.M. ZAMBOLIM[1], L. ZAMBOLIM[1,4], A.A. PEREIRA[3,4]

[1]Universidade Federal de Viçosa/DFT, 36570-000 Viçosa, MG, Brazil
[2]Embrapa Café, Brasília, Brazil
[3]EPAMIG, Viçosa, Brazil
[4]CNPq researcher

SUMMARY

RAPD and microsatelite DNA fingerprinting was performed to investigate the introgression of *C. canephora* into Híbrido de Timor genome. A set of 57 genotypes was analyzed, including five *C. arabica*, five *C. canephora*, and 47 Híbrido de Timor. It was observed that the genomes of Híbrido de Timor plants are mainly composed by *C. arabica* and a smaller portion of *C. canephora* genome. Híbrido de Timor CIFC 4106 is likely to descend from at least one generation of natural backcrossing with *C. arabica*, not a F1 hybrid between *C. arabica* and *C. canephora*. Híbrido de Timor accesses carried variable levels of *C. canephora* introgressions, which are lower than presumed before, yet very important genetic variability for *C. arabica* breeding. Financial support: CBP&DCafé, FAPEMIG, CNPq, and CAPES.

INTRODUCTION

Hibrido de Timor, the interespefic hybrid between *C. arabica* and *C. canephora* used as source of resistance gene for economically important diseases and pests of coffee such as coffee leaf rust (*Hemilia vastatrix*), coffee berry disease (CBD) caused by *Colletotrichum Kahawae*, root knot nematode (M*eloidgyne exigua*) and bacteriosis caused by *Pseudomonas syringae* pv garçae (Bettencourt, 1973). Since Hibrido de Timor is an important source of gene for resistance to disease and pests and used to a large extent in the breeding program of coffee in the world, understanding the genome introgression from its origin (*C. arabica* and *C. canephora*) is very important. Therefore, analyzing the genome introgression of *C. canephora* into Hybrid Timor using different types of molecular markers such as microsatellite (SSR) and RAPD, including large number of Hybrid Timor lines, originally introduced Hibrid de Timor lines (CIFIC 4106) in comparisons with *C. arabica* and *C. canephora* cultivars gave more information to understand the genome introgression. Therefore this work was realized with the objective of investigating the level of introgressed genome of *C. arabica* and *C. canephora* into Hibrido de Timor lines currently used in the breeding program of coffee at Univesidade Federal de Viçosa (UFV), Brazil using molecular marker technique microsatellite (SSR) and RAPD (Randomized Amplified Polymorphism DNA).

MATERIAL AND METHODS

A total of fifty seven genotypes obtained form the germplasm banc of UFV/EPAMIG were used in this study: 5 *C. Arabica*, 5 *C. canephora*, 4 Híbrido de Timor clones (CIFC 832/1,832/2, 4106 and 1341/269), and 43 Hibrido de Timor from seeds (Table 1). To analyse the genome introgression and genetic diversity 52 primer of RAPD and 18 primers of SSR

(microsatlite). Only weel defined marker band were considered for this analysis. The data was scored as 1 for the presence of band and 0 for absence of band. Marker bands present in C. arabica and absent C. canephora were considered from C. arabica genome, and vice-versa. These markers were used to study the introgression of *C. canephora* genome into Hibrido de Timor genome.

Table 1. Accessions of *C. arabica*, *C. canephora* and Hibrid de Timor genotypes.

	Genotype	Species		Genotype	Species
1	UFV 2144	*C. arabica*	29	408-18	Hibrido de Timor
2	Catuai Vermelho 44	*C. arabica*	30	408-26	Hibrido de Timor
3	Típica c117(UFV 2945)	*C. arabica*	31	408-28	Hibrido de Timor
4	Burbon -UFV 2952	*C. arabica*	32	427-01	Hibrido de Timor
5	Burbon -UFV 535-1	*C. arabica*	33	427-09	Hibrido de Timor
6	Conillon 3751	*C. canephora*	34	427-15	Hibrido de Timor
7	Conillon 3580	*C. canephora*	35	427-22	Hibrido de Timor
8	Guarani 513	*C. canephora*	36	427-55	Hibrido de Timor
9	Guarani 514	*C. canephora*	37	427-56	Hibrido de Timor
10	Robusta C 2258	*C. canephora*	38	427-65	Hibrido de Timor
11	CIFIC 832/1	Hibrido de Timor	39	427-90	Hibrido de Timor
12	CIFIC 832/2	Hibrido de Timor	40	438-52	Hibrido de Timor
13	CIFIC 4106	Hibrido de Timor	41	439-02	Hibrido de Timor
14	CIFIC 1343/269	Hibrido de Timor	42	440-22	Hibrido de Timor
15	376-01	Hibrido de Timor	43	442-108	Hibrido de Timor
16	376-04	Hibrido de Timor	44	443-03	Hibrido de Timor
17	376-05	Hibrido de Timor	45	446-08	Hibrido de Timor
18	376-35	Hibrido de Timor	46	445-46	Hibrido de Timor
19	376-37	Hibrido de Timor	47	428-04	Hibrido de Timor
20	376-52	Hibrido de Timor	48	432-07	Hibrido de Timor
21	376-57	Hibrido de Timor	49	433-11	Hibrido de Timor
22	376-79	Hibrido de Timor	50	435-11	Hibrido de Timor
23	377-01	Hibrido de Timor	51	437-06	Hibrido de Timor
24	377-02	Hibrido de Timor	52	441-03	Hibrido de Timor
25	377-23	Hibrido de Timor	53	447-48	Hibrido de Timor
26	377-24	Hibrido de Timor	54	448-69	Hibrido de Timor
27	377-34	Hibrido de Timor	55	449-20	Hibrido de Timor
28	379-07	Hibrido de Timor	56	450-61	Hibrido de Timor
			57	451-41	Hibrido de Timor

RESULTS AND DISCUSSION

The genome introgression analysis of *C. canephora* into Hibrido de Timor lines ranges from 2-12% and 0-14% for RAPD and SSR molecular markers (Figure 1). Considering all the marker bands observed the introgression of *C. canephora* genome into Hibrido de Timor reached up to 25% for SSR and 24% for RAPD molecular marker. The individual genome introgression analysis between originally introduced materials from CIFC showed that CIFC 4106 presented 6% and 12% genome introgression of *C. canephora,* which is lower than the proportion expected for a F1 plant. However based on other molecular maker data CIFC 4106 was previously assumed to be a F1 interspecific hybrid between *C. arabica* and *C. canephora* (Lashermes et al., 2000). The molecular analysis also showed that the maximum genome

introgression considering all of the Híbrido de Timor lines not exceed 25%. Those remaining introgressions are very important sources of variability for breeding of arabica coffee. We concluded that: 1) CIFC 4106 has likely descended from at least one generation of natural backcrossing with *C. arabica*, not a F1 hybrid between *C. arabica* and *C. canephora*; and 2) Híbrido de Timor accesses carried variable levels of *C. canephora* introgressions, which are lower than presumed before, yet very important genetic variability for *C. arabica* breeding.

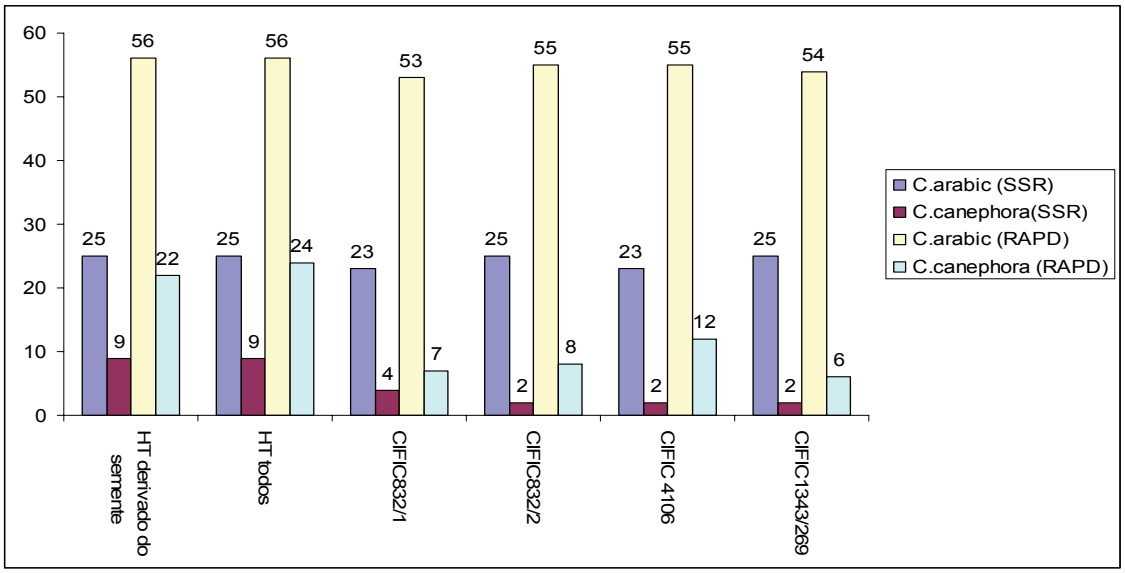


Figure 1. Number of RAPD and SSR marker bands of *C. arabica* and *C. canephora* amplified among lines of Hibrid de Timor using molecular marker RAPD and SSR.

REFERENCES

Bettencourt A. (1973) Considerações gerais sobre o 'Hibrido de Timor'. Instituto Agronomico de Campinas, Circular No. 31 (285 p), Campinas, Brasil.

Lashermes P., Andrzejewski S., Bertrand B., Combes M.C., Dussert S., Graziosi G., Trouslot P., Anthony F. (2000) Molecular analysis of introgressive breeding in coffee (*Coffea arabica* L.). Theor Appl Genet 100: 139-146.

Identification of Coffee Genotypes More Adapted to Areas with Water Deficit During Fall and Winter Time

C.H.S. CARVALHO[2*], M.A. BENTO[1], J.B. MATIELLO[1], S.R. ALMEIDA[1], R.A. FERREIRA[1], L. PADILHA[2], A.F. VILELA[1]

[1]MAPA/Fundação Procafé, Alameda do Café, 1000, 37026-400, Varginha, MG, Brazil
[2]Embrapa Café, Alameda do Café, 1000, 37026-400, Varginha, MG, Brazil.
[*]E-mail: carlos.carvalho@embrapa.br

SUMMARY

Although most of the Brazilian coffee growing areas are located in regions with enough rainfall, a significant amount is cultivated in regions subjected to water deficit during fall and winter time. This situation reduces coffee yield, since Brazilian coffee cultivars are not adapted to this condition. This work was conducted to evaluate the response to irrigation and aiming to select coffee genotypes that are more adapted to environments subjected to water constrain. The work was carried out in Coromandel, MG, Brazil, a region characterized as marginal to coffee cultivation, due to the shortage of rainfall during fall and winter time. A field experiment was set in January of 2004 using a randomized block designed, with 30 genotypes, six plants per plot and four replications, with and without drip irrigation. During the first 2.5 years all the treatments were irrigated to allow proper plant growth and better homogeneity. Yield (kg/tree) was evaluated in July 2007 and, in average, fruit production was increased in 50% due to irrigation in all the genotypes. The highest response to irrigation was observed in the late maturity genotypes, such as Red Obatã, Yellow Catucaí (late) line 30 cv. 2 and Yellow Bem-te-vi, with 223.3%, 120.9% and 110.9% of yield increase, respectively. On the other hand, the irrigation did not significantly increased fruit production of early maturity genotypes, as observed for Catucaí 785-15, Yellow Catucaí 24/137 (early) cv. 900 and Siriema. Since the early maturity cultivars differentiate flowers in the beginning of the year, when water availability is still not a limiting factor, and the late maturity cultivars flower differentiation occurs later on, during the dry season, it is suggested that the early maturity cultivars are more adapted to those conditions. These data indicates that early maturity genotypes may be an interesting genetic source for the development of coffee cultivars more adapted to regions with low rainfall during fall/winter time.

INTRODUCTION

Most of the Brazilian arabica coffee growing areas are located in regions with enough rainfall, but a significant amount is cultivated in regions were water availability is not enough, particularly during fall and winter time. Furthermore, even in regions considered good for coffee cultivation, periods of water deficit are very common. This situation reduces coffee yield, since Brazilian coffee cultivars are not adapted to severe water stress. This work was conducted to evaluate irrigation response aiming the selection of arabica coffee genotypes that are more adapted to environments subjected to water constrain.

MATERIALS AND METHODS

The work was carried out in Coromandel, MG, Brazil, a region characterized as marginal to coffee cultivation, due to the shortage of rainfall during fall and winter time. A field

experiment was set in January of 2004 (3.80 x 0.80m) using a randomized block designed, with 30 genotypes, six plants per plot and four replications, with and without drip irrigation. During the first 2.5 years all the treatments were irrigated to allow proper plant growth and better homogeneity. After May of 2006 irrigation was removed from the plants in the treatments without irrigation, but kept in the irrigated ones. Yield (kg/tree) was evaluated in July 2007.

Table 1. Yield in kg of cherries per tree, of 30 arabica coffee cultivars grown with and without drip irrigation, in Coromandel, MG. 2007.

Cultivar	Yield (kg/tree)		Yield increase duo to irrigation (%)
	Sequeiro	Irrigado	
Yellow Catucaí 2SL cv. 50	5.0	8.3	67.5
Yellow Catucaí 24/137 (early) cv. 900	5.5	5.6	2.2
Red Catucaí 36/6 470 cv 488	3.2	3.6	11.1
Red Catucaí 20/15 cv. 885	2.5	5.0	100.0
Yellow Catucaí cv. 434	3.1	5.5	75.0
Yellow Catucaí 2SL 446 (late), cv. 788	4.2	5.4	28.6
Acauã	2.9	4.0	37.5
Yellow Catucaí 20/15 479 cv. 1106	4.4	4.6	4.1
Yellow Catucaí 3 SM cv. 937	2.9	5.6	93.8
Red Catucaí cv. 61	4.4	5.5	24.7
Red Catucaí 24/137 cv. 81	3.5	4.9	39.7
Yellow Sarchimor	3.9	6.2	58.5
Red Obatã	1.9	6.2	221.9
IBC-Palma 1	2.7	4.7	75.6
IBC-Palma 2	3.2	6.0	85.2
Yellow Catucaí 2SL MF	4.3	5.6	30.6
Sabiá 398	3.4	5.0	50.0
Yellow Catucaí cv. 612	3.6	5.6	55.0
Yellow Catucaí 3.5	3.4	5.9	73.7
Yellow Bem-te-vi	2.4	5.0	110.0
Red Bem-te-vi	3.5	6.2	74.6
Yellow Catucaí (late) line 30, cv. 2	2.6	5.7	120.9
Red Catucaí 36/6 cv. 365	4.3	6.2	43.1
Red Catucaí 785-15	4.0	4.2	6.1
Yellow Catucaí R	2.7	5.5	102.2
Yellow Bourbon	3.7	4.7	27.4
Paraíso: H. 514-7-10-9-3-1	3.7	5.0	36.1
Paraíso	3.6	4.1	15.0
Red Catuaí IAC 99	4.2	5.8	37.1
Siriema	3.8	4.1	6.3
Mean	3.5	5.3	50.8

RESULTS AND DISCUSSION

In average yield was increased in 50.8% due to irrigation in all the cultivars (Table 1). The highest response to irrigation was observed in the late maturity cultivars, such as Red Obatã, Yellow Catucaí (late) line 30 cv. 2 and Yellow Bem-te-vi, with 223.3%, 120.9% and 110.9% of yield increase, respectively. On the other hand, the irrigation did not significantly increased

fruit production of early maturity genotypes, as observed for Catucaí 785-15, Yellow Catucaí 24/137 (early) cv. 900 and Siriema. According to the water balance data of Coromandel region (data not shown), water availability was considered enough until March 2007, a critical period for flower differentiation in that region. The early maturity cultivars usually differentiate flowers in the beginning of the year, when water availability is not a limiting factor. On the other hand, flower differentiation of the late maturity cultivars seems to happen later on, during the dry season. Since this situation usually occurs every year, it is suggested that the early maturity cultivars are more adapted to these conditions and may be an interesting genetic source for the development of coffee cultivars more adapted to regions with low rainfall during fall/winter time. However, the results presented here are considered preliminary and complementary studies of floral differentiation and fruit set and development are under way for conclusive results.

Contribution of Changes in ABA Levels in Root and Leaves and Its Contribution to Drought Tolerance in *Coffea canephora*

V.A. SILVA[1], W.C. ANTUNES[1], B.L.S. GUIMARÃES[1], P. DIMASCIO[2], F.M. DAMATTA[1], A.M. ALMEIDA[1], M.E. LOUREIRO[1*]

[1]Departamento de Biologia Vegetal, Universidade Federal de Viçosa, Viçosa, MG, Brazil, 36570-000. [*]E-mail: mehlers@ufv.br
[2]Instituto de Química, Univesidade de São Paulo, São Paulo, Brazil

SUMMARY

The root system could has an important role in drought tolerance, and use of tolerant rootstocks has been a successful strategy to improve productivity of economically important woody plants under drought, but none is known about this potential use in coffee. We perform reciprocal gratings experiments to evaluate the relative contribution of root system to drought tolerance, using *Coffea canephora* Pierre var. Conilon 120 clone (tolerant) and clone 109 (sensible) and studied the relationship between leaf and root ABA and oxidative damage. Under severe drought stress (-3 Mpa), all plants show a dramatic increase in ABA root levels, but ABA levels are higher in the plants 120, 120/120 and 109/120 that in the plants 109,109/109 and 120/109, inclusive in absence of water stress. Despite sharper reduction in g_s in plants 120, 120/120, 120/109, and 109/120 at -0.5 MPa, no increase in leaf ABA was observed. However, progressive increase in leaf ABA was observed from lower to moderate drought stress (-1 to -1,5 Mpa), and leaf ABA content was higher in the plants 120/120, 109/120 and 120/109 that in plants 109/109, and were no correlated with differences in stomata conductance, but were correlated with lower levels of oxidative damage. The higher levels of ABA in 120/109 plants indicate that ABA synthesis or mobilization in leaves could have important role in determining the concentration of this hormone in this organ. Between moderate and severe water stress, increase in leaf ABA was observed only for plants 109/109 and 120/109, and were paralleled by higher level of oxidative damage. Altogether these results do not shown a clear link between ABA levels and an earlier reduction in g_s in the tolerant clone, but indicates an important role of root ABA in decrease oxidative damage and to postpone water deficit in leaves, indicating the potential of the use tolerant coffee rootstocks to improve productivity under drought conditions.

INTRODUCTION

The root system could have an important role in drought tolerance, and use of tolerant rootstocks has been a successful strategy to improve productivity of economically important woody plants under drought, but none is known about this potential use in coffee. In Brazil the drought is a major factor affecting productivity, and also the more important factor affecting the international prices of this commodity.

Drought tolerance is a quantitative trait, and previous work published by our group, have already identified the contribution of antioxidative mechanisms, efficient stomata closure, changes in carbon allocation and portioning and depth of the root system to coffee water deficit tolerance. The relative contributions of each of these factors varied between genotypes.

However, until now, we still do not know the relative contribution of root or shoot system to this tolerance.

Use of grafting of drought tolerant rootstocks was already a successful strategy in apple production under field conditions, and the potential use of this technological alternative has been barely explored in other woody plants. In order better to know the relative importance of the root system and ABA metabolism, and to explore the potential of the use of water deficit tolerant rootstocks to improve productivity under field condition, we performed reciprocal and non-reciprocal grafting between tolerant (120) and sensitive (109A) *Coffea canephora* Pierre var. *Conilon* clones and evaluated physiological parameters under drought responses in three different water deficit conditions.

MATERIAL AND METHODS

The plants used in these experiments were allografted 120/109A, 109A/120 and autografted 120/120,109A/109A and ungrafted control plants 109A and 120. All plants were cultivated in 12 L pots for 6 months, under greenhouse conditions. After a growth of for six months, half of the plants remained irrigated, whereas the other half was subjected to water stress by withholding irrigation. The experiment was designed in full factorial (6 x 2) randomized blocks with five repetitions. The grafting experiments were: 1) 120/109A: Sensible rootstock (109A) x tolerant scion (120);2) 109A/120: Tolerant rootstock (120) x sensible scion (109A);3) 120/120: Tolerant self-grafted (120);4) 109A/109A: Sensitive self-grafted (109A); 5) 109A: non-grafted 109A; 6) 120: non-grafted 120. Were evaluated Abscisic Acid (ABA) concentration in roots and leaves and electrolyte leakage.

RESULTS AND DISCUSSION

ABA concentration in leaves was higher in the plants 120 and 109A/120 (Figure 1). Independently of water deficit, ABA concentration in roots were higher in plants 120, 120/120 e 109A/120 when compared to plants 109A, 120/109A e 109A/109A (Figure 2). The higher levels of ABA in 120/109A plants indicate that ABA synthesis or mobilization in leaves could have important role in determining the concentration of this hormone in this organ.

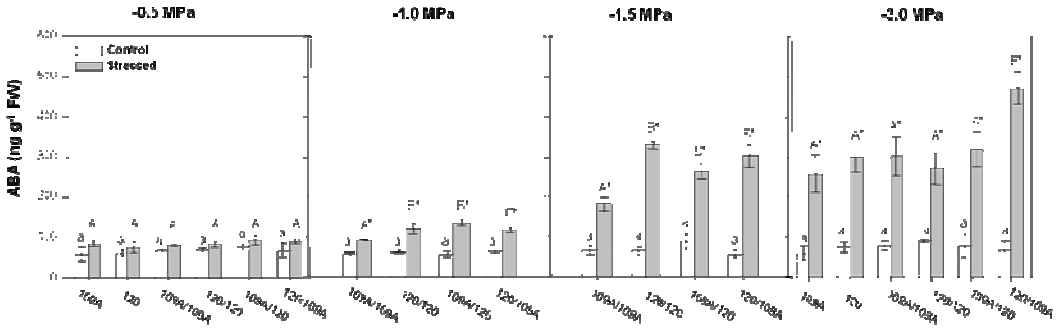


Figure 1. Abscisic Acid (ABA) concentration in ungrafted, autografted and allografted plants of *C. canephora* clones. Irrigated (white) and submitted to progressive hydric deficits (grey), Ψ_{am} = -0.5, -1.0, -1.5 and -3.0 MPa. Different small letters means significant differences among media of irrigated plants. Different capital letters means significant differences among media of stressed plants. (Newman-Keuls, $p \leq 0.05$). An asterisk denotes significant differences between stressed to control irrigated plants (*F* test $p \leq 0.05$). n = 5 +/- SE.

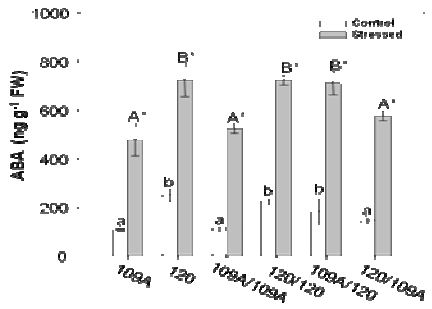


Figure 2. Abscisic acid (ABA) concentration in roots of ungrafted, autografted and allografted of *C. canephora* clones. Irrigated (white) and submitted to hydric deficit (grey), Ψ_{am} = -3.0 Mpa. See others details in Figure 1.

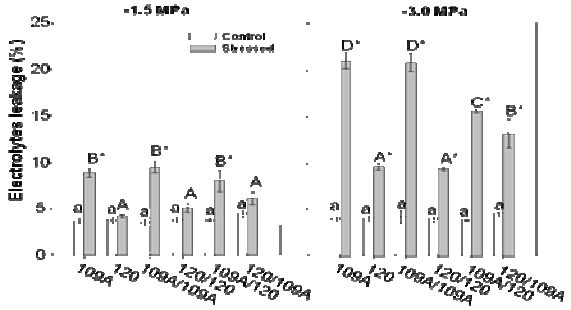


Figure 3. Electrolytes leakage (%) in leaves of ungrafted, autografted and allografted of *C. canephora* clones. Irrigated (white) and submitted to hydric deficit (grey), Ψ_{am} = -1.5 and -3.0 Mpa. See others details in Figure 1.

Under severe water stress the ion leakage increase in all plants, but were higher in the plants 109A and 109A/109A (Figure 3). However, in the plants 109A/120, the tolerant rootstock was able to reduce the ion leakage in the sensible genotype, a fact that was paralleled by an increase in leaf ABA levels under moderate stress.

In conclusion**,** rootstock of tolerant plants (120) can increase ABA concentration in leaves of sensitive scion under moderate drought stress with concomitant reduction in electrolyte leakage. Altogether these results do not shown a clear link between ABA levels and an earlier reduction in stomata aperture in the tolerant clone, but indicates an important role of root ABA in decrease oxidative damage and to postpone water deficit in leaves, indicating the potential of the use tolerant coffee rootstocks to improve productivity under drought conditions

ACKNOWLEDGEMENTS

We thank to CNPq, FAPEMIG and CAPES to financial support to this research.

Contribution of Root System to Adjustment of Photosynthetic Responses to Drought in *Coffea canephora*

V.A. SILVA, W.C. ANTUNES, B.L.S. GUIMARÃES, A.T. CORDEIRO, F.M. DAMATTA, A.M. ALMEIDA, M.E. LOUREIRO*

Departamento de Biologia Vegetal, Universidade Federal de Viçosa, Viçosa, MG, Brazil, 36570-000. *E-mail: mehlers@ufv.br

SUMMARY

Drought tolerance is a quantitative trait, and previous work published by our group in coffee., have already identified the contribution of antioxidative mechanisms, efficient stomata closure, changes in carbon allocation and portioning and depth of the root system to water deficit tolerance The relative contributions of each of these factors varied between genotypes. Drought stress responses usually involve decrease in stomata aperture induced by increased ABA levels or response. However, in parallel with ABA signals, other chemical or non-chemical signals as electrical and hydraulic long-distance signals are further ways how physiological communication and integration occurs between roots and leaves for orchestration of drought responses. However, until now, we still do not know the relative contribution of root or shoot system to this tolerance. We analyze here the effect of reciprocal grafting between contrasting genotypes (clone 120, tolerant; 109, sensible) under four different levels of water deficit, in order to detect the possible occurrence of other root signals that could contribute to drought tolerance. At -0.5MPa, g_s and carbon isotopic discrimination ($\delta^{13}C$) of plants 109/120 were similar to plants 120, 120/120 and 120/109, but different from 109 and 109/109, although no changes were observed for net photosynthetic (A) and C_i/C_a rates. Under predawn leaf water potential (ψ_{am}) of -3,0 MPa, whereas in one side, plants 120 and 120/120 shown reduction of 55% in A and 93% in g_s., and in other side, plants 109 and 109A/109A shown reductions of 75% and 96%, plants 120/109A e 109A/120 shown intermediate reductions of 60% and 95%, respectively. No effect of drought and grafting treatments on photochemical parameters were observed in all levels of water deficit analyzed. Although an indistinct decrease in sucrose and starch were observed for all gratings, increase of glucose were observed only for the plants 109, 109/109 and 109/120 under severe drought, concomitant with a stronger decrease in A. This parallelism suggests that increase in glucose levels under drought could be a reason why the clone 109 is more sensible to this abiotic stress, but this change seems not to be regulated by a root signal. Altogether these results suggest that other signal that ABA produced in the root of tolerant clone could be responsible to reduce the stomata aperture from leaves of the sensible genotype increasing the drought tolerance of this clone.

INTRODUCTION

The CO_2 uptake of plants occurs through the stomata of the leaves and is accompanied by water loss. Drought stress responses usually involve decrease in stomata aperture induced by increased ABA levels or response. However, in parallel with ABA signals, other chemical or non-chemical signals as electrical and hydraulic long-distance signals are further ways how physiological communication and integration occurs between roots and leaves for orchestration of drought responses.

Drought tolerance is a quantitative trait, and previous work published by our group, have already identified the contribution of antioxidative mechanisms, efficient stomata closure, changes in carbon allocation and portioning and depth of the root system to coffee water deficit tolerance. The relative contributions of each of these factors varied between genotypes. However, until now, we still do not know the relative contribution of root or shoot system to this tolerance.

In order better to know the relative importance of the root system on gas exchange and carbon metabolism, and to explore the potential of the use of water deficit tolerant rootstocks to improve productivity under field condition, we perform reciprocal and non-reciprocal grafting between tolerant (120) and sensible (109A) *Coffea canephora* Pierre var. *Conilon* clones and evaluate physiological parameters under drought responses in different water deficit conditions.

MATERIAL AND METHODS

The plants used in these experiments were allografted 120/109A, 109A/120 and autografted 120/120,109A/109A and ungrafted control plants 109A and 120. All plants were cultivated in 12 L pots for 6 months, under greenhouse conditions. After growth for six months, half plants remained irrigated, whereas the other half was subjected to water stress by withholding irrigation. The experiment was designed in full factorial (6 x 2) in randomized blocks with five repetitions. The grafting treatments analyzed were: 1) 120/109A: Rootstock sensible (109A) x tolerant scion (120); 2) 109A/120: Tolerant rootstock (120) x sensitive scion (109A); 3) 120/120: Tolerant self-grafted (120); 4) 109A/109A: Sensitive self-grafted (109A); 5) 109A: non-grafted 109A; 6) 120: non grafted 120.

The following parameters were evaluated: predawn leaf water potential (Ψ_{pd}), duration of the experiment, gas exchange evaluation at 9 h both in different predawn leaf water potential and ^{13}C composition and sugars contents in leaves at -3.0 MPa.

RESULTS AND DISCUSSION

After suppression of irrigation both 109A or 109A/109A plants show down ψ_{pd} as fast as 120 or 120/120 plants (Figure 1 A and B). When compared to ungrafted or autografted 109A plants the combination of 120/109A and 109A/120 have the ψ_{pd} diminished slowly and they achieved ψ_{pd} = -3.0 MPa in 22 and 25 days respectively, (Figure 1C).

At -0.5 MPa g_s of leaves of the plants 120, 120/120 were undersized when compared to 109, 109A/109A plants, although little changes for net photosynthetic rate (A) were observed, (Figure 2 A and B).

Under ψ_{pd} of -3,0 MPa, whereas in one side, plants 120 and 120/120 shown reduction of 55% in A and 93% in g_s, and in other side, plants 109A and 109A/109A shown reductions of 75% and 96%, plants 120/109A e 109A/120 shown intermediate reductions of 60% and 95%, respectively, (Figure 2 A and B).

Carbon isotope composition was reduced in 109A and 109A/109A when compared to 120 or 120/120 plants. The 109A/120 plants had similar carbon isotope composition as 120 resistant to hydric deficits, (Figure 3), indicative of increase of water use efficiency.

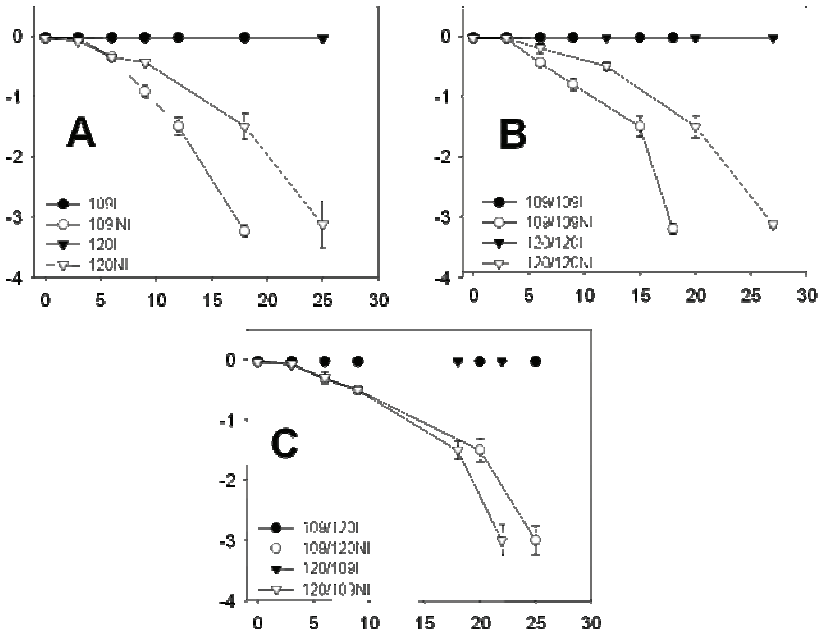


Figure 1. Time course of predawn leaf water potential (Ψ_{pd}) in ungrafted (A), autografted (B) and allografted (C) *C. canephora* plants at irrigated (I - black symbols) and non-irrigated (NI - open symbols). n = 5 +/- SE.

Although an indistinct decrease in sucrose and starch (not shown) were observed for all gratings, increase of glucose was observed only for the plants 109A, 109A/109A and 109A/120 under severe drought, concomitant with a stronger decrease in A (Figure 4). This parallelism suggests that increase in glucose levels under drought could be a reason why the clone 109A is more sensitive to this abiotic stress, but this change seems not to be regulated by a root signal.

In conclusion, the use of reciprocal grafting between genotypes with contrasting tolerance to water deficits allow us to observe contribution of tolerant rootstock 120 in repress water deficit in sensible scion 109A. It was observed that aerial part of tolerant clone has also mechanisms that contribute to drought tolerance, showing the 120 scion always smaller stomata conductance, independently of the rootstock used, a fact that was translated in higher ^{13}C composition, reinforcing the importance of stomata movements to increase the drought tolerance. These results suggest the viability of the use of grafting using tolerant rootstocks for improvement of drought tolerance of more drought sensible genotypes that have other important agronomical traits in coffee.

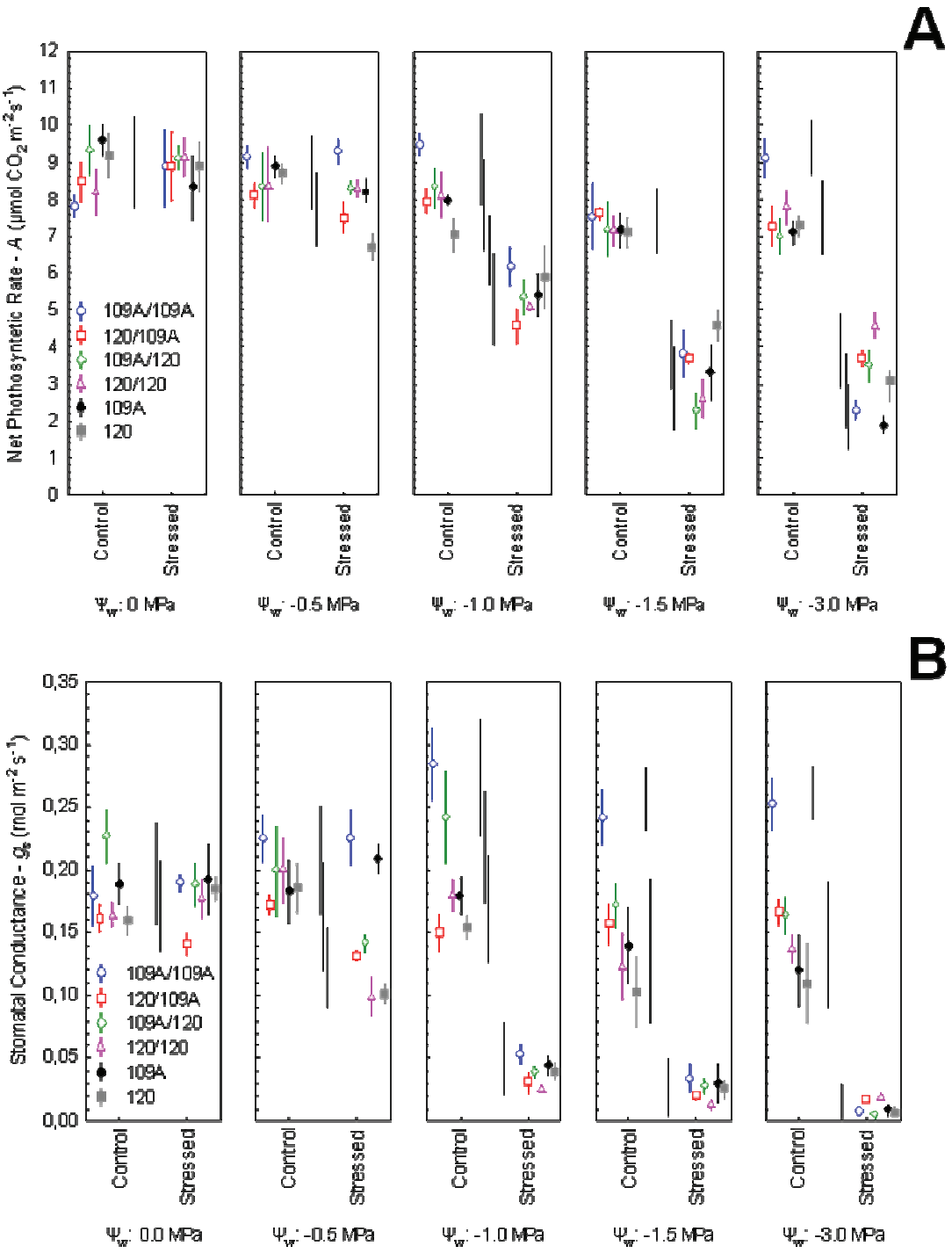


Figure 2. Net photosynthetic rate - *A* (A) and stomatal conductance – g_s (B) in ungrafted, autografted and allografted plants of *C. canephora* clones submitted to progressive hydric deficits (Ψ_{pd} = 0, -0.5, -1.0, -1.5 and -3.0 MPa). The points (mean) was briefly dislocated horizontally to facilitate observation of spreads that means standard error of mean. Points inside the same vertical black line do not differ statistically (p > 0,05) at Newman-Keuls test. n = 5 +/- SE.

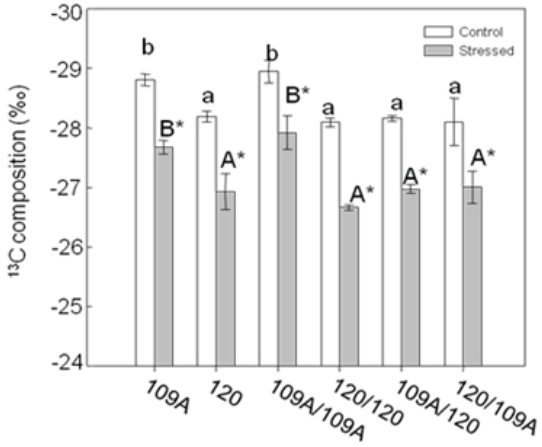


Figure 3. Carbon isotope composition (‰) in leaves of ungrafted, autografted and allografted of *C. canephora* clones. Irrigated (white) and submitted to hydric deficit (grey), Ψ_{pd} = -3.0 Mpa. Equal minor or capital letters do not differ statistically (p > 0,05) among control (irrigated) and stressed plants at Newman-Keuls test. Asterisks means statistical difference (p < 0,05; *F* test) between control and stressed plants.

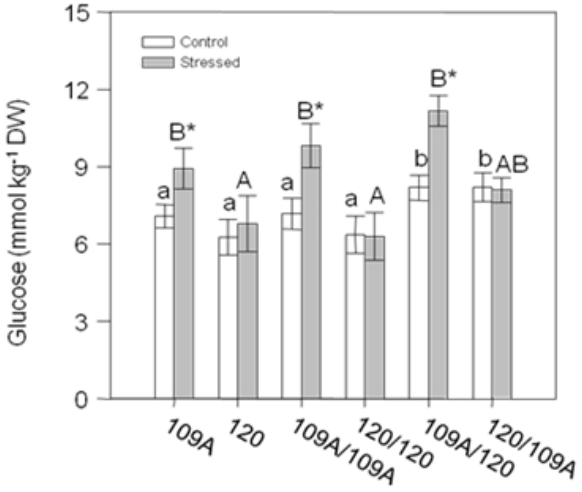


Figure 4. Glucose content (mmol kg^{-1} DW) in leaves of ungrafted, autografted and allografted of *C. canephora* clones. Irrigated (white) and submitted to hydric deficit (grey), Ψ_{pd} = -3.0 Mpa. Equal minor or capital letters do not differ statistically (p > 0,05) among control (irrigated) and stressed plants at Newman-Keuls test. Asterisks means statistical difference (p < 0,05; *F* test) between control and stressed plants.

ACKNOWLEDGEMENTS

We thank to CNPq, FAPEMIG and CAPES by the financial support to this research.

PEG Osmotic Stress on Coffee Seedlings

V.E. ROSA JR.[1], J.A.S. ALMEIDA, P.B. GALLO[2], C. COLOMBO[1], W. GONÇALVES[1], L.C.S. RAMOS[1*]

[1]APTA. [*]E-mail: lcramos@iac.sp.gov.br
[2]Instituto Agronomico, Campinas, SP, Brasil

SUMMARY

Osmotic stress was induced on coffee (*Coffea arabica* and *C. canephora*) seedlings by using PEG (polyethylene glycol 6000). Initial experiments were performed with *C. arabica* cv. Catuai individual seedlings on glass tubes, to establish a lethal solution concentration; 160 grams per liter was selected and used as a discriminatory level afterwards. The osmotic experiments were conducted at room temperatures (25 °C) and under indirect fluorescent light condition. Seedling stress symptoms observed were cotyledon withering and rolling as well as a brown constriction in the upper part of the hypocotyls. These symptoms appeared within 24 to 48 hours, being irreversible, as the seedlings did not recover in water; however, some of them did not show symptoms at all. Thus, we considered as susceptible the first ones and tolerant the later ones. *C. canephora* PEG selected seedlings as above were grown in the field along non selected ones and observations were made in a dry and hot season. The PEG selected plants did not show leaf wilt symptoms but the non selected ones did. Field plant infrared temperatures were measured on both plant types, but they did not differ. Leaf disk desiccation tests, measured at room conditions, with relative water content, were also performed and did not show different rates between the two groups. Progeny tests will be carried on with the selected PEG plants. Conversely, progeny seedlings from a F_2 arabusta plant observed to display visual field tolerance to drought relatively to others showing withering symptoms in a very dry year, were assayed also with PEG. It turned out that a proportion of those seedlings were tolerant. These preliminary results suggest that the PEG seedling test could be useful and further assays are planned to access the potential as a selection tool for drought stress tolerance in coffee.

INTRODUCTION

Coffee is one most important cash crop in Brazil. Despite this fact and that Brazil is the largest world producer, productivity still remains one of the most important breeding goal. Among productivity constraints, water availability can be considered one of the most important followed by fertilizing, diseases and pest control. Thus any improvement regarding to water restraints would reflect in productivity. However, breeding towards a better withstanding against water stress seems not to be simple because of lack of efficient selection tools.

Levitt (1972) defines defense mechanisms against water stress as drought *avoidance* – drought endurance with high internal water content – and drought *tolerance* – drought endurance with low internal water content – along with drought escape. Drought avoidance can be achieved by a well developed root system; whereas drought tolerance could be achieved by withstanding lower tissue hydratation, for instance.

The effects of water stresses on plants will determine the crop yield potential lastly. However, the mechanisms by which plants respond to this constraint have been long studied. Newmann (2008) hypothesed that roots could sense water deficits by transmitting signals to the shoots. He proposed the plant hormone abscisic acid as one candidate because it inhibits stomatal opening and possibly growth in water-stressed leaves.

Although the concepts of Levitt (1972) are not strictly followed, Van der Vossen (2001) indicated that higher tolerance to water stress in Arabica is partly supported by a well developed root system, outstanding plant vigour and ability to retain leaves under water stress as compared to Robusta. Also drought tolerance of Robusta based on the ability to yield under water stress could be explained by a higher root mass to leaf area ratio and deeper roots according to Pinheiro et allii (2005).

The use of polyethylene glycol (PEG) can be helpful to maintain rooting media at predetermined values in the study of water stress in plants. According to Lawlor (1970), PEG can be used successfully to decrease the water potential of plants as long as it does not enter the roots. PEG is a highly water-soluble neutral polymer and several studies have been utilizing this polyether to induce hydric stress in plants in order to select favorable traits related to plant tolerance in limited water supply conditions (Zgallai et al., 2005).

Although to our knowledge no studies have been focused the utilization of PEG to observe drought symptoms in coffee seedlings, some studies have been conducted to understand the influence of drought in coffee growth and development. Coffee plant retain water under drought conditions, being considered a dehydrating tolerant species (Nunes, 1976; Josis et al., 1983), and this characteristic have been proposed to be a consequence of an efficient stomatal control on transpiration and low cell-wall elasticity (Nunes and Duarte, 1969; DaMatta et al., 2003).

In Robusta, leaf shedding occurs from older leaves to younger ones after drought stress and drought-sensitive clone have greater leaf extention (DaMatta, 2004). Osmotic adjustment in both Arabica and Robusta coffee resemble not to be a general trait observed (Meinzer et al., 1990). Besides, some genotypes show lower variance in its turgor suggesting that osmotic adjustment may not be an effective mechanism of drought tolerance in coffee as occur in other woody species (Fan et al., 1994).

Reduced stomatal conductance (RSC) has been reported to be an early indicator of water shortage in Arabica coffee, showing decreases as soon as one-third of the available soil water has been depleted (Nunes, 1976). Also, RSC declines sharply with increasing evaporative demand irrespective of the leaf water status (Gutiérrez et al., 1994; Tausend et al., 2000). By contrast, Robusta coffee appears to exhibit poorer stomatal control on transpiration than Arabica (DaMatta et al., 1997).

A few studies have focused the limit point of tolerance in plants, which could be useful to select plants highly tolerant to water stress and to verify the physiological effects under extreme conditions. Selection efficiency would be better if done as early as possible in coffee, considering the long plant life cycle, up to three years. Attempts to correlate seedling measurements to adult field responses was already done, indicating feasibility (Ramos, 1997). With this aim tests using osmotic solutions were carried out to access coffee seedling responses.

In this work we analyzed different concentrations of PEG (6000) solutions to identify the maximum osmolarity level that could discriminate seedlings of *C. arabica* and thus to explore the variability on other genotypes.

MATERIAL AND METHODS

Coffea arabica cv. Catuai, cv. Mundo Novo and cv. Bourbon Vermelho and *C. canephora* cv Robusta seedlings were grown on a sandy glass covered micronursery up to the cotyledonary stage. The micronursery was 88 cm wide, 70 cm length, 55 and 25 cm of back and front height, with 40 cm deep washed sand. Seeds were sown and irrigated daily and the germination occurred around 45 days. The seedlings were used when the cotyledonary leaves expanded in 30 days, approximately. PEG (polyethylene glycol 6000) osmotic solutions were made with distilled water and used to induce stress on the seedlings.

Initial experiments were performed with *C. arabica* cv. Catuai individual seedlings on 20 mm glass tubes with 30 ml solutions, to establish a lethal and/or semi-lethal concentration of PEG. Leaf withering, constriction of hypocotyl and seedling death, were considered as visual stress symptoms for all experiments. The visual symptoms were observed and ranked on a time scale, up to 48 hours. The osmotic experiments were usually conducted at room temperatures (25^{o} C) and under indirect fluorescent light condition. To determine a suitable concentration of PEG necessary and sufficient to lead seedlings to death, solutions of PEG 6000 (0, 25, 50, 100, 130, 160, 200, 230, 260 and 300 g/L) were tested with Catuaí seedlings. The seedlings were carefully harvested from sand, with entire root system and transferred to a Becker with water to wash the roots. Nextly the seedlings were brought to the lab and five of them were placed in glass tubes with PEG solutions.

Surviving *C. canephora* cv Robusta seedlings screened on 160 g/L PEG solutions were further raised in a regular nursery for about an year, till they achieved size for field planting. Three years later, the plants were visually evaluated along the normal plants regarding to their appearance, when a season was particularly dry and warm. Leaf temperatures were sampled in the mid-afternoon of a day with clear skies, with the help of an infrared thermometer. Leaf disk desiccation tests were made by measuring their relative water content (RWC) on the PEG selected and non-selected neighbor plants. The RWC was assayed as Barr and Weatherley (1962).

Arabusta seedlings germinated in the same way as before, originated from selected field plants of a F_2 population family, previously observed to display “drought field tolerance” in a very dry season, by showing turgid leaves along others with wilted or scorched ones. About 30 F_3 seedlings from one plant were PEG tested as before. Response classes were (1) acute, with constriction as in Figure 3; (2) acute; (3) intermediary; (4) minor symptom and (5), no symptoms. After PEG challenging, the seedlings were transferred do water for recovering and the surviving ones were grown in the field.

RESULTS AND DISCUSSION

Visual seedling stress symptoms observed in *C. arabica* cv. Catuai were cotyledon withering and rolling as well as a brown constriction in the upper part of the hypocotyls (Figure 1). The severe symptoms appeared within 24 to 48 hours, being irreversible, as the seedlings did not recover in water. Results (Table 1.) indicated that concentration of 100 g/L of PEG caused severe damage on the seedlings, causing further death. From this experiment we choose the concentration of 160 grams per liter using it as a discriminatory level afterwards. Thus, we considered as susceptible the seedlings which died at this level and tolerant the ones which survived. The *C. arabica* cv. Mundo Novo and cv. Bourbon Vermelho were also observed to be susceptible on this test.

Figure 1. Effect of curving and drying of the hypocotyl below to the cotyledons of *C. Arabica* seedlings in response to PEG treatment.

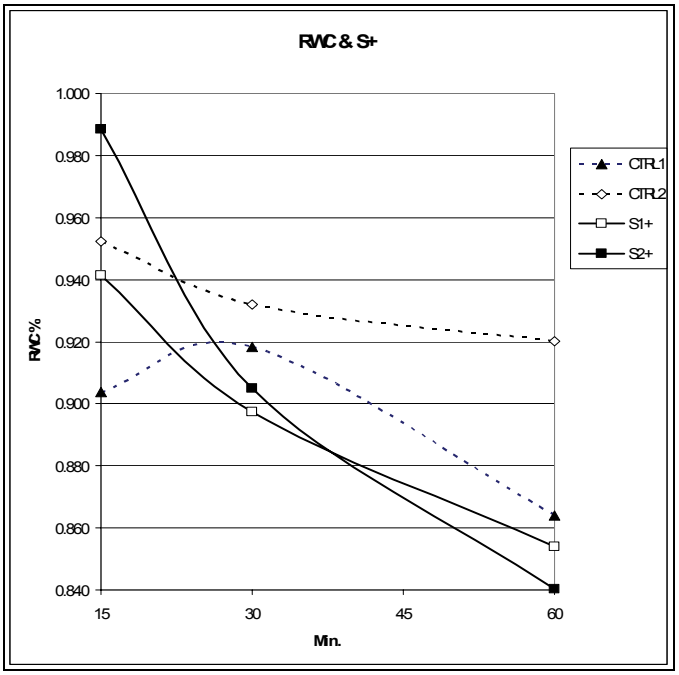
Table 1. Visual evaluation of the effects of different concentrations of PEG 6000 in 30 days seedlings of *C. Arabica* sp cv Catuaí. Seedlings were observed concerning to the effects of leaf withering, leaf drying and death after 48 h.

PEG g/L	Symptom	Description
0	No symptoms	No visual alterations
25	No symptoms	No visual alterations
50	Moderated symptoms	Withering leaves
100	severe symptoms	Dried leaves
130	severe symptoms	Dried leaves
160	death of seedlings	Dried leaves and constriction of the hypocotyl
200	death of seedlings	Dried leaves and constriction of the hypocotyl
230	death of seedlings	Dried leaves and constriction of the hypocotyl
260	death of seedlings	Dried leaves and constriction of the hypocotyl
300	death of seedlings	Dried leaves and constriction of the hypocotyl

C. canephora PEG selected plants as above were grown in the field along non selected ones and observations were made in dry season. The PEG selected plants did not show leaf wilt symptoms but the non selected ones did. Field plant infrared temperatures were measured on both plant types, but they did not differ, either for full exposed to the sun or shaded leaves. Plant leaves cool while loosing water; if the PEG tolerant plants were transpiring more water, their temperature could be lower, or the reverse, if were transpiring less. Leaf disk desiccation tests, measured at room conditions with relative water content, were also performed for one hour and did not show different rates between the two groups (Figure 2). If one type holds weight longer, it could be an evidence of stomatal control. Perhaps the plant system control of water loss may lie on the roots, as suggested by Newmann (2008). Progeny tests will be carried on with the selected PEG plants, to check if this control is of genetic nature.

Conversely, progeny seedlings from F_2 Arabusta plants, known to display visual field tolerance to drought relatively to others showing withering symptoms in a very dry year, were assayed also with PEG. It turned out that a proportion of those seedlings did not show the PEG symptoms at all (Figure 3) and stem constriction did not occurred in all susceptible seedlings. The surviving seedlings were saved and grown in the field. Because the proportion

of tolerant seedlings were rather small it suggests a recessive nature. Progeny tests will also be carried out later.



Obs.: 15 minutes initial time to bring leaves from field to begin experiment in lab

Figure 2. Relative water content (RWC) from leaves of drought tolerant plants (S1+, S2+) and from controls (CTRL1, CTRL2) in one hour desiccation test.

Drought tolerance have been reported associated with high yield by Anim-Kwapong and Adu-Ampomah (2004). This study was done in field conditions, as the one of Shimber et al (2004). Although careful statistical planning can avoid local bias, results may affected by drought avoidance, achieved by a well developed root system genotype (Levitt, 1972). The PEG test should not have this effect since the entire seedling root system is submerged in solution.

These preliminary data suggest that the PEG seedling test could be useful and further assays are planned to access the potential as a selection tool for drought stress tolerance in coffee.

REFERENCES

Anim-Kwapong E. and Adu-Ampomah Y. 2004. Agronomic Characteristics of Drought Tolerant Robusta Coffee Genotypes. XX International Congress of "Assoc. Scientifique Intenationale du Café" – ASIC. Bangalore, India. October 11-15, 2004. 20: 1081-1084.

Barr H.D. and Weatherley P.E. 1962. A re-examination of the relative turgidity technique for estimating water deficit in leaves. Aust. J. Biol. Sci. 15:413-428

DaMatta F.M., Maestri M., Barros R.S.: Photosynthetic performance of two coffee species under drought. Photosynthetica 34: 257-264 (1997).

DaMatta F.M., Chaves A.R.M., Pinheiro H.A., Ducatti C., Loureiro M.E.: Drought tolerance of two field-grown clones of Coffea canephora. Plant Science 164: 111-117 (2003).

DaMatta F.M. Exploring drought tolerance in coffee: a physiological approach with some insights for plant breeding Braz. J. Plant Physiol. vol.16 no.1 (2004)

Fan S.H., Blake T.J., Blumwald E.: The Relative Contribution of Elastic and Osmotic Adjustments to Turgor Maintenance of Woody Species. Physiologia Plantarum 90: 408-413 (1994).

Fischer R.A. and Maurer R. Drought Resistance in Spring Wheat Cultivars. I Grain Yield Responses. Australian Journal of Agricultural Research. 1978, 29, 897-912

Gutierrez M.V., Meinzer F.C.: Estimating Water-Use and Irrigation Requirements of Coffee in Hawaii. Journal of the American Society for Horticultural Science 119: 652-657 (1994).

Lawlor D.W.: Absorption of Polyethylene Glycols by Plants and Their Effects on Plant Growth. New Phytologist 69: 501-& (1970).

Levitt J. (1972) Responses of Plants to Environment Stresses. Academic Press. New York. 607 p.

Josis P., Ndayishimiye V., Rénard C. (1983) Étude des relations hydriques chez Coffea arabica L. II. Évaluation de la resistance à la secheresse de divers cultivars à Ghisa (Burundi). Café Cacao Thé 27:275-282.

Meinzer F.C., Grantz D.A., Goldstein G., Saliendra N.Z.: Leaf Water Relations and Maintenance of Gas-Exchange in Coffee Cultivars Grown in Drying Soil. Plant Physiology 94: 1781-1787 (1990).

Neumann P.M. 2008. Coping Mechanisms for Crop Plants in Drought-prone Environments. Annals of Botany 101 (7): 1-7 2008.

Nunes M.A., Duarte N.T. (1969) Resistência à secura em Coffea arabica - estudo comparativo de duas selecções. Garcia de Orta, Sér. Est. Agron. 17:83-96.

Nunes M.A. (1976) Water relations in coffee: significance of plant water deficits to growth and yield: a review. J. Coffee Res. 6:4-21

Pinheiro H.A., DaMatta F.M., Chaves A.R.M., Loureiro M.E., Ducatti C.: Drought tolerance is associated with rooting depth and stomatal control of water use in clones of Coffea canephora. Annals of Botany 96: 101-108 (2005).

Ramos, L. C. S. and Carvalho, A. Shoot and root evaluations on seedlings from Coffea genotypes. Bragantia, Campinas, 56(1): 59-68, 1997.

Shimber T., Ismail M.R., Mahmood M., Ramlan M.F. 2004. Growth and Physiological Processes in Coffea Canephora Clones in Response to Water Deficit Stress. XX International Congress of "Assoc. Scientifique Intenationale du Café" – ASIC. Bangalore, India. October 11-15, 2004. 20: 1142-1146.

Tausend P.C., Goldstein G., Meinzer F.C.: Water utilization, plant hydraulic properties and xylem vulnerability in three contrasting coffee (Coffea arabica) cultivars. Tree Physiology 20: 159-168 (2000).

van der Vossen H.A.M. Agronomy I: Coffee Breeding Practices, In: Coffee Recent Developments. Ed. RJ Clarke and OG Vitzthum. Blackwell Science. 2001. pp 184-201

Zgallai H., Steppe K., Lemeur R.: Photosynthetic, physiological and biochemical responses of tomato plants to polyethylene glycol-induced water deficit. Journal of Integrative Plant Biology 47: 1470-1478 (2005).

Analysis of Phenotypic Plasticity in Response to Water Constraints in Coffee Plants Growing Under Field Conditions

P. MARRACCINI[1,3], G.C. RODRIGUES[2], O.C. ROCHA[2], F.A. GUERRA[2], A.C. ANDRADE[3], T. LEROY[1], D. POT[1,4], C. JOURDAN[5], J-M. GION[6]

[1]CIRAD UMR DAP, Montpellier SupAgro, 2 place Viala - 34060 Montpellier, France
[2]EMBRAPA Cerrados, CP 08223, 73310-970 Planaltina-DF, Brazil
[3]EMBRAPA Recursos Genéticos e Biotecnologia, LGM-NTBio, Parque Estação Biológica, CP 02372, 70770-900 Brasília-DF, Brazil
[4]Instituto Agronômico do Paraná, LBI-AMG, CP 481, 86001-970 Londrina-PR, Brazil
[5]CIRAD, UPR 80, Montpellier, France
[6]CIRAD, UPR 39/UMR BIOGECO-INRA, 69 route d'Arcachon, 33612 Cestas, France

SUMMARY

In a context of climate change, adaptation of perennial plantations to water constraints becomes a major concern for wood and fruit productivity. Adaptation depends on the level of genetic diversity in breeding and natural populations, as well as their plasticity. This project plans to describe adaptive mechanisms under water constraints for three perennial plants of temperate and tropical regions, including *Pinus*, *Eucalyptus* and *Coffea,* through a combined analysis of plant architecture, physiology, anatomy and molecular responses to drought stress.

EXPERIMENTAL APPROACH

Germplasm

The coffee field trial has been set up in the experimental fields at Embrapa Cerrados (Planaltina-DF, Figure 3) located near Brasilia, because that region is always subjected to a long and regular dry season during the winter (june to august). Field trials will be conducted with cultivars Iapar59 and Rubi MG 1189 of *Coffea arabica*, the former considered more tolerant to drought than the latter (M.A.G. Ferrão, personal communication) (Figure 1).

Field experiment

Three water treatments will be applied (Figure 2): T1, an unlimited watering treatment (always irrigated during the dry season), T2, a limited watering treatment (not irrigated during the dry season) and T3, limited watering in the dry season of year 1 and unlimited watering in year 2 (recovering). There will be six measurement points (P1 to P6) over the two years of the experiment (2008-2009).

Planned activities

For all conditions, molecular plasticity will be investigated for leaves, stems and roots (Figure 4) by analysing expression of candidate genes known to be involved in plant responses to drought by quantitative PCR (qPCR). Gene expression experiments will be also performed using total RNA from meristematic cells isolated by laser-assisted microdissection to hybridize microarrays coated with coffee ESTs. Excavated plants (Figure 5) will be used to

analyse phenotypic plasticity by measuring leaf area index (LAI), leaf thickness and stomata number for example. On an anatomical level, xylem vessel structure and parenchyma thickness will be assessed by cross section of coffee tissues. These data will be correlated with ecophysiological measurements such as biomass estimations (for leaves, stems and roots), hydraulic conductivity, stomatal conductance, and water-use efficiency (δC^{13}). Leaf stomatal density will also be compared between the two cultivars and the different water treatments (Figure 6). In order to estimate the effects of drought on coffee plant development, we will also characterize the root and aerial architecture of coffee plants.

Figure 1. Plants (7 years old) of *C. arabica* var. Iapar59 (left) and Rubi (right) cultivated in field condition (Embrapa Cerrados) without irrigation and subjected to more than 200 days of drought. Note that under these conditions, leaves are still present for Iapar59 but not for Rubi (date 13/09/2007).

A multidisciplinary network for technological and technical innovations

A network of scientists and technicians in molecular biology, genetics, ecophysiology, anatomy and developmental biology from different research institutions and universities is involved in this project. This will permit to monitor the experimental field trials for two growing periods of the project. The molecular plasticity will be checked with microarrays for particular cells sampled by microdissection.

Expected results

Overall, the results should enable (1) the identification of dynamic changes on molecular, morphological and ecophysiological levels, (2) an analysis of their correlations, and (3) identification of GxE interactions for coffee plants. The understanding of ecophysiological and molecular mechanisms for water stress adaptation should help us to identify adaptive traits and candidate genes for adaptation to water stress.

In order to see if common mechanisms are found between coffee plants, *Pinus* and *Eucalyptus,* the results of this experiment will be compared to those obtained for the two other model plants.

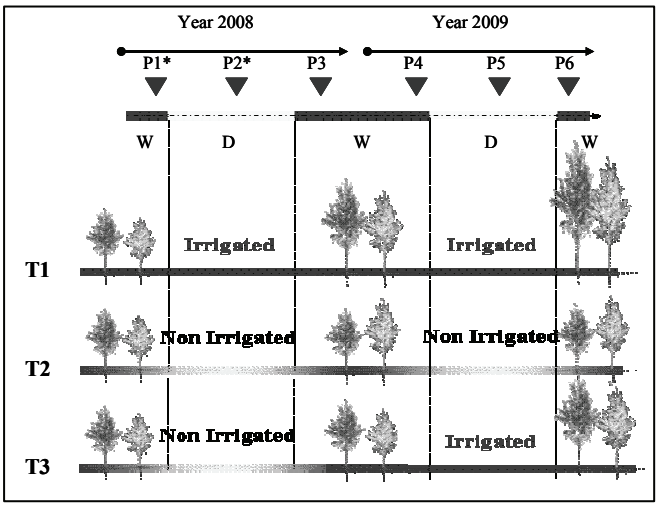


Figure 2. Schematic representation of water treatments applied to coffee plantlets. W: wet season (October to April); D: dry season (May to September). The different colours of trees symbolize the two cultivars (tolerant and susceptible to drought) of *C. arabica*. P1 to P6: analysis points. *points already measured

Figure 3. Field trial (Embrapa Cerrados-Planaltina-DF) with plantlets of *C. arabica* (date 08/05/2008).

Figure 4. Tissues (leaves, stems, roots and meristems) are collected for anatomical and molecular analyses (date 07/05/2008).

Figure 5. Plant excavation for biomass and architectural analyses (date 08/05/2008).

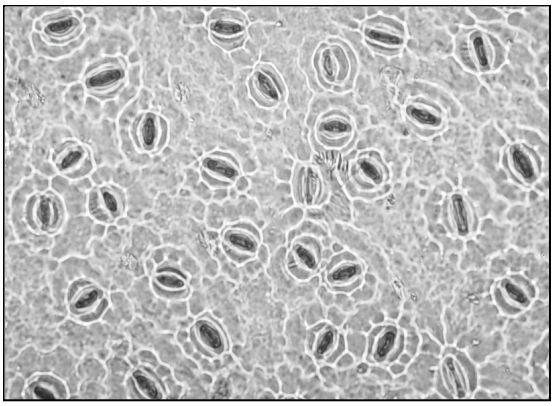


Figure 6. Determination of leaf stomatal density by optical microscopy (x 40).

PARTENSHIPS

EMBRAPA Recursos Genéticos e Biotecnologia
EMBRAPA Cerrados
Cirad UMR DAP
Cirad UMR AMAP
Cirad UPR 80
INRA UMR BIOGECO
University of Bordeaux

This work was supported by cirad (atp 2007/01) and inra.

Prospection of Tissue Specific Promoters in Coffee

J.D. DE ALMEIDA[1], L.M.G. BARROS[1], D.B.M. SANTOS[1], M.G. COTTA[1], E.A. BARBOSA[1], S.B. CAÇÃO[3], M.T.S. EIRA[4], G.S.C. ALVES[1], F. VINECKY[1], L.F.P. PEREIRA[4], F.R. DA SILVA, A.C. ANDRADE[1], P. MARRACCINI[1,2], M. CARNEIRO[1]

[1]Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, BR
[2]Cirad UMR-DAP, Montpellier, FR
[3]IAPAR, Londrina PR Brasil
[4]Embrapa Café Brasília, DF, Brasil

SUMMARY

The majority of transgenic organisms reported in the literature have been made using constitutive promoters. However, there are economic, environmental and biosecurity related restrictions involving indiscriminate (constitutive) expression of heterologous genes. The usage of tissue-specific and induced promoters can resolve those issues by limiting the expression of a transgene to the necessary tissues and conditions. The promoters currently used at Embrapa are transnational properties, burdening the research and causing technological dependence. Therefore, the objective of this work was to find and characterize tissue and organ specific promoter in Coffea spp. We have used the Coffee genome database *in silico* tools to find genes preferentially expressed in root, leaf and fruit. In this way we found 72 organ-specific candidates: 18 apparently preferentially expressed on leaves, 14 on roots and 40 on fruits. Some of those candidates were tested *in vitro* using RT-PCR, semi-quantitative PCR, northern blotting and qPCR assays. All four leaf-specific candidates tested (GCFo1, GCFo2, GCFo3 and GCFo4) and at least one of the two fruit-specific candidates tested (GCFr1 e GCFr2) where confirmed to be preferentially expressed on their respective organs. Temporal and spatial expression assays showed that GCFr2 has its expression peak at the endosperm, 180 days after flowering. The highest expressed genes of leaf (GCFo3 and GCFo4) and fruit (GCFr2) were used as probes to isolate its respective promoter through a BAC libraries screening or using the Genome Walker Universal Kit (Clontech). Results concerning gene expression and the molecular characterization of these genes will be presented.

INTRODUCTION

Coffee culture is facing several problems that can drastically compromise its production in Brazil. In this scenario, the Brazilian Coffee Genome Project had the intention to supply information on coffee genome aiming at developing improved varieties. Once *Coffea arabica*, the most important variety, is perennial, tetraploid and has low genetic variability, the transgenia appears like a good way to obtain plants more suitable to different purposes. In fact, there are some established protocols of coffee transformation especially in *C. canephora* (Ribas et al., 2006) and promising experiences with *C. arabica* (Alpizar et al., 2008). Despite this, transgenics in general undergoes a lot of restrictions in part because of the indiscriminated expression of the transgene guided by constitutive promoters like 35S. The transgene expression in an organ-specific or condition specific manner is required for plant molecular breeding and could be addressed using adequate promoters (Wally et al, 2008 and Marraccini et al., 2002). With the aim to identify organ specific genes (leaf, roots and fruit)

that could be used as a probe to isolate its respective promoters, we performed an *in silico* analysis using the data basis of the Coffee Genome Project (Vieira et al., 2006). Our electronic northern has identified 18 leaf genes, 14 roots genes and 40 fruit genes. Some of these genes were assayed through RT-PCR, RT qPCR and northern blot approaches. In this way we confirm the organ preferentially expression of 4 genes of leaf, here named GCFo1, GCFo2, GCFo3 and GCFo4 and 1 gene of fruit, the GCFr2. Nowadays we are isolating its respective promoters to test then in model plants and, in the future, in coffee.

MATERIALS AND METHODS

In silico analysis

Based on the UniGene coffee databank (Vieira et al., 2006), ESTs libraries of only one tissue (leave, root or fruit) were grouped and contrasted against another group containing all the other libraries through an Accurate Fisher Test. The genes witch presented preferential expression in roots, leaves or fruit were selected. Among these were chosen those stronger and unpublished.

Expression analysis

Northern blot

Total RNA from *Coffea arabica* root, leaf and fruit were hybridized in SSPE to probes corresponding to partial sequence of the candidate genes labeled with ^{32}P dCTP.

RT Real Time PCR

RNA from root, leaf and fruit was treated with DNase and converted to cDNA. The PCR reaction was prepared using SYBR Green and specific primers and performed in a 7500 PCR systems (Applied Biosystems) as described by Bustin (2002). Normalization was done against ubiquitin gene.

Promoter isolation

Genome Walker approach

Specific reverse primers were designed at the 5´ sequence of the leaf candidates to amplify the 5´ promoter region from genomic DNA template by genome-walking method (Genome Walker Universal Kit, Clontech) following manufacturers protocols.

Screening of BAC libraries

Nitrocellulose membranes containing 18.432 genomic fragments of high molecular weight cloned in BACs were hybridized with the GCFr1 probe. Positive clone was digested with several restriction enzymes, subcloned and sequenced for promoter region isolation.

The promoters sequences obtained through the strategies described above were analyzed in PLACE database (http://www.dna.affrc.go.Jp/htdocs/PLACE/, Higo et al., 1999) and PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantCARE, Prestridge,1991) for identification of cis-elements

RESULTS AND DISCUSSION

The electronic northern blot analysis has revealed 103 UniGene tissue-specific. Among these, 18 refer to leaves, 40 to fruits, 14 to root and 31 to floral buttons. Those, 84% are homologous to known sequences and 16% are unknown. The first step to confirm the tissue specificity expression of candidate genes identified in the virtual analysis was a qualitative RT-PCR study, performed with total RNA samples extracted from leaves, roots and fruit and converted to cDNA. RT-PCR results showed that GCFo1, GCFo2, GCFo3 and GCFo4 transcripts are detected preferentially in leaves and GCFr1 was detected preferentially in fruit (Figure 1).

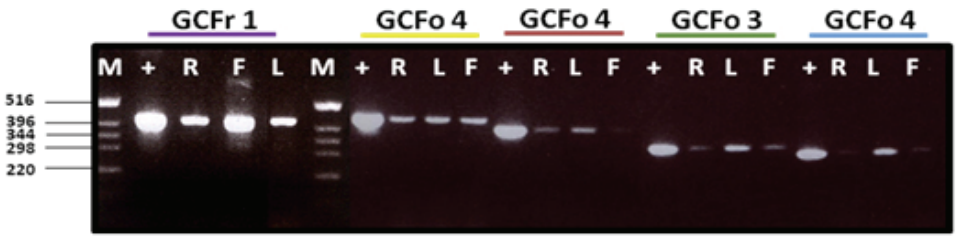


Figure 1. RT-PCR analysis for GCFo1, GCFo2, GCFo3 and GCFo4. Templates used in the reactions: (+) positive control, (R) root cDNA; (L) leaf cDNA; (F) fruit cDNA.

A quantitative analysis was then performed in order to access the expression ratio of these genes at each tissue. Data presented here are based in a relative quantification in comparison with the constitutive ubiquitine gene. The preferential expression in the target tissue was confirmed (Figure 2).

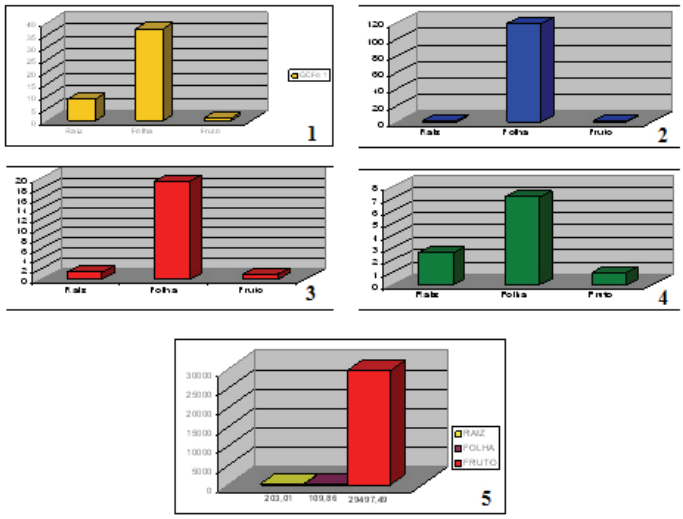


Figure 2. RT Real Time PCR relative analysis. GCFo1 (1), GCFo2 (2) GCFo3 (3), GCFo4 (4) and GCFr1 (5). Ubiquitine gene has been used as a constitutive control.

For the initial promoters isolation experiments the GCFo4 in the case of leaves and the GCFr1 in the case of fruit were chosen. The criteria adopted were: high expression level, the ineditism and the tissue specificity trait. In order to isolate these 2 promoters, two different approaches were used: genome walker method (GCFo4) and screening of BAC libraries (GCFr1) (Figure 3). The physical clones were obtained, sequenced and analyzed (Figure 4). In the case of GCFo4 promoter, sequence analysis by the PlantCare and PLACE showed the basic cis elements like TATA box and CAT box and, beside, putative elements responsive to

dehydration stress (ABRELATERD1) (Simpson et al., 2003), recognition sequence of *Arabidopsis* Athb-1 protein, which is characterized by the presence of a homeodomain (HD) with a closely linked leucine zipper motif (Zip) (CAATWATTG) (Sessa et al., 2003).

Our results show that the suggested strategy to isolate specific promoters is efficient. Expression assays *in vivo* are necessary to confirm the tissue specificity of the isolated promoters.

Once validated these promoters could be very useful to obtain transgenics to different conditions like drought, pathogen resistance and cup quality. These promoters could be assayed in other species to evaluate its performance in a universal way. The development of new promoters with tissue specific expression patterns is important for the future development of crops.

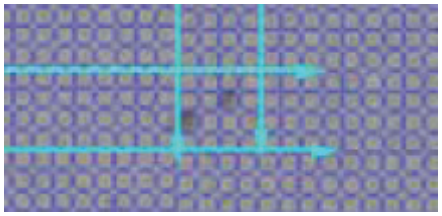


Figure 3. BAC libraries screening through hybridization with GCFr1 probe. Positive clones are delimited by green arrows.

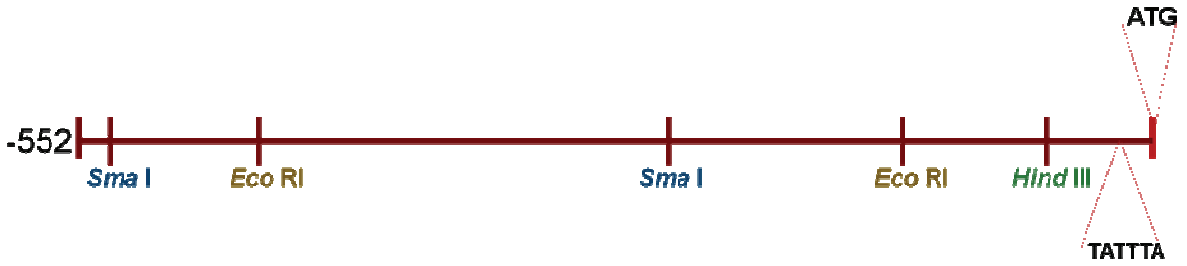


Figure 4. GCFo4 promoter restriction map.

REFERENCES

Alpizar E. et al. (2008). Annals of Botany; 101: 929-940.

Marraccini P. et al. (2003). Plant Physiology and Biochemistry; 41:17-25.

Ribas A.F. et al. (2006). Brazilian Arquives of Biology and Technology; 49 (1): 11-19.

Sales R.M.O.B. et al. (2005). In: Anais do IV Simpósio de Pesquisa dos Cafés do Brasil. Londrina, Brasil. CD-ROM.

Sales R.M.O.B. and Silva F.R. (2005). In: Anais do X Encontro do Talento Estudantil da Embrapa Recursos Genéticos e Biotecnologia. Brasília-DF.

Sessa G., Morelli G., Ruberti I. (1993). EMBO Journal;12(9):3507-17.

Simpson S.D. et al. (2003). Plant Journal 33(2):259-70.

Vieira L.G.E. et al. (2006) Brazilian Journal of Plant Physiology; 18: 95-108.

Wally O. et al. (2008). Plant Cell Report; 27: 279-287.

Potential Utilization of Functional Molecular Markers as Predictors for Partial Resistance to Leaf Rust (*Hemileia vastatrix* Berk. & Br.) in Coffee (*Coffea arabica* L.)

J.C. HERRERA*, G. ROMERO, G. DE LA TORRE, G. ALVARADO

Centro Nacional de Investigaciones de Café, CENICAFE. AP 2427 Manizales (Caldas), Colombia. *E-mail: juanc.herrera@cafedecolombia.com

SUMMARY

Genetic markers in plants represent an useful tool, not only for gene isolation but also for marker-assisted introgression of favorable alleles and for variety protection. While random DNA markers (RM) are derived at random from polymorphic sites in the genome, functional markers (FM) are derived from polymorphic sites within genes causally involved in phenotypic trait variation. Systematic analysis of plant resistance or defense genes in linkage disequilibrium with resistance, may allow identification of suitable targets for marker-assisted selection, cloning or both. Therefore, the challenge of FM development is to associate sequence polymorphisms with phenotypic variation. In this report we describe an strategy to develop FM markers linked to partial resistance to leaf rust in coffee. Markers were detected by using: (i) primer pairs amplifying conserved domains of resistance genes (RG) involved on initial recognition of pathogens, (ii) primers for defense response genes (DR), and (iii) primers for genes encoding transcriptional regulatory factors. Markers were screened by using a well characterized population derived from a cross between the susceptible variety Caturra and the resistant introgressed line DI.200, issued from the Timor Hybrid as resistant source. The parents as well as the most resistant and susceptible individuals of the population were analyzed. A preliminary screening by using genomic DNA from both the susceptible and the resistant parents allowed identification of 40 different genomic coffee sequences exhibiting strong nucleotide homology to either RG or DR well-known genes. Based on the similarities of their aminoacid sequences, seven RG candidate families were identified (i.e. fm1 to fm7). Deduced amino acid sequences indicated that six of the seven families showed strong sequence similarity with previously described R-genes of the NBS-non-TIR class. Further, a new family (i.e. fm1) belonging to the TIR-class of R-genes was for the first time described in coffee. An additional group of sequences exhibited strong aminoacid homology with pathogenic related proteins (PR) of the PR5 class, involved in antifungal activity. Two of PCR products derived from amplification of either LRR or NBS regions in candidate genes, exhibited clear polymorphism between the resistant and the susceptible parents. Furthermore, BLAST comparisons with coffee databases allowed the identification of almost five expressed sequence tags (ESTs) clones, three of which were previously identified in *C. canephora* and only present in the resistant parent DI.200. These results represent a preliminary approach towards the identification of functional markers linked to partial leaf rust resistance in coffee.

INTRODUCTION

Genetic resistance and particularly a combination of specific and non-specific resistances, has been advocated by coffee researchers as the most effective alternative for rust control. However, progress in breeding has been hampered by a lack of effective screening methods, the diversity of the rust fungus (*H. vastatrix*) and the limited information on the inheritance

and adequate components of resistance. Implementation of a marker assisted selection strategy in coffee has great promise to drastically increase the efficiency of breeding programs, especially for complex traits such as partial resistance. Lately, some molecular markers linked to major specific resistance locus (i.e. *S_H3, Mex1, Ck1*) have been identified in coffee. Nevertheless, no reports on the development of molecular markers for quantitative traits, like partial rust resistance, are available so far in this species.

Genetic markers in plants represent an useful tool, not only for gene isolation but also for marker-assisted introgression of favorable alleles. While random DNA markers are derived at random from polymorphic sites in the genome, functional markers (Andersen and Lubberstedt, 2003) are derived from polymorphic sites within genes causally involved in phenotypic trait variation. Therefore, functional markers seems to be more useful for precise identification and localization of genes conditioning quantitative resistance (QTLs) and for rapid identification of suitable targets for marker-assisted selection, cloning or both. The candidate gene approach is based on the hypothesis that known-function genes (the candidate genes) could correspond to loci controlling traits of interest. This strategy has been applied to co-localizing candidate genes with disease resistance QTLs in several pathosystems. Candidate genes involved in defense responses can be classified as: (i) resistance genes (RG), involved in the initial recognition, (ii) defense response genes (DG), that allow pathogen inhibition, and (iii) regulatory genes which regulate the coordinate expression during plant defense response. Using the candidate gene approach, both the RG as well as the DG genes have been found associated with QTL regions conditioning resistance to different diseases caused by fungus, bacteria and viruses (Lashermes et al., 1997; Pflieger et al., 2001).

The aim of this work is to develop gene-derived markers tightly linked to partial resistance to leaf rust in coffee, towards an strategy of marker assisted selection. The hypothese that we want to test with this strategy is that genetic markers derived from candidate resistance or defense response genes are adequate predictors of partial resistance in coffee.

MATERIAL AND METHODS

Plant materials

For marker screening a F2 population derived from a cross between the susceptible variety Caturra and the *C. arabica* introgressed line DI.200, as resistant parent, was studied (Figure 1). The DI.200 parent corresponds to a well-characterized F_4 inbred line, which exhibits a high level of partial resistance to coffee leaf rust (*H. vastatrix*). This line was derived by pedigree selection from a cross between the *C. arabica* cv. Caturra and the CIFC1343 accession of the Timor Hybrid (Alvarado et al., 2002). The parents as well as the most resistant and susceptible individuals of the population were analyzed. Additional accessions of the Timor hybrid (CIFC 1343) and the *C. canephora* and *C. liberica* species, were also included as controls.

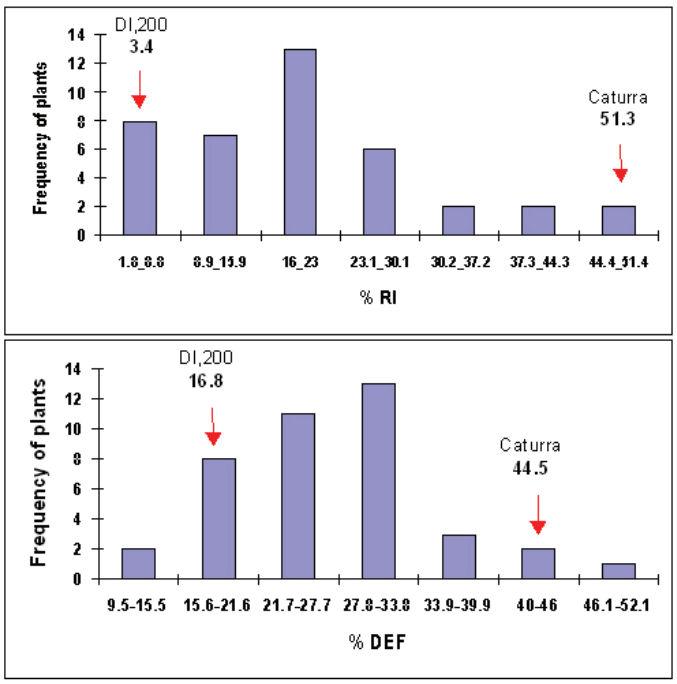


Figure 1. Frequency distribution of rust resistance estimated as the percentage of rust incidence, RI (above) and desfoliation, DEF (below) in adult coffee plants in field. Arrowz indicate the mean values for parental genotypes.

Evaluation of disease progress

Partial resistance was evaluated in the field y measurement of percentage of rust incidence (RI) and defoliation (DEF) due to rust. Information of disease progress was collected during three consecutive years (2003-2005).

Identification of resistance and defense gene analogous

Genomic DNA from the susceptible var. Caturra (P1) and the resistant line DI.200 (P2) were obtained from fresh leaves. A total set of sixteen primer combinations including degenerate as well as non-degenerate primers, were evaluated. All primers used in this study were previously designed based on conserved motifs and domains in the aligned amino acid sequences derived from known resistance and defense genes. In order to improve PCR amplification, specific conditions were adjusted to each primer combination. Amplified fragments PCR products were separated on a 1.5% (w/v) low melting agarose gel. Bands having the expected size were excised from the gel and cloned into the pGEM-T vector system I (Promega). For each transformation event, eight to ten white colonies were selected for further sequencing. After purification, inserts having appropriate size were sequenced in a single direction using universal primer SP6. Sequences obtained were edited manually to further verify the sequence and, using Codon code Aligner v.1.2.3. software, to remove the primer and vector sequences. Amino acid sequences were searched for their similarity to either resistance or defense related genes cloned in plants (including coffee) by using the BLASTX algorithm. Only the amino acid sequences without interruption and exhibiting intact open reading frame (ORF) were considered for multiple sequence alignments. Multiple-sequence alignments were performed using ClustalW 1.83 software. Sequences that were at least 50% identical were considered to be potentially members of the same cluster. Original sequences reported here have been assigned the following GeneBank accession: EU196026 to EU196037. A phylogenetic tree was constructed using the PAUP package version 4.0b10 for

Unix. The neighbor-joining method was employed to evaluate the reliability of each branch of the tree by using 10000 bootstrap sample steps. Phylogenetic relationships between amino acid sequences obtained in this work and cloned gene products of selected resistance genes in coffee as well as in other plant species, like tobacco, flax, Arabidopsis and tomato, were investigated. Original sequences of these genes were obtained from GenBank.

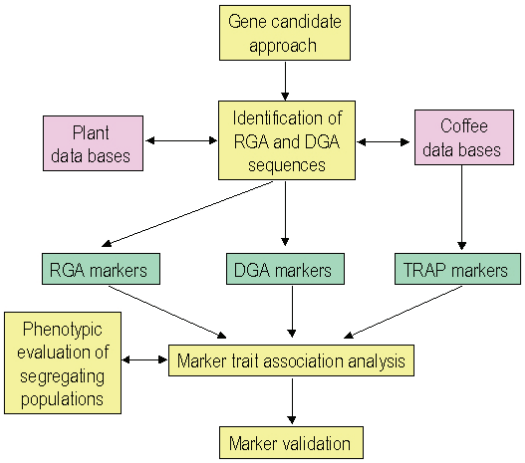
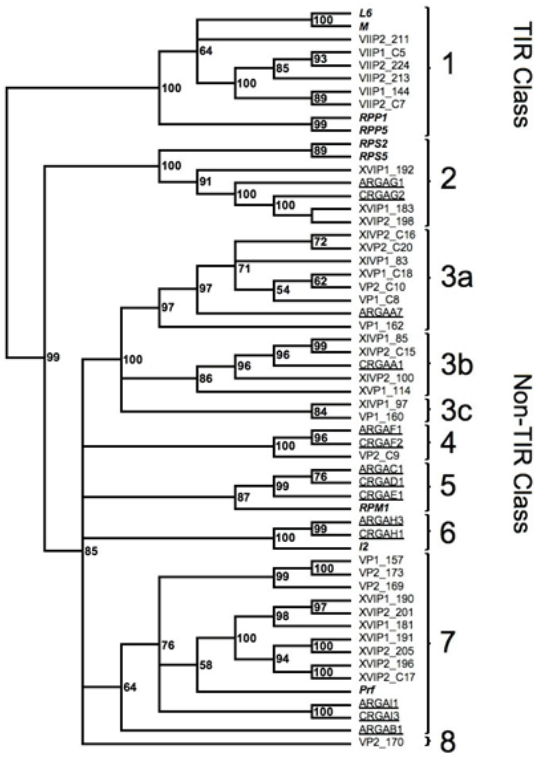


Figure 2. Flowchart of a general strategy for development of functional-markers linked to resistance leaf rust in coffee.

Marker identification was carried out by using different approaches (Figure 2) involving PCR amplification of: (i) conserved domains of candidate resistance genes (i.e. resistance gene analog polymorphism, RGAP), (ii) conserved domains of candidate defense response genes (DR), and (iii) genomic regions around target candidate gene sequences (i.e. target region amplification polymorphism, TRAP). The RGAP markers were obtained using degenerate primers reported by Mutlu et al. (2006) and designed from highly conserved motifs, kinase-1a (K) and hydrophobic domain (HD) of NBS-LRR type resistance genes. In order to target the gene family, the NBS-LRR sequences were obtained from known R-genes and EST data bases of dicots. In this work, a total of 100 primer combinations were tested in order to identify RGAP polymorphism between the two parents (Caturra and DI.200). The TRAP markers were developed according to Hu and Vick (2003). In short, this technique uses 2 primers to generate markers. One of the primers, the fixed primer, is designed from a target EST sequence in the database; the second primer, the arbitrary primer, is an arbitrary sequence with either an AT- or GC- rich core to anneal with intron or exon, respectively. The candidate RG and DG sequences isolated from the resistant line DI.200 were used as target for identification of coffee ESTs presents in the CENICAFE database throughout multiple-sequence alignment. In order to visualize the different markers, PCR products derived from the different strategies were separated on either agarose or denaturing polyacrylamide (6%) gels.

RESULTS AND DISCUSSION

In this report we present the initial results of a strategy towards the development of functional molecular markers based on putative candidate genes involved on partial resistance response to the coffee leaf rust. Candidate gene sequences were isolated and then used as source for molecular marker development.



Fugure 3. Neighbor-joining tree based on an alignment of amino acid sequences from the resistant parental line DI 200 and the NBS domains of previously characterized R genes from: coffee (Noir et al., 2001, underlined) and other plant species (italic bold). RGA clusters (subfamilies) are separated by brackets. Labels from 1 to 8 correspond to subfamilies of closely related RGA sequences. The numbers on the branches indicate bootstrap values (10000 replications).

Identification and isolation of RG and DR analogous sequences

A total of 137 clones were sequenced but only 98 were selected for further analysis. Sequence analysis of these 98 sequences revealed that 40 of them showed an uninterrupted ORF (open reading frame). As observed in Figure 3 the RGC sequences were grouped into 8 clusters or subfamilies (1-8), all of which were highly supported by bootstrap values. The number of identified amino acid sequences for each subfamily ranged from 1 to 10, except for subfamilies 5 and 6 where no representative sequences were isolated. Defined subfamilies were separated into two distinct groups. The first group contains six predicted amino acid sequences analogous to the NBS-TIR proteins L6, M, RPP1 and RPP5. These sequences represent the first NBS-TIR RGC proteins reported in coffee. The second group includes predicted amino acid sequences analogous to NBS no-TIR RGC proteins previously identified in coffee (Noir S. et al., 2001) as well as other known no-TIR NBS-RGC plant proteins. Among the no-TIR NBS subfamilies identified, the subfamily 3 appears more diverse with three different subgroups (3a, 3b and 3c). An additional group of sequences, exhibiting strong amino acid homology with pathogenic related proteins (PR) were also found. Interestingly the PR proteins and particularly the PR5 class has been involved in anti-fungal activity. Although the DI.200 line exhibits a low level of introgression compared with similar lines introgressed by the Timor hybrid, it might be possible that the RGCs isolated here represent only a subset of the NBS-TIR and no-TIR sequences present in this line. Nevertheless, the candidate gene approach showed to be particularly productive for tagging a wide range of resistance and defense genes in coffee.

Detection of useful polymorphism associated to rust resistance

RGA and DGA derived makers

Although RGAP markers derived from RGA sequences showed low polymorphism, some combinations exhibited clear polymorphic bans between resistant and susceptible individuals. Similarly, among the DGA homologous sequences isolated from coffee, one showing elevated homology with the *PR3* gene, a chitinase type gene from rice, exhibited clear polymorphic bands in resistant genotypes including the DI.200 parent, some Timor hybrid accessions, and some F1 individuals. This kind of markers are interesting because they are less expensive and faster than other PCR- based markers. Indeed, they do not require cloning and its products could be separated on a 1.2% agarose gel.

TRAP markers

Table 1. Annotation of some coffee EST clones homologous of the RGA sequences isolated in this work.

RGA cluster (subfamily) [a]	EST accession [b]	EST annotation	Identity	e-value
3b	Cl_CEN82239	Disease resistance-like protein - *Coffea arabica* (Coffee)	80	3.00E-41
3b	Ca_CEN114972	Disease resistance-like protein - *Coffea arabica* (Coffee)	81	8.00E-11
3a	Cl_CEN82239	Disease resistance-like protein - *Coffea arabica* (Coffee)	98	0
3a	Cl_CEN82239	Disease resistance-like protein - *Coffea arabica* (Coffee)	98	0
4	Ca_CEN135208	Disease resistance-like protein - *Psilanthus bengalensis*	86	3.00E-78
4	Ca_CEN132890	Putative disease resistance protein At1g50180 - *Arabidopsis thaliana*	92	3.00E-32
3a	Cl_CEN82239	Disease resistance-like protein - *Coffea arabica* (Coffee)	97	0
3a	Cl_CEN82239	Disease resistance-like protein - *Coffea arabica* (Coffee)	96	1.00E-155
3b	Cl_CEN82239	Disease resistance-like protein - *Coffea arabica* (Coffee)	87	2.00E-81
3b	Ca_CEN139668	Disease resistance-like protein - *Coffea canephora* (Robusta coffee)	82	3.00E-13
3b	Cl_CEN82239	Disease resistance-like protein - *Coffea arabica* (Coffee)	86	1.00E-15
3b	Ca_CEN129533	Disease resistance-like protein - *Psilanthus travancorensis*	79	1.00E-09
3b	Cl_CEN82239	Disease resistance-like protein - *Coffea arabica* (Coffee)	87	2.00E-81
3b	Ca_CEN139668	Disease resistance-like protein - *Coffea canephora* (Robusta coffee)	82	3.00E-13
3a	Cl_CEN82239	Disease resistance-like protein - *Coffea arabica* (Coffee)	97	1.00E-162
3b	Cl_CEN82239	Disease resistance-like protein - *Coffea arabica* (Coffee)	87	5.00E-27
3b	Ca_CEN139668	Disease resistance-like protein - *Coffea canephora* (Robusta coffee)	82	3.00E-13
3b	Cl_CEN82239	Disease resistance-like protein - *Coffea arabica* (Coffee)	90	1.00E-122
3b	Ca_CEN129533	Disease resistance-like protein - *Psilanthus travancorensis*	83	9.00E-17
3a	Cl_CEN82239	Disease resistance-like protein - *Coffea arabica* (Coffee)	98	0
3a	Cl_CEN82239	Disease resistance-like protein - *Coffea arabica* (Coffee)	98	0
7	Ca_CEN138533	Late blight resistance protein, putative - *Solanum demissum* (Wild potato)	93	1.00E-103
7	Cl_CEN79387	Disease resistance protein, putative - *Solanum demissum* (Wild potato)	97	2.00E-08
7	Ca_CEN132443	P0431G06.4-like - *Solanum tuberosum* (Potato)	96	5.00E-06
2	Ca_CEN136771	NBS-LRR type disease resistance protein - *Populus trichocarpa* (poplar)	87	3.00E-35
7	Ca_CEN138533	Late blight resistance protein, putative - *Solanum demissum* (Wild potato)	92	4.00E-99
7	Cl_CEN79387	Disease resistance protein, putative - *Solanum demissum* (Wild potato)	94	5.00E-09

[a] *Number correspond to subfamilies of closely related RGA sequences identified in the DI200 parent (Figure 3).*

[b] *Accession number of ES Ts retrieved in the CENICAFE data base. Cl, C. liberica, Ca, C. arabica, and Cc, C. canephora.*

Multiple sequence comparisons between RG sequences and coffee databases allowed the identification of ESTs which were derived not only from the *C. arabica* species, but also from the *C. canephora* and *C. liberica* diploid species (Table 1). Recovered EST sequences were used for construction of the specific forward and reverse TRAP primers. To date a total of 49 combinations of TRAP markers have been evaluated. Among reproducible markers, a number of ten markers (20.4%) have showed clear polymorphism associated with related genotypes exhibiting rust resistance (Figure 4). TRAP markers have several advantages over anonymous

markers. Indeed, it have been observed that TRAP markers results in a moderate number of codominant loci, exhibiting similar profiles for different populations. Further, a similar number of reproducible bands are observed when the same DNA samples are run in independent experiments. In conclusion, a new strategy for identification of functional markers in coffee is presented. To date different types of PCR–based markers have been developed, including RGAP, DGA and TRAP markers which target coding sequences in the genome. Overall, our results represent a preliminary approach towards the identification of functional markers linked to partial leaf rust resistance in coffee.

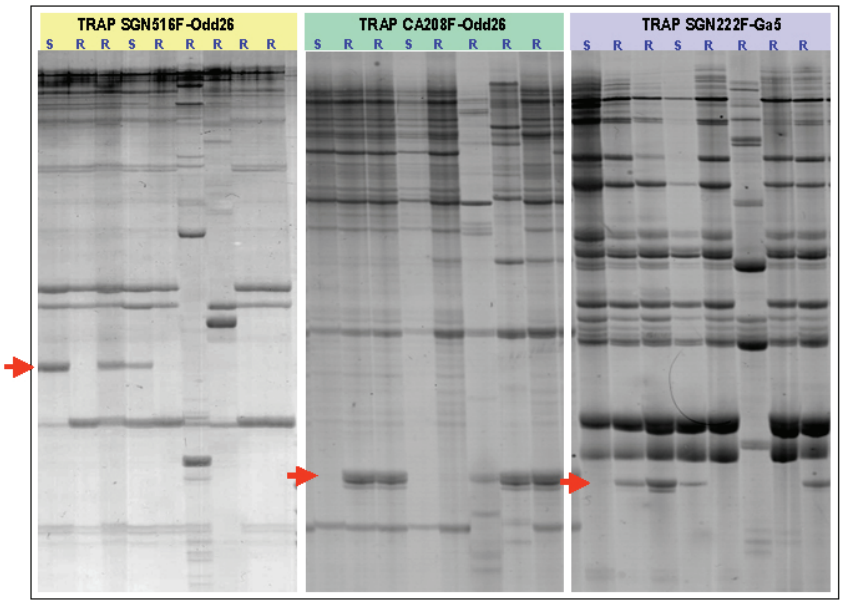


Figure 4. Representative molecular profiles obtained by using the TRAP primers. R, restistant; S, susceptible genotypes.

REFERENCES

Alvarado G. et al., (2002) Caracteres agronómicos y resistencia incompleta a *Hemileia vastatrix* en progenies de Caturra x híbrido de Timor. Cenicafé, 53: 7-24.

Andersen Jr. and Lubberstedt T. (2003) Functional markers in plants. Trends Plant Sci., 8: 554-560.

Hu J. and Vick B.A. (2003) Target Region Amplification Polymorphism: A novel marker technique for plant genotyping. Plant Mol Biol. Reporter, 21: 289-294.

Lashermes et al., (1997) Molecular marker-assisted selection: a powerful approach for coffee improvement. XVII Colloque ASIC. Nairobi, Kenya.

Mutlu N. et al., (2006) Resistance gene analog polymorphism (RGAP) markers co-localize with disease resistance genes and QTL in common bean. Mol Breed., 17: 127-135.

Noir S. et al., (2001) Origin, diversity and evolution of NBS-type disease-resistance gene homologues in coffee trees (*Coffea* L.) Mol Genet Genomics, 265: 654-662.

Pflieger S. et al., (2001) The candidate gene approach in plant genetics: a review. Mol Breed., 7: 275-291.

Transcript Profiles in Compatible and Incompatible Host-Coffee Leaf Rust Interactions

F. CARDENAS[1], N. GALEANO[1], K. O'BRIEN[2], M. YEPES[3], M. GEOFFROY[4], R. BUELL[4], A. GAITAN[1]

[1]Colombian National Coffee Research Center (CENICAFE), Chinchiná, Caldas, Colombia
[2]School of Medicine University of Maryland, Baltimore, MD, USA
[3]Department of Plant Pathology, Cornell University, Geneva, NY, USA
[4]Department of Biology, Michigan State University, MI, USA. Research Co-sponsored by the Colombian Coffee Growers Federation, the Colombian Ministry of Agriculture, and the US National Science Foundation Developing Country Collaborations

SUMMARY

Coffee leaf rust caused by *Hemileia vastatrix* continues to be a limiting disease around the world, including in the Americas since its arrival from Africa in 1970. Complete rust resistance bred in commercially available cultivars is currently being lost to new pathogen races, although a background of strong incomplete resistance persists on some derivatives of the Timor hybrid (*Coffea arabica* x *C. canephora*). A better understanding of plant host responses to coffee rust at the molecular level is essential to obtain durable resistance to control the disease. The main objective of this study was to perform a global transcriptome profiling of rust interactions with resistant and susceptible genotypes. A spotted cDNA microarray was constructed at TIGR containing a 36,480 probe set from normalized libraries from *C. arabica* and *C. liberica*, representing 21,373 unigenes. For the genotypes *C. arabica* var Caturra (susceptible) and Timor Hybrid (resistant), mRNAs were collected from leaves of 6-month old plantlets after 30, 70 or 120 hours of inoculation with uredospores of *H. vastatrix*. Control leaves were sprayed with water, and for each treatment 5 biological replicates were included. Microarray hybridizations revealed 1,644 clones associated with the host pathogen interaction that consistently shifted their expression in all of the experiments. Of those, 715 corresponded to sequences with significant homology to previously annotated genes. Challenge with rust uredospores upregulated expression of 232 genes in the resistant genotype, 343 in the susceptible, and 241 in both, although delayed for Caturra. In contrast, rust inoculation induced the downregulation of 350 genes in Timor hybrid, 241 in Caturra and 237 in both. From the annotated genes, the main metabolic pathway activated involved in plant defense was ROS (Reactive Oxygen Species), and several changes occurred related to cell wall and cytoskeleton metabolism, as well as in sugar transferases and invertases, and protein degradation, probably required to adjust plant metabolism to fungal nutrition. These pathways are important candidate targets to provide race independent resistance to coffee rust.

INTRODUCTION

Coffee breeding programs around the world have been introgressing rust resistance mainly from accessions of the Timor Hybrid. The complete resistance exhibited by progenies of this parental during more than 15 years is being lost against new races of the pathogen, although a strong background of incomplete resistance remains in commercial varieties (Alvarado, 2004). A deeper knowledge of the resistance mechanisms is required in order to design better strategies leading to durable resistance under field conditions. This experiment is an approach to *Coffea* sp. genetic profiles elucidated during the interaction with *Hemiliea vastatrix* when

using three pos-inoculation times (30, 60 y 120). A cDNA microarray was constructed to evaluate more than 30,000 EST's and the Real Time PCR technique was used to corroborate expression of candidate genes.

MATERIALS AND METHODS

Planlets from *Coffea arabica* var. Caturra (compatible) and Timor Hybrid 1343 (incompatible) were kept in a greenhouse. A set of equally-aged plants was inoculated with a spore concentration of $4x10^7$ *H. vastatrix* genotype CU975 spores/ml (0.5 mg/ml) by spraying on the back side of first and second leaf pairs of both genotypes. Plants were maintained during 24 hours in the dark and at a 100% relative humidity. Control plants were sprayed with distilled water. The leaves were collected at different hours post-inoculation (30, 70, 120 hpi), frozen immediately in liquid nitrogen and macerated. Biological replicates were done at two-week intervals.

Total RNA was extracted from coffee leaves using the RNAeasy Plant Maxi Kit (Qiagen, USA) complemented with DNAse treatment. RNA samples were quantified using RiboGreen (INVITROGEN, USA) by means of a Turner Biosystems 380 fluorometer. On Corning glass slides, 37,632 plasmid minipreps were spoted, distributed as follows: 19,074 spots corresponded to a normalized library of *C. arabica* (5 tissue RNA mixture), 7,169 to a *C. liberica* normalized fruit library and 7,020 to *C. liberica* normalized leaf library, for a total of 33,000 effective spots. 3217 spots corresponded to sequences that were discarded out of the Transcript Assembly pipeline. Slides were scanned using an Axon 4000B scanner (Axon Instruments, Union City, CA), simultaneously for both channels (635nm and 532nm) at 100% laser power, at a 10 micron resolution. Images were saved in a non-compressed TIFF file format for both channels. For background correction and data normalization for each hybridization the raw intensities were loaded into the limma package of Bioconductor (www.bioconductor.org). Using PERL scripts and R software, statistical differences at 0.01% were found for the intensities of spots consistent among biological replicas.

For RT-PCR, first-strand cDNAs were synthesized from 300ng of the same RNA used to hybridize the microarrays in a final volume of 12,5 ul, using RT for PCR kit Advantage (CLONTECH) following the manufacturer's instructions. 10 specific gene primer-pairs were designed from the cDNA sequences of each gene to be analyzed using Oligoanalyzer and synthesized by IDT. The Ubiquitin gene (Fernandez et al., 2004) was used as an internal constitutively expressed control (reference gene). PCR reactions were performed in 20 ul with 2ul of cDNA, 2ul of each primer (1uM) and 14ul of DyNamo HS SYBR Green qPCR (FINNZYMES**).** Two technical replicates and three biological replicates of the PCR assay were used for each sample. Real-time quantitative PCR was conducted on a Chromo4 (BioRad). PCR cycles were as follows: 1 cycle for 10 min at 94 °C, followed by 40 cycles each of 15s at 96 °C , and 20s at 55 °C, and 1min at 72 °C. Absolute transcript levels were determined from standard curves, obtained using serial dilutions of samples containing know concentrations.

RESULTS AND DISCUSSION

Microarray hybridizations revealed 1,644 clones associated with the host pathogen interaction that consistently shifted their expression in all of the experiments. Of those, 715 corresponded to sequences with significant homology to previously annotated genes. Challenge with rust uredospores upregulated expression of 232 genes in the resistant genotype, 343 in the susceptible, and 241 in both, although delayed for Caturra. In contrast, rust inoculation

induced the downregulation of 350 genes in Timor hybrid, 241 in Caturra and 237 in both (Figure 1).

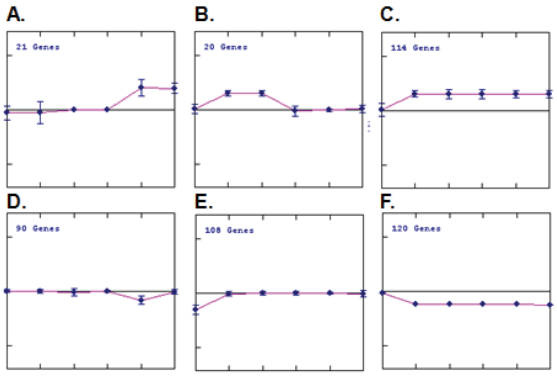


Figure 1. General expression Profiles in Caturra and Timor Hybrid 1343 through three different post-inoculation times. Each profile shows the ratio between rust and water inoculation, summarizing the unique expression patterns depending on the genotype and the post-inoculation time. From left to right: Caturra inoculated 30, 60 and 120 hpi, Timor Hybrid 1343 inoculated 30, 60 and 120 hpi.

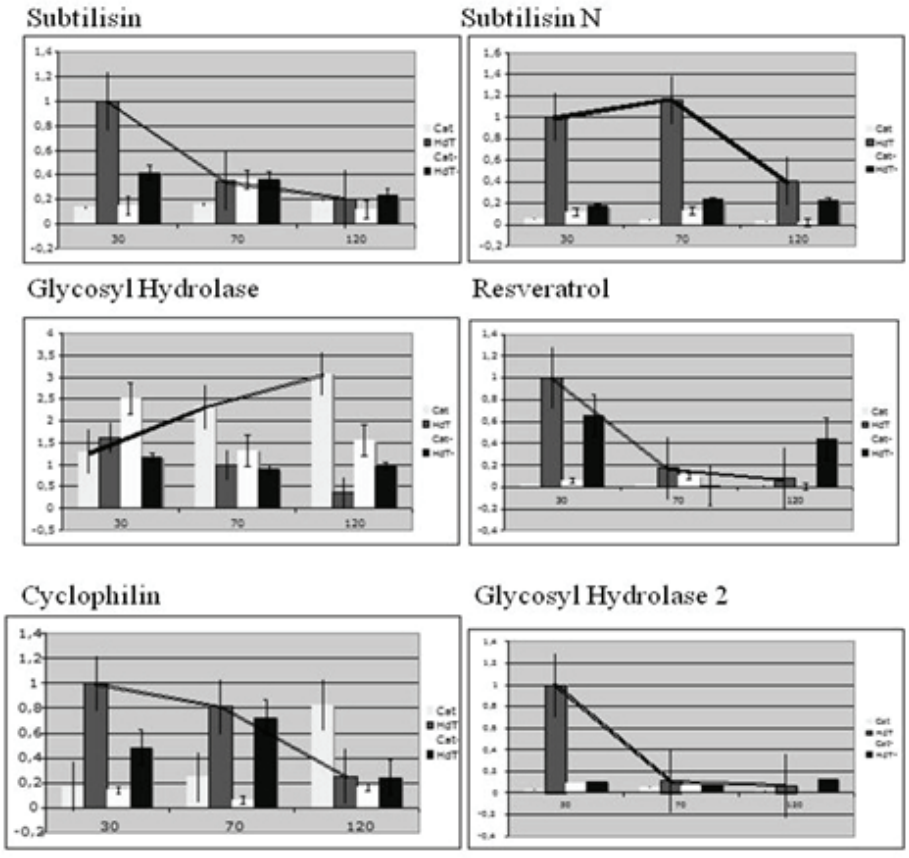


Figure 2. General expression Profiles in Caturra and Timor Hybrid 1343 through three different post-inoculation times. Each profile shows the ratio between rust and water inoculations, summarizing the unique expression patterns depending on the genotype and the post-inoculation time. From left to right: Caturra inoculated 30, 60 and 120 hpi, Timor Hybrid 1343 inoculated 30, 60 and 120 hpi.

The relative changes in gene expression are presented below. Six genes showed changes that were detected between control and inoculated plants at 30 and 60 hpi in HDT, however after that their expression returned to the basal levels. Glycosyl Hydrolase changed its expression from 30 to 120 hpi. Four out of ten genes did not show any expression. Quantitative RT-PCR confirmed that all genes tested presented similar relative expression on three biological replicates (Figure 2). The timing reflects cellular developments according to histological descriptions (Silva et al., 2002).

The main metabolic pathway activated involved in plant defense was ROS (Reactive Oxygen Species), and several changes occurred related to cell wall and cytoskeleton metabolism, as well as in sugar transferases and invertases, and protein degradation, suggesting mechanisms by which the fungus transports nutrients across cell wall structures in the haustorial complex similar to those observed in *Uromyces* (Voegele et al., 2003). The transcriptional changes probably reflect the necessary adjustments required by a biotrophic pathogen in order to adjust plant metabolism to fungal nutrition. These pathways are important candidate targets to provide race-independent resistance to coffee rust. Six out of ten genes showed the expected expression profiles at different hours and genotypes; indicating the multiple responses through several genes towards *H. vastatrix* at different periods. It is necessary to continue evaluating genes and corroborate the expression profiles observed in the microarrays.

REFERENCES

Alvarado G. Colombia, cultivar compuesto de café con resistencia durable a la roya - atributos actuales. Fitotecnia Colombiana 4:18-27. 2004.

Fernandez D., Santos P., Agostini C., Bon M., Petitot A., Silva M., Guerra-Guimarães L., Ribeiro A., Argout X., Nicole M. Coffee (Coffea arabica L.) genes early expressed during infection by the rust fungus (Hemileia vastatrix). Molecular Plant Pathology, 5: 527-536. 2004

Silva M., Nicole M., Guimaraes Jr. L., Rodrigues C. Hypersensitive cell death and post-haustorial defence responses arrest the orange rust (*Hemileia vastatrix*-race II) growth in resistant coffee leaves. Physiol. Mol. Plant Pathol. 60:169-183. 2002.

Voegele R.T. Mendgen K. Rust haustoria: nutrient uptake and beyond. New Phytologist, 159: 93-100. 2003

Differential Proteomic Analysis Indicates That Modulation in the Expression of Photosystem II Proteins May Be Involved in Differential Drought Response in *Coffea canephora* Genotypes

A.M. ALMEIDA[1*], B.L.S. GUIMARÃES[1], C.Q.G. SOARES[1], V.A. SILVA[1], M.A. VIEIRA[1], R.H. VALENTE[2], M.C. BARACAT[3], E.P.B. FONTES[3], M.E. LOUREIRO[1]

[1]Departamento de Biologia Vegetal, Universidade Federal de Viçosa, Viçosa – MG, Brazil.
[*]E-mail: amalmeida@ufv.br
[2]Laboratório de Toxinologia , Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro-RJ, Brazil
[3]Departamento de Bioquímica e Biologia Molecular, Universidade Federal de Viçosa, Viçosa, MG, Brazil

SUMMARY

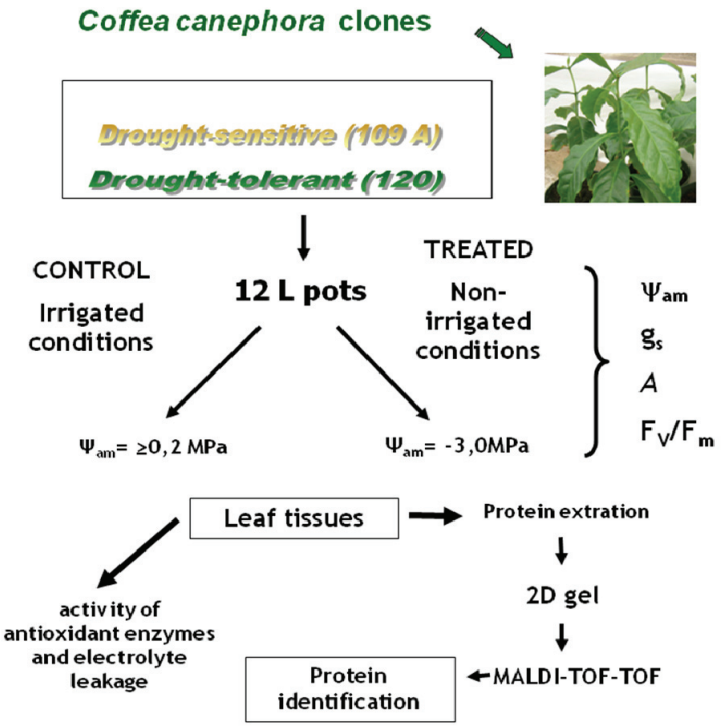
Severe drought stress in coffee is associated with sharp reduction in net photosynthetic rate (*A*), with no changes in electron transport rate (ETR) and non-photochemical *quenching*, which could potentially lead to increased formation of reactive oxygen species (ROS) and photoinhibition. However, in *Coffea sp*., no photoinhibition could be detected by chlorophyll fluorescence parameters. Damaged photosystem II (PSII) centers are usually subjected to repair, which include partial disassembly of the PSII, replacement of damaged D1 protein, reassembly and photoactivation of PSII. The aim of this work was the elucidation of the role of PSII repair in *Coffea canephora* drought response. It was observed detectable expression of PSII PsbO protein only in sensitive genotype under drought, while decreased expression of PS II PsbP was observed only in the tolerant genotype. PsbO plays an important role in PSII repair mechanism by regulating turnover of the D1 protein. The increased expression of PsbO correlates with higher level of oxidative damage and lower photosynthesis in the sensitive clone. These results suggest that increased PsbO protein levels reflect the degree of oxidative damage, being part of a drought acclimatation mechanism. PsbP plays an important role in the photoactivation of assembled functional PSII, being PSII activity linearly correlated with the amount of the PsbP protein. Since that PsbP protein levels decreased only in tolerant clone where lower oxidative damage was observed, we suggest that reduction in PSII activity could be part of a drought tolerance mechanism reducing oxidative damage under drought. The absence of changes in estimated ETR could be tentatively explained by associated increased levels of NADPH quinone oxidoreductase protein and a postulated increased cyclic electron flux between PSI and PSII.

INTRODUCTION

Photosystem II (PSII) is a large pigment-protein complex embedded in the thylacoid grana membranes that catalyses light-induced electron transfer from water to plastoquinone. The oxygen-evolving complex (OEC), located on the lumenal side of PSII is responsible for water oxidation and produces molecular oxygen as a by-product. The OEC consists of a manganese-calcium cluster and several extrinsic-proteins. Plant PSII presents 4 oxygen-evolving extrinsic proteins: PsbO, PsbP, PsbQ and PsbR. Damaged PSII centers are usually subjected to repair, which include partial disassembly of the PSII, replacement of damaged D1 protein,

reassembly and photoactivation of PSII. All these process are controlled by these oxygen-evolving extrinsic proteins. Severe drought stress in coffee is associated with sharp reduction in net photosynthetic rate (*A*), with no changes in electron transport rate and non-photochemical *quenching*. Thus, over-reduction of photosynthetic electron chain may result in production of reactive oxygen species (ROS) that can lead to photoinhibitory and photooxidative damage. However, in *Coffea sp*., no photoinhibition could be detected by chlorophyll fluorescence parameters. The aim of this work was the elucidation of the role of PSII repair in *Coffea canephora* drought response.

MATERIAL AND METHODS



Severe water deficit was imposed in plants of clone 120 (drought-tolerant) and 109A (drought-sensitive) and Leaf Ψ_{pd} (water potential at predawn) was measured using Scholander-Type pressure chamber. Net CO_2 assimilation (*A*), g_s (stomata conductance) was measured used IRGA (infrared gas analyzer) LCA-4, ADC, Hoddesdon. UK. Fluorescence parameters measured with Fluorometer (FMS2, Hansatech, King´s Lynn, Norfolk, UK). Leaf protein from three independent plants of each clone was extracted and analyzed in 2-D electrophoresis. Differential expressed proteins were excised from the gels, digested with trypsin and peptides were sequenced by mass spectrometry (MALDI-TOF-TOF).

RESULTS

Expression of PSII PsbO protein was observed only in sensible clone (109A) upon drought (Fig.1). This increased expression correlates with higher level of oxidative damage and lower photosynthesis in this genotype. As PsbO is related to D1 protein repair, increase in protein level may reflect the degree of oxidative damage, being part of a drought acclimatation mechanism in this genotype. Decreased expression of PS II PsbP protein was observed only in the tolerant genotype (Figure 1). PsbP plays an important role in photoactivation of

assembled functional PSII, being PSII activity linearly correlated with the amount of the PsbP protein. Data show that PsbP protein decrease only in tolerant 120 clone (Figure 1A e 1B) that showed lower oxidative damage, suggest that reduction in PSII activity could be part of a drought tolerance mechanism reducing oxidative damage under drought.

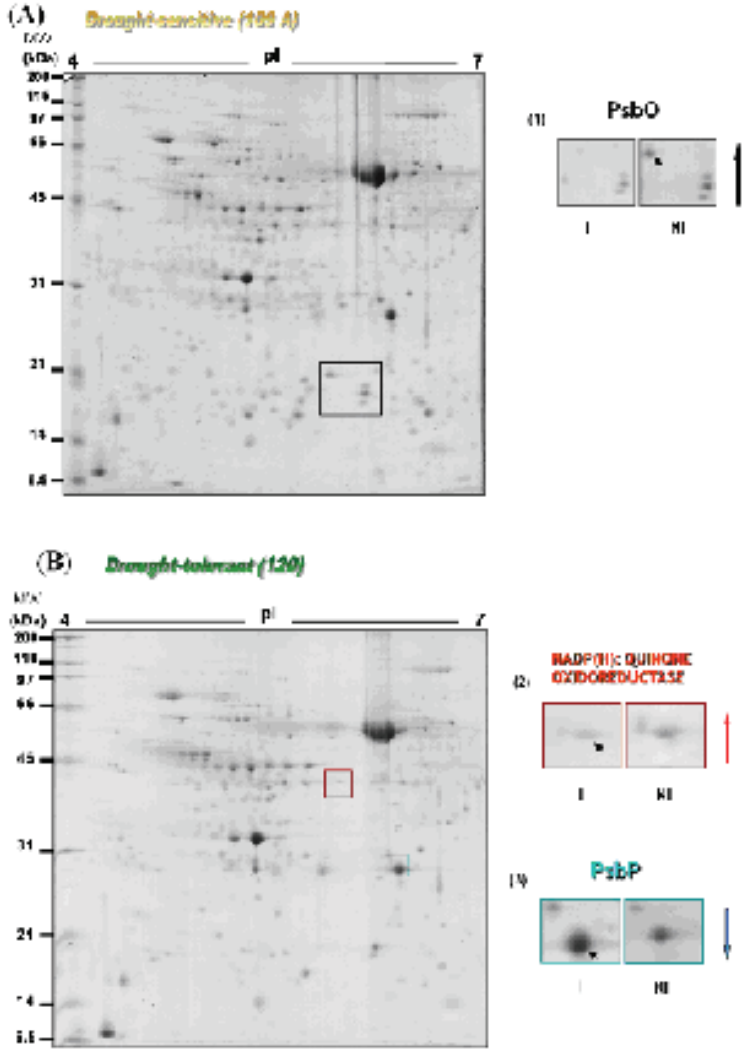


Figure 1. Changes in abundance of leaf proteins in clones of *Coffea canephora* submitted to water deficit (-3 MPa). Total leaf protein was separated by isoeletric focalization in immobilized pH 4-7, and subsequently separated by SDS-PAGE. Image analysis was performed by Image Master 2-D platinum. (A) gel containing protein from clone 109A leaves. Spot (1), PsbO (33kDa precursor protein of oxygen-evolving complex). (B) gel containing protein from clone 120 leaves; spots (2) NADP(H):quinone oxidoreductase, (3) PsbP (23kDa polypeptide of water-oxidizing complex of photosystem II). (I) Irrigated; (NI) Non-Irrigated.

Photosynthesis carbon assimilation (*A*) decrease upon drought stress on both correlates with stomata conductance decrease (g_s) (Table 1). No changes in maximum photochemical efficiency of PSII (Fv/Fm), photochemical yield (Φ) and non-photochemical *quenching* (NPQ) (Table 1) could be tentatively explained by associated increased levels of NADPH:quinone oxidoreductase protein (Figure 1) and a postulated increased cyclic electron flux between PSI and PSII.

Table 1. Effect of suspension of irrigation on leaf water potential at predawn (Ψ_{pd}), net carbon assimilation rate (A), stomata conductance (g_s), maximum photochemical efficiency of PSII (F_v/F_m), photochemical yield (Φ) and non-photochemical *quenching* (NPQ) in *C. canephora* clones.

	109A clone		120 clone	
Parameters	**Control**	**Water stressed**	**Control**	**Water stressed**
days after suspension of irrigation	12 days	12 days	14 days	14 days
Ψ_{pd} (MPa)	-0.19Aa	-3,27Ab	-0,14Aa	-3,28Ab
A (μmol m^{-2} s^{-1})	6,70Aa	0,76Ab	6,37Aa	0,94Ab
g_s (mol m^{-2} s^{-1})	0.073Aa	0.003Ab	0.099Aa	0.012Bb
F_v/F_m	0.804Aa	0.762Aa	0.821Aa	0.812Aa
Ø PSII	0,500Aa	0,3156Ba	0,447Aa	0,350Ba
q_p	0,750Aa	0,529Ba	0,616Aa	0,484Ba
NPQ	1,729Aa	1,500Aa	1,500Aa	1,789Aa

Financial support: Brazilian consortium of coffee research and development.

Differential Root Proteomic Analysis Indicates Several Proteins Involved in Drought Stress Response in *Coffea canephora* Genotypes

A.M. ALMEIDA[1*], C.Q.G. SOARES[1], B.L.S. GUIMARÃES[1], R.H. VALENTE[2], M.A. VIEIRA[1], E.A. SOARES, S.H.C. ARAÚJO, V.A. SILVA[1], E.P.B. FONTES[3], M.E. LOUREIRO[1]

[1]Departamento de Biologia Vegetal, Universidade Federal de Viçosa, Viçosa – MG, Brazil.
[*]E-mail: amalmeida@ufv.br
[2]Laboratório de Toxinologia, Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro-RJ, Brazil
[3]Departamento de Bioquímica e Biologia Molecular, Universidade Federal de Viçosa, Viçosa, MG, Brazil

SUMMARY

In this work, we performed a differential root proteomic analysis in order to elucidate the basis of complex biological processes such as tolerance and adaptation to drought stress. Tolerant clone appeared to postpone tissue dehydration to a remarkable greater extent than sensitive clone.

INTRODUCTION

Plants have long-distance root-to-shoot signaling mechanisms that generate integrative responses. Plant responses under water stress conditions involve a series of biochemical and morphological changes in the different organs. Chemical or non-chemical signals are further ways how physiological communication and integration occurs between roots and leaves for orchestration of adaptive responses. In *Coffea canephora* plants, the morpho/physiological drought responses are well characterized. In this work, we performed a differential root proteomic analysis in order to elucidate the basis of complex biological processes such as tolerance and adaptation to drought stress in coffee.

MATERIAL AND METHODS

Leaf xylem pressure potential at predawn (Ψ_{pd}) was measured using Scholander-type pressure chamber on mature leaves. Root proteomic analyses of two *Coffea canephora* clones, sensitive (109A) and tolerant (120) to water deficit, were performed upon severe water deficit (Ψ_{pd} = -3,0 MPa). Root protein extracts were separated by two dimensional gel electrophoresis. Gel images were analyzed using Image Master 2-D Platinum software and spots with differential abundance upon water deficit were sequenced. The spectra of spots sequenced by mass spectrometry (MALDI-TOF-TOF) were processed using MASCOT® MS/MS Ion Search and SCAFFOLD® for confident protein identification. Data mining was done in NCBI non-redundant (Viridiplantae) protein database and EST Coffea bank.

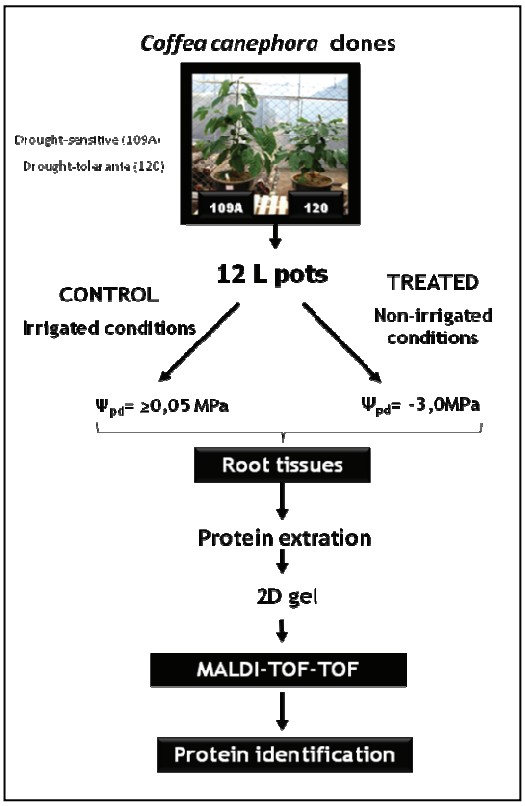


Figure 1.

RESULTS AND DISCUSSION

Figure 2 shows 2D-gels containing proteins from roots of drought susceptible 109A clone an tolerant 120 clone. Comparative proteome analysis revealed more than 250 protein spots reproducibly detected, 81 presented differential expression under drought stress conditions.

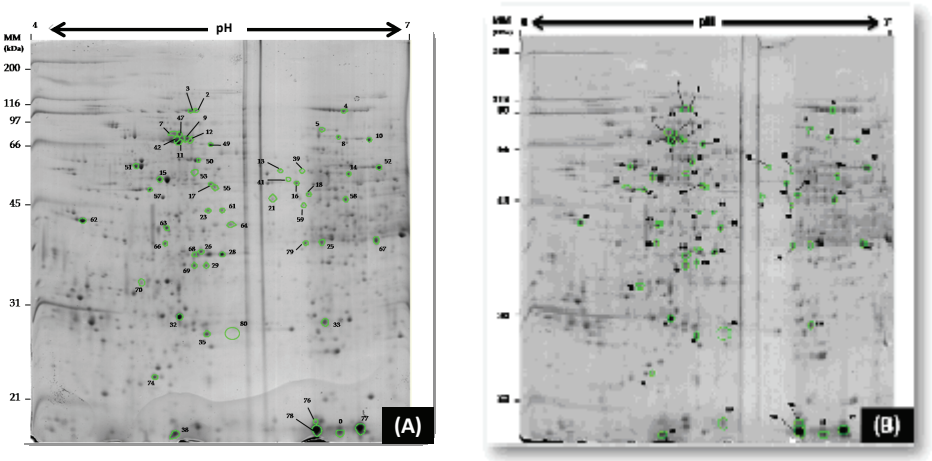


Figure 2. Root proteins with changes in abundance in clones of *Coffea canephora* in response to water deficit (Ψ_{pd}= -3 MPa). Total root protein was separated by isoelectric focalization in immobilized pH 4-7, and subsequently separated by SDS-PAGE. Image analysis was performed using Image Master 2-D Platinum software. (A) Gel containing protein from clone 109A roots irrigated and (B) gel containing protein from clone 120 roots irrigated.

From the sequenced spots, proteins from protein processing and defense and oxidative stress classes were the most representative, about 28 and 26% respectively (Figure 3). It was also observed a general decrease in abundance protein processing enzymes upon water deficit condition, that could be also related to programmed cell death events. On the defense and oxidative stress class it was observed abundance increase in dehydrin, mitochondrial aldehyde dehydrogenase, monodehydroascorbate reductase and aldo/keto reductase are related to detoxification of reactive oxygen species and dehydration prevention.

It was also sequenced proteins related to carbohydrate metabolism (18%). A general decrease in abundance of enzymes of glycolysis and tricarboxylic acid cycle (Krebs cycle) was observed on both clones in response to water deficit. This may indicate changes in the metabolic fluxes for supply of intermediates to other biosynthetic pathways and the oxidative pentose phosphate pathway. Synthesis of amino acids from deviated carbon intermediates can contribute to osmotic adjustment in roots and/or long-distance root-to-shoot signaling molecules.

Differential abundance of actins and proteins involved in the biosynthesis of ethylene and gibberellins (growth and development - 10% of sequenced spots, Figure 3). may reflect changes in root architecture.

About 18% of sequenced spots showed no defined function. This study provide a dynamic view of the proteins involved in the severe hydric stress for this species giving new insights into drought stress response in coffee roots, and demonstrates the power of the proteomic approach in plant biology studies to understand mechanism involved in abiotic stress.

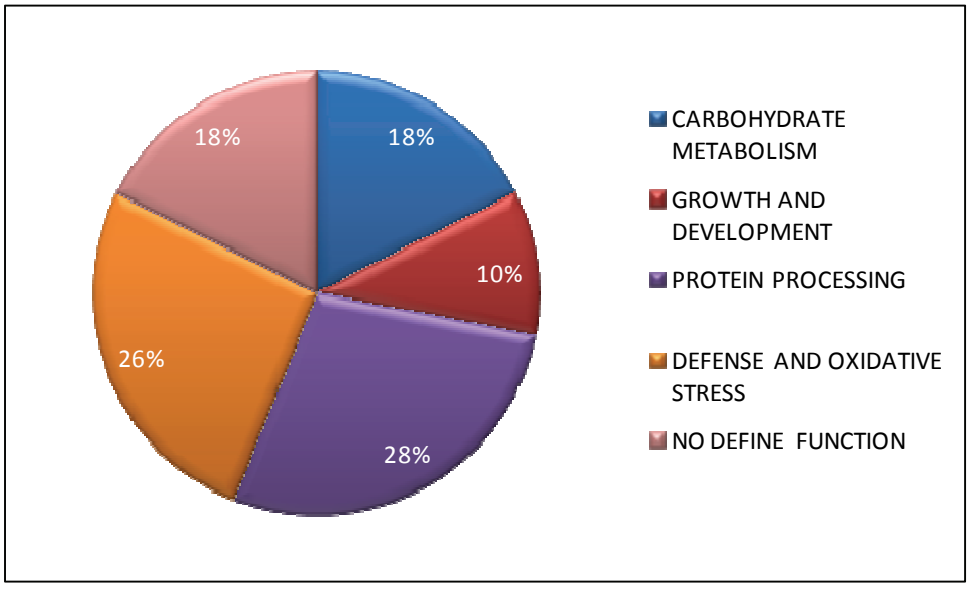


Figure 3. Functional classifications of differential expressed proteins identify in *Coffea canephora* roots on hidric stress conditions.

Financial support: Brazilian consortium of coffee research and development

Photorespiration as a Drought Tolerance Mechanism in *Coffea canephora*

B.L.S. GUIMARÃES[1], C.Q.G. SOARES[1], V.A. SILVA[1], M.A. VIEIRA[1], R.H. VALENTE[2], M.C. BARACAT[3], E.P.B. FONTES[3], M.E. LOUREIRO[1], A.M. ALMEIDA[1*]

[1]Departamento de Biologia Vegetal, Universidade Federal de Viçosa, Viçosa, MG, Brazil.
[*]E-mail: amalmeida@ufv.br
[2]Laboratório de Toxinologia, Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro-RJ, Brazil
[3]Departamento de Bioquímica e Biologia Molecular, Universidade Federal de Viçosa, Viçosa, MG, Brazil

SUMMARY

Drought is one of the major limitations to crop yield, and will become increasingly important adverse factor in regions of the globe due to changes in global climate. In this work, we performed a differential proteomic analysis in order to elucidate further mechanisms involved in water deficit tolerance in *Coffea canephora*.

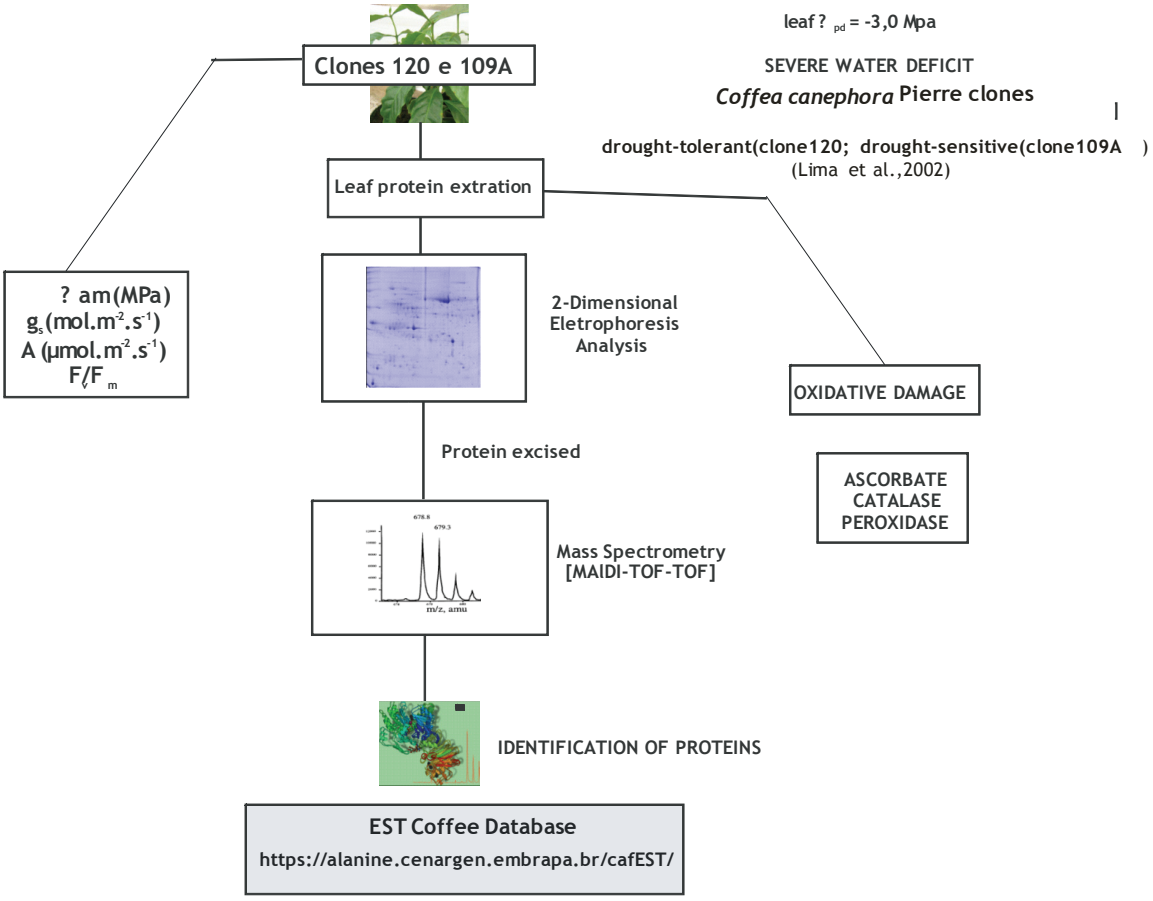
Reduction in net photosynthetic rate (*A*) was not followed by changes in electron transport, thermal dissipation or photoinhibition. Increase in the larger rubisco subunit (rbcL) levels were of 175% and 106% in tolerant clone (isoforms 2 and 8, respectively), concomitantly with a decrease of 50% in isoform 4 expression. These results suggest the importance of post-translational changes in Rubisco protein expression, which could also be highlighted due to presence of just one contig for this gene between 240 thousand coffee sequenced ESTs. In the other hand, the sensible clone has shown increase of 88% just for isoform 8. This average higher level of rbcL accumulation in clone 120 was associated with higher levels of *A*. Parallels to unaltered protein expression for glutamine synthase (GS) for clone 120, an opposed 39% reduction was observed for clone 109, which also shown oxidative damage 230% higher than that observed for clone 120 upon severe water deficit. Altogether, the clone 120 has shown lower levels of oxidative damage and no general reductions in photorespiratory enzymes, in contrast with the 109 clone. These results suggest the importance of photorespiration to drought tolerance in coffee, contributing to drain the excess of reduction power associated with water deficit in leaves.

INTRODUCTION

Drought is one of the major limitations to crop yield, and will become increasingly important adverse factor in regions of the globe due to changes in global climate. Clones of *Coffea canephora* Pierre with contrasting tolerance to drought stress have been chosen on the basis of their productivities under rainfed conditions. In this work, we performed a differential proteomic analysis in order to elucidate further mechanisms involved in water deficit tolerance in *C. canephora*.

MATERIAL AND METHODS

Severe water deficit (leaf Ψ_{pd} of -3MPa) was imposed to two contrasting *C. canephora* drought response clones (120 tolerant and 109 sensible) and leaf protein was analyzed in 2 dimensional electrophoresis (2DE) using biological triplicates. Identification of differential expressed proteins was done through protein sequence by mass spectrometry (MALDI-TOF-TOF).



RESULTS

Reduction in net photosynthetic rate (*A*) was not followed by changes in electron transport (ETR), thermal dissipation or photoinhibition (Fv/Fm) (Table 1). Increase in the larger Rubisco subunit (RbcL) levels in tolerant clone suggests the importance of post-translational modifications in Rubisco protein, which could also be highlighted due to presence of just one contig for this gene among 240 thousand coffee EST sequences (Figure 1A). Glutamine synthase (GS) protein expression presented constant in clone 120 (Figure1A) on both irrigated and non-irrigated conditions while 39% reduction was observed in non-irrigated clone 109 plants (Figure 1B) which also showed high oxidative damage. Patterns of RbcL and GS expression together with lower level of oxidative damage in clone 120 suggest that this clone presents higher levels photorespiration compared with clone 109. These results suggest the importance of photorespiration to drought tolerance in coffee, contributing to drain the excess of reduction power associated with water deficit in leaves.

Table 1. Effects of supension of irrigation on leaf water potencial (Ψ_{pd}) at predawn, net carbon assimilation rate (A), stomatal condutance (g_s), maximum photochemical efficiency of PSII (F_v/F_m) in *C. canephora* clones.

	Clone 109A		Clone 120	
Parameters	**Control**	**Water stress**	**Control**	**Water stress**
days after suspending irrigation	12 days	12 days	14 days	14 days
Ψ_{pd} (MPa)	-0.19Aa	3,27Ab	-0,14Aa	3,28Ab
A (μmol m^{-2} s^{-1})	6,70Aa	0,76Ab	6,37Aa	0,94Ab
g_s (mol m^{-2} s^{-1})	0.073Aa	0.003Ab	0.099Aa	0.012Bb
F_v/F_m	0.804Aa	0.762Aa	0.821Aa	0.812Aa

Different small letters represent statistical between means for each parameter within each clone. Different capital letters represent statistical significance between means to each parameter within each watering regime ($p \leq 0,05$). Each value represent the mean of 4 replicates.

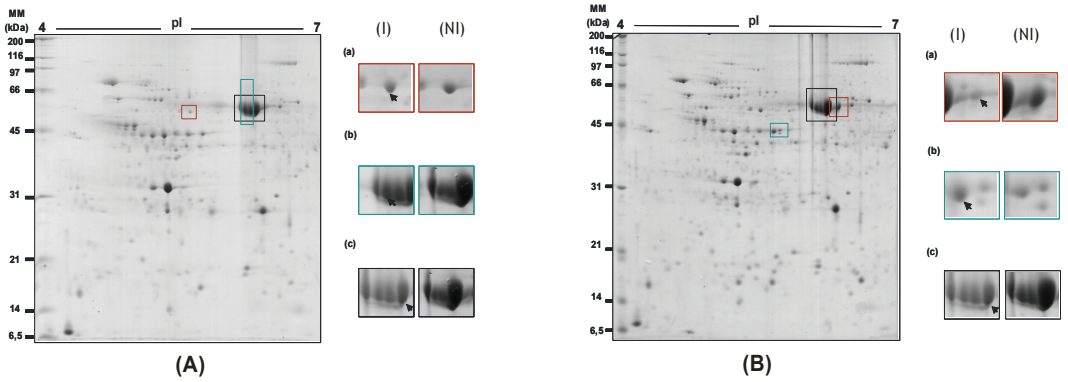


Figure 1. Changes in abundance of leaf proteins in clones of *Coffea canephora* submited to water deficit (-3MPa). Total leaf protein was separated by isoeletric focalization in imobilized pH4-7, and subsequently separated by SDS-PAGE. Image analysis was performed by Imagemaster 2-D platinum. (A) gel containing protein from clone 120 leaves; spots (a), (b) and (c) are rbcL (Ribulose-1,5-biphosphatate carboxylase large subunit). (B) gel containing protein from clone 109 leaves; spots (a) and(c) are rbcL, and (b) is glutamine synthase protein. (I) Irrigated; (NI) Non-Irrigated.

Financial support: Brazilian consortium of coffee research and development.

Galactinol synthase Expresssion By Heat Stress in *Coffea arabica* L.

T.B. SANTOS[1], C.J. MARUR[2], C.L.O. PETKOWICZ[3], F. REICHER[3], L.F.P. PEREIRA[4], L.G.E. VIEIRA

[1]Laboratório de Biotecnologia Vegetal, Instituto Agronômico do Paraná – IAPAR, Londrina – PR, Brazil
[2]Área Ecofisiologia – IAPAR, Londrina – PR, Brazil
[3]UFPR, Dep. de Bioquímica, Curitiba – PR, Brazil
[4]EMBRAPA – Café - IAPAR- Londrina – PR, Brazil

SUMMARY

Plants in nature encounter a combination of environmental conditions that may include stresses such as drought or heat shock. The response of plants to a combination the stress abiotc induction of a large number of defense genes, and changes in genes involved in sugar metabolism. Galactinol synthase (GolS) is a key enzyme in the raffinose and stachyose biosynthetic pathway (RFOs family). This protein functions a galactosyl transferase, which catalyses the key regulatory reaction utilizing UDP-galactose and *myo*-inositol as substrates to form galactinol and UDP. *GolS* genes have often been identified as stress responsive genes in many plant species. To study the function of different isoforms of *GolS* during heat stress in *Coffea arabica*, this work was carried out with the objective to characterize the expression of three *Galactinol synthase* isoforms: *CaGolS1*, *CaGolS2* and *CaGolS3*.

INTRODUCTION

The study of abiotic stress in plant has advanced considerably in recent years. Abiotic stress in plants results in major alterations in sugar status and hence affects the expression of various genes by down and up-regulating their expression. Raffinose family oligosaccharides (RFOs) have diverse roles in plants, being used for the transport and storage of carbon and as compatible solutes for protection against abiotic stress (Taji et al., 2002). Galactinol synthase (GolS) catalyses the first step in the biosynthesis of RFOs and plays a key regulatory role in carbon partitioning between sucrose and RFOs (Saravitz et al., 1987). Galactinol is formed from UDP-galactose and *myo*-inositol by galactinol synthase (EC 2.4.1.123; GolS), which belongs to the glycosil transferase 8 family (Campbell et al., 1997). The GolS contains a characteristic carboxyl terminal pentapeptide in various organisms, APSAA (Sprenger and Keller, 2000). Expression of *GolS* isoforms is increased during drought and cold exposure in *Arabidopsis thaliana* and *GolS* overexpression increased drought tolerance (Cunningham et al., 2003). In *Arabidopsis*, seven *GolS* genes were identified three of them were stress responsive. *AtGolS1* and *AtGolS2* were induced by drought and high-salinity stress, while *AtGolS3* was induced by low temperature. In *Cucumis melo* it was demonstrated that *CmGolS1* transcription occurs in mature leaves and seeds during plant development, while *CmGolS2* transcription was observed only in mature leaves (Volk et al., 2003). Nishizawa et al. (2008) suggest a novel function galactinol and raffinose as scavengers of hydroxyl radicals to protect plant cells from oxidative damage caused by MV (methylviologen) treatment, salinity or chilling. Also, a study in *Coptis japonica* identified one *GolS* cDNA clone that conferred tolerance to the toxic compound berberine, (Takanashi et al., 2008). The acquisition of tolerance to heat stress is correlated with induction of heat shock protein expression. In

Arabidopsis, 21 different heat shock transcription factors (HSF) genes have been identified (Nover et al., 2001). *AtGolS1* is one of the genes that are heat-inducible in wild type *Arabidopsis* but showed constitutive mRNA levels in transgenic plants expressing heat shock factor 3 (*HSF3*) gene. The conclusion that *AtGolS1* is a true target of HSF regulation was confirmed by the investigation of transgenic plants carrying *AtGolS*-promoter *GUS*-reporter constructs. It appears that HSFs may be involved in drought- and salinity-induced *AtGolS1/AtGolS2* expression whereas the expression of cold inducible *GolS3* is regulated by DREB1/CBF (Panikulangara et al., 2004). The objective of this study was to increase the knowledge on the transcriptional activity of *GolS* genes in coffee plants under heat stress and to access the expression each isoform on coffee thermotolerance.

MATERIALS AND METHODS

Three *galactinol synthase* isoforms, *CaGolS1*, *CaGolS2* e *CaGolS3* were identified the Coffee Genome Brazilian Project database (http://www.lge.ibi.unicamp.br/café/). The contigs formed by ESTs from different tissues of stressed plants were analyzed using BlastP and BlastX. Six month-old plants of *Coffea arabica* cv. IAPAR-59, cultivated in 10L pots were submitted to different heat stress conditions: control non stressed (plants maintained 7 days in growth chamber at 24 °C), 3 and 5 day at constant temperature of 37 °C in growth chamber (day 3 and day 5, respectively). The plants were evaluated total water potential, osmotic potential and photosynthetic rates. The total RNA was extracted according to Geromel et al. (2006) for Northern blot analysis to detect the *galactinol synthase* transcripts in leaves submitted to heat stress. Galactinol, raffinose and stachyose quantification was performed using in high-pressure liquid chromatography (HPLC) Shimadzu, Japan) with a Supelcogel Ca (Supelco-USA), 30 cm x 7.8 mm, column and Supelcogel Ca, 5cm x 4.6 mm, pre-column. The temperature of analysis was 80 °C, flow of 0.5 mL / min, using water as eluent. Calibration curves for absolute quantification were made with standards, which were used of carbohydrates in the samples.

RESULTS AND DISCUSSION

Three full-length *galactinol synthase* isoforms were identified: *CaGolS1*, *CaGolS2* and *CaGolS3*. These isoforms have 1005, 1026 and 1017 bases pair (bp), respectively, coding for proteins of 314, 341 and 338 amino-acids (aa). All the three isoforms present the glycosyl transferase domain pfam01501. In plants kept in a growth chamber for five days at 37°C, the *CaGolS1* isoform presented an increased transcriptional activity on the third day of heat stress, decreasing on the fifth day of heat stress (Figure 1).

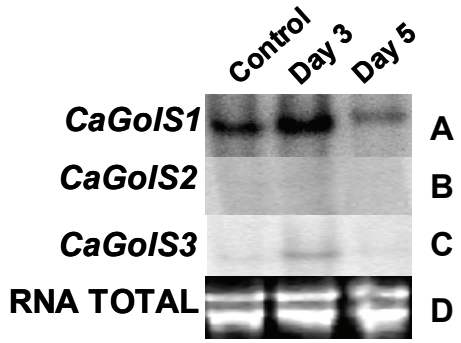


Figure 1. Northern blot analysis of total RNA from *C. arabica* cv. IAPAR-59 leaves hybridized with CaGolS1, 2 and 3 probes. A-B-C. RNA from leaves of control plants and under heat stress (3 and 5 days). D. Total RNA loading control is represented under the blots.

The expression of isoform *CaGolS2* was barely periods of stress tested. Regarding *CaGolS3,* the presence of this isoform was detected only during the third day of stress in leaves of *C. arabica* cv. IAPAR- 59. Panikulangara et al. (2004) also observed the expression of the *galactinol synthase* isoforms when dealing with heat stress. The combination of high-light and heat stress or treatment with hydrogen peroxide also induced the transcription of two *AtGolS1* and *AtGolS2* (Nishizawa et al., 2006), low and similar amounts of galactinol were observed during heat stress in comparison the levels of raffinose and stachyose in both non stressed and heat shock treatments (Figure 2).

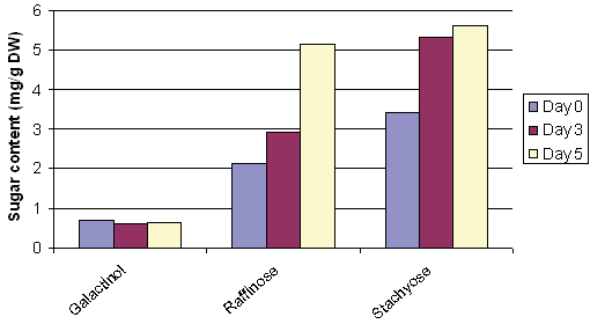


Figure 2. Quantification of galactinol, raffinose and stachyose in leaves of heat stressed *C. arabica* cv. IAPAR 59 plants by HPCL.

Galactinol synthase probably should be regulating the levels of reserve oligosaccharides in parts of the plant, as in leaves. The accumulation of galactinol in coffee leaves did not occur by the heat stress treatment, but instead it was used a key precursor in the formation of raffinose and stachyose. Thus, the high GolS transcription did not reflect the amount of galactinol, as specific glycosyltransferases most likely used galactinol to produce raffinose and stachyose, as suggested by the increase of raffinose and stachyose during the stress period. Our data agrees with early reports showing the correlation between expression of *GolS* and the synthesis of RFOs, suggesting an important role of this gene in stress-induced osmolyte synthesis in vegetative tissue and the role of this pathway in environmental stress responses (Panikulangara et al., 2004).

REFERENCES

Campbell J.A., Davies G.J., Bulone V., Henrissat B. A classification of nucleotide-diphospho-sugar glycosyltransferase based on amino acid sequence similarities. Biochem J., 326: 929-942, 1997.

Cunningham S.M., Nadeau P., Castonguay Y., Laberge S., Volenec J.J. Raffinose and stachyose accumulation, *galactinol synthase* expression, and winter injury of contrasting alfalfa germplasms. Crop Science., 43: 562-570, 2003.

Geromel C., Ferreira L.P., Guerreiro S.M.G., Cavalari A.A., Pot D., Pereira L.F.P., Leroy T., Vieira L.G.E., Mazzafera P., Marraccini P. Biochemical and genomic analysis of sucrose metabolism during coffee (*Coffea arabica*) fruit development. Jour. of Exp Bot., 55: 3243-3258, 2006.

Nishizawa A., Yabuta Y., Shigeoka S. Galactinol and Raffinose constitute a novel function to protect plants from oxidative damage. Plant Physiol., 147: 1251-1263, 2008.

Nishizawa A., Yabuta Y., Yoshida E., Maruta T., Yoshimura K., Shigeoka S. *Arabidopsis* heat shock transcription factor A2 as a key regulator in response to several types of environmental stress. Plant J., 48: 535-547, 2006.

Nover L., Bharti K., Doring P., Mishra S.K., Ganguli A. and Scharf K.D. *Arabidopsis* and the heat stress transcription factor world. How many heat stress transcription factors do we need?; Cell Stress Chaper., 6: 177-189, 2001.

Panikulangara T.J., Eggers-Schumacher G., Wunderlich M., Stransky H. and Schöffl F. *Galactinol synthase1*. A novel heat shock factor target gene responsible for heat induced synthesis of raffinose family oligosaccharides in *Arabidopsis*; Plant Physiol., 136: 3148-3158, 2004.

Saravitz D.M., Pharr D.M., Carter T.E. Galactinol synthase activity and soluble sugars in developing seeds of four soybean genotypes; Plant Physiol., 83: 185-189, 1987.

Sprenger N., Keller F. Allocation of raffinose family oligosaccharides to transport and storage pools in *Ajuga reptans*: the roles of two distinct *galactinol synthases*. Plant J. 21: 249-258, 2000.

Taji T., Ohsumi et al. Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana*. Plant J., 29: 417-426, 2002.

Takanashi K., Shitan N., Sugiyama A., Kamimoto Y., Hamamoto M., Iwaki T., Takegawa K., Yazaki K. Galactinol synthase gene of *Coptis japonica* is involved in berberine tolerance. Biosci Biotechnol Biochem, 72: 398-405, 2008.

Volk G.M., Haritatos E.E., Turgeon R. *Galactinol synthase* gene expression in melon. J Am Soc Hortic Sci., 128: 8-15, 2003.

In Silico Characterization and Transcription Analysis of Two Alpha-Expansins Isoforms in *Coffea arabica* L.

I.G.F. BUDZINSKI[1], L.F.P. PEREIRA[2], T. SERA[1], L.G.E. VIEIRA[1]

[1]Plant Biotechnology Laboratory, Agronomic Institute of Paraná (IAPAR), Londrina, PR, Brazil
[2]Embrapa – Café, Brasília, DF, Brazil

SUMMARY

The development and maturation of coffee fruit reflects on grain size, cup quality and consequently better prices for the coffee produced. It is characterized by intense cell division, elongation and softening of cell walls in different fruit tissues. Those processes are related with the action of different proteins, including expansins. The objective of this work was to identify α-expansins genes expressed during coffee fruit development and maturation. Through *in silico* analysis in the Brazilian Coffee Genome Project database we selected two α-expansins genes present in fruit cDNA libraries. We observed that both isoforms (*CaEXPA1* and *CaEXPA2*) have signal peptide and specific N- terminal and C-terminal domains for expansins proteins. To study gene expression, we collected tissues (root, plagiotropic shoots, leaf, bud flower and flower) from *C. arabica* cv. IAPAR-59 and fruits from both cv. IAPAR-59 and cv. IAPAR-59 Graúdo (a genotype with larger fruit/grain size). Fruits were monthly collected after flowering. The transcripts of coffee *CaEXPA*1 and *CaEXPA*2 were observed at different coffee tissues and at different fruit ripening stages, indicating spatial and temporal differences on both isoforms transcription pattern. *CaEXPA*1 was mainly expressed during the early stages of fruit development and at the end of the fruit ripening period. On the other hand, *CaEXPA2* was only expressed in the last steps of fruit ripening.

INTRODUCTION

Coffee fruit growth is an asynchronous process, resulting in the presence of fruits in different stages of ripening in the same plant. The presence of green fruits and over-ripened fruits in the same batch of grains changes the acidity, the bitterness and consequently the quality of the product (Pereira et al., 2005). The early stages of coffee fruit development and ripening are characterized by intense cell division, perisperm development, elongation and softening of cell walls. Those processes are related with the action of different proteins, including expansins, pectin methylesterases, xyloglucanases. Expansins are plant cell-wall loosening proteins that induce cell wall extension and stress relaxation at acidic pH condition (McQueen-Manson et al., 1992). These proteins play roles in a diverse range of developmental process including fruit development and ripening (Rose et al., 1997; Brummell et al., 1999; Vidhu et al., 2005; Dotto et al., 2006; Vidhu et al., 2007; Ishimaru et al., 2007). Expansins represent a protein superfamily, that are formed by four families designated α-expansin (EXPA), β-expansin (EXPB), expansin-like A (EXLA) and expansin-like B (EXLB) (Kende et al., 2004). Members of the EXPA and EXPB families are known to have wall-loosening activity (Cho and Kende, 1997; Cosgrove et al., 1997), whereas the other two families have been identified only from sequence homology, but protein function analysis has not been reported (Lee et al., 2001; Li et al., 2002).

MATERIALS AND METHODS

Through analyses on the Brazilian Coffee Genome Project database (http://www.lge.ibi.unicamp.br/cafe/), we selected two α-expansin isoforms named *CaEXPA*1 and *CaEXP*2, both containing sequences from fruit cDNA libraries. Predicted amino acid sequences were obtained using ORF finder at NCBI (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). ScanProsite (http://www.expasy.org) was used to verify the two domains specific for mature expansin protein. The pollen allergen domain was verified for each isoform using BlastP and the signal peptide region was identified using SignalP (http://www.cbs.dtu.dk/services/SignalP/). To gene expression analysis, fruits were monthly collected, after flowering, from *Coffea arabica* cv. IAPAR-59 and *C. arabica* cv. IAPAR-59 Graúdo, at the experimental station of the Agronomic Institute of Paraná (IAPAR, Londrina, BR). We also collected tissues (root, plagiotropic shoots, leaf, bud flower and flower) from cv. IAPAR-59. Total RNA was isolated from different tissues and fruits at different stages of maturation from cv. IAPAR 59 and cv. IAPAR 59 Graúdo, according to Chang et al. One μg of total RNA was used to produce cDNA with Thermoscript^{TM} oligo DT System (Invitrogen) to amplify the two α-expansin genes, which were used as probes for transcript analysis. For Northern Blot analysis 15 ug of total RNA was transferred to nylon membranes and hybridized using UltraHyb solution. The specificity of each probe was tested through dot-blot analysis (date not shown).

RESULTS AND DISCUSSION

Through *in silico* analysis we observed that both *CaEXPA*1 and *CaEXPA*2 isoforms have a signal peptide, ranging from 21 to 30 aminoacids. They have the specific domains for expansins proteins, an N- terminal distantly related to the catalytic domain of glycoside hydrolase (GH-45) and a C-terminal domain distantly related to group-2 grass pollen allergens. *CaEXPA*1 and *CaEXPA*2 presented high identity (from 80% to 86% and from 79% to 85%, respectively) with expansins from other plants according to BlastX analysis. Northern blot from different tissues of coffee showed for *CaEXPA*1, transcripts in roots and bud flowers (Figure 1A) and for *CaEXPA*2 transcripts were observed in shoots (Figure 1B).

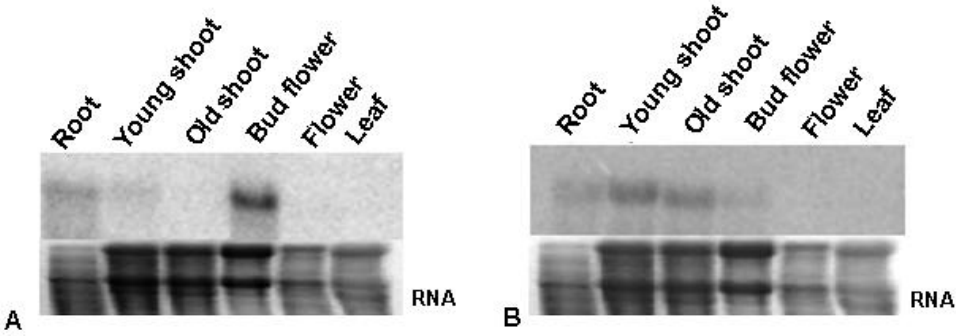


Figure 1. Northern blot analysis of two *C. arabica* α-expansin genes. Total RNA from root, young shoot, old shoot, bud flower and flower from *C. arabica* cv. IAPAR-59. A – Hybridization using *CaEXPA*1 as probe. B – Hybridization using *CaEXPA*2 as probe.

*CaEXPA*1 was similarly transcribed in both genotypes (Figure 2). Transcripts were detected during the early stages of fruit development and in the last steps of fruit ripening. For cv. IAPAR-59, increased transcripts were observed in November (60 days after maturation – DAF), a period of rapid expansion of the fruit and perisperm development (Figure 2). Interestingly, in the genotype cv. IAPAR-59 Graúdo, we also observed transcripts at 60 DAF; however, higher accumulation of transcripts was detected at 90 DAF (Figure 2). This

expression pattern of *CaEXPA*1 in cv. IAPAR-59 Graúdo probably indicates that in this genotype fruit expansion and perisperm development continues until 90 DAF, a longer period of time when compared to cv. IAPAR-59. Increased transcripts were also observed in April (210 DAF) for both cultivars. These results probably suggest the participation of *CaEXPA*1 in the fruit development and ripening process.

Out	Nov	Dec	Jan	Feb	Mar	April
Fruit initial expansion (Perisperm elongation)			Endosperm growth		Fruit maturation	

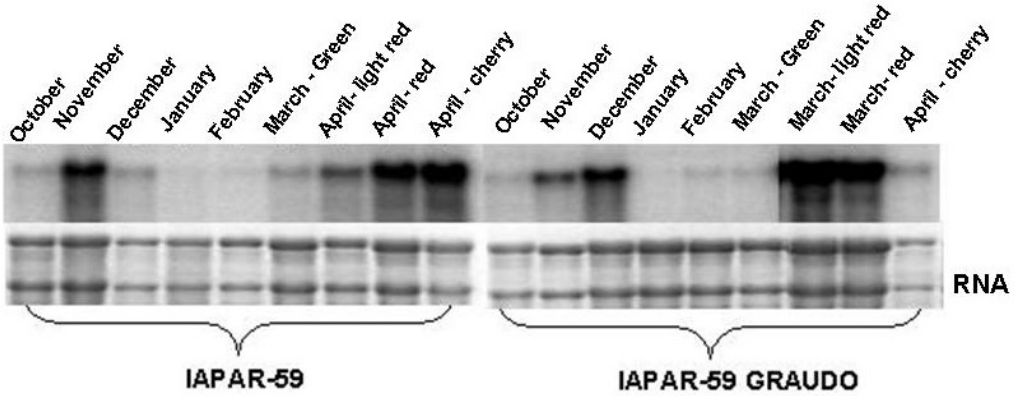


Figure 2. Northern blot of *CaEXPA1* during fruit development. Total RNA from the whole fruit of *C. arabica* cv. IAPAR-59 and cv.IAPAR-59 Graúdo in different stages of fruit development and ripening. Fruits were monthly collected after flowering: October (30 DAF), November (60 DAF), December (90 DAF), January (120 DAF), February (150 DAF), March (green - light red - red, 180 DAF) and April (light red - red and cherry, 210 DAF).

Out	Nov	Dec	Jan	Feb	Mar	April
Fruit initial expansion (Perisperm elongation)			Endosperm elongation		Fruit maturation	

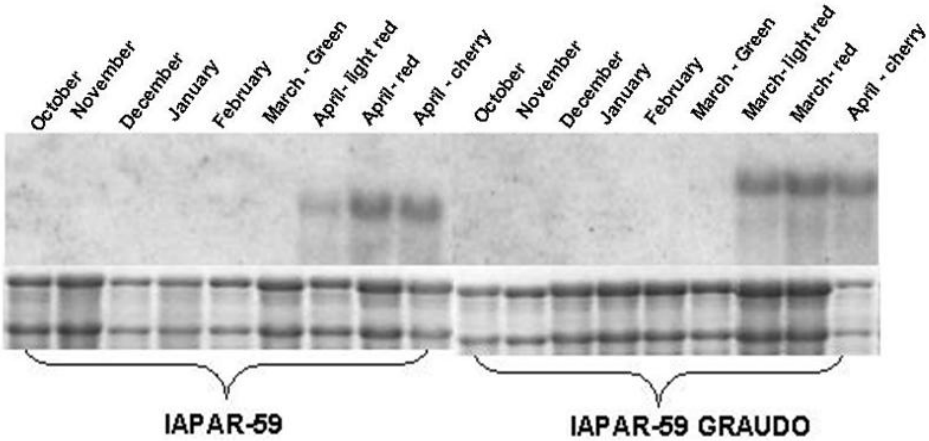


Figure 3. Northenr blot analysis of *CaEXPA2* during fruit development. Total RNA from the whole fruit of *C. arabica* cv. IAPAR-59 and cv.IAPAR-59 Graúdo in different stages of fruit development and ripening. Fruits were monthly collected after flowering: October (30 DAF), November (60 DAF), December (90 DAF), January (120 DAF), February (150 DAF), March (green - light red - red, 180 DAF) and April (light red - red and cherry, 210 DAF).

On the other hand, the isoform *CaEXPA2* (Figure 3), showed specific expression during the later stages of fruit ripening for cv. IAPAR-59 and cv. IAPAR-59 Graúdo, suggesting the involvement of this isoform in the process which involves the cell wall disassembly of pericarp. In climacteric fruit, as the case of *C. arabica*, fruit softening is found to be ethylene-dependent overall, which may imply that CaEXPA2 is probably regulated by ethylene. Some expansins are expressed in several organs and tissues type, whereas others shows very tight specificity in their spatial and temporal expression patterns (Rose et al., 1997; Cho and Kende, 1997), indicating that regulation of cell wall extensibility could be controlled at least in part by differential regulation of the different expansin genes (Reinhardt et al., 1998).

The findings presented in this work provided basic information about the participation of expansin in coffee fruit development and maturation and can be used for more detailed experiments involving coffee fruit. Our group is currently studying the pattern of transcription of *CaEXPA*1 and *CaEXPA*2 in each fruit tissue (pericarp, perisperm and endosperm), looking in detail for the spatial and temporal characterization of those genes.

ACKNOWLEDGMENTS

This research was supported by the Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café (CBP&D-Café).

REFERENCES

Brummell D.A., Harpster M.H., Civello P.M., Palys J.M., Bennett A.B., Dunsmuir P. (1999) Modification of expansin protein abundance in tomato fruit alters softenbing and cell wall polymer metabolism during ripening. Plant Cell 2:2203-2216.

Cho H.T., Kende H. (1997) Expression of expansin genes is correlated with growth in deepwater rice. Plant Cell 1997, 9:1661-1671

Cosgrove D.J., Bedinger P., Durachko D.M. (1997) Group I allergens of grass pollen as cell wall-loosening agents. Proc Natl Acad Sci USA 94:6559-6564.

Dotto M.C., Martinez G.A., Civello P.M. (2006) Expression of expansin genes in strawberry varieties with contrasting fruit firmness. Plant Physiology and Biochemistry 44: 301-307.

Ishimaru M., Smith D.L., Gross K.C., Kobayashi S. (2007) Expression of three expansin genes during development and maturation of Kyoho grape berries. Journal of Plant Physiology 164: 1675-1682.

Kende H., Bradford K., Brummell D., Cho H.T., Cosgrove D.J., Fleming A., Gehring C., Lee Y., Queen-Mason S., Rose J., Voesenek L.A. (2004) Nomenclature for members of the expansin superfamily of genes and proteins. Plant Mol Biol 55:311-314.

Lee Y., Choi D., Kende H. (2001) Expansins: ever-expanding numbers and functions. *Curr* Opin Plant Biol 4:527-532.

Li Y., Darley C.P., Ongaro V., Fleming A., Schipper O., Baldauf S.L. (2002) Plant expansin are a complex multigene family with an ancient evolutionary origin. *Plant* Physiol 128:854-864.

McQueen-Manson S., Durachko D.M., Cosgrove D.J. (1992) Two endogenous proteins that induce cell wall expansion in plants. Plant Cell 4:1425-1433.

Pereira L.F.P., Galvão R.M., Kobayashi A.K., Cação S.M.B., Vieira L.G.E. (2005) Ethylene production and acc oxidase gene expression during fruit ripening of *Coffea araica* L. Brazilian Journal of Plant Physiology, v. 17: 283 – 289.

Reinhardt D, Witter F, Mandel T, Kuhlemeier C. (1998) Localized upregulation of a new expansin gene predicts the site of leaf formation in the tomato meristem. *Plant Cell* 10:1427-1437.

Rose J.K., Lee H.H., Bennett A.B. (1997) Expression of a divergent expansin gene is fruit-specific and ripening-regulated. Proceedings of the National Academy of Science 94:5955-5960.

Vidhu A.S., Chourasia A., Pravendra N. Softening in mango (*Mangifera indica* cv. Dashehari) is correlated with the expression of an early ethylene responsive, ripening related expansin gene, MiExpA1 (2005) Postharvest Biology and Technology 38: 223-230.

Vidhu A.S., Aniruddha S.P., Pravendra N. (2007) Multiple forms of α-expansin genes are expressed during banana fruit ripening and development. Postharvest Biology and Technology 45: 184-192.

Identification and Characterization of Gene Expression of Different Members of Snrk Kinase Family in *Coffea canephora* and *Coffea arabica*

R.M.C. PAIVA, R.B. FREITAS, F.J.F FAGUNDES, M.E. LOUREIRO

Laboratório de Fisiologia Molecular de Plantas - Departamento de Fisiologia Vegetal – Universidade Federal de Viçosa

SUMMARY

The plant hormone abscisic acid (ABA) has an important role in the regulation of plant responses to several abiotic stresses, as salinity, drought and cold. Drought responses involve protein phosphorylation as signaling events, and several kinases could be involved in this process. Some SnRK kinases (SNF1 related kinases) have important role in the stress and ABA signaling pathways. The objective of this work is to begin to characterize the SnRK coffee gene family and identify the members involved in drought responses. Extensive analysis of homology in several coffee cDNA banks have allowed us to identify 16 members of this family, distributed in all three SnRK families (SnRK1, 2 and 3). Fourteen primers pairs were design to analyze the changes in gene expression under drought. RNA extracted from leaves and roots of *Coffea canephora* clones contrasting in drought tolerance (120, tolerant; 109, susceptible that were submitted to severe water stress (Ψ_W = -3MPa), first strand cDNA was produced for Real Time PCR analysis of gene expression. Independent from stress, the majority of the SnRK genes are more expressed in leaves that in roots. In leaves of clone 120, stress was not linked to changes in expression of SnRK3 and SnRK1 members, whereas for SnRK2 members an increase was observed only for clone 109 under stress. In roots, also no changes were observed for SnRK3 and SNRK1 genes under stress, whereas a decrease for SnRK2 was observed for both clones under water deficit. These results suggest that changes in gene expression in family SnRK2 and not in SnRK3 and SnRK1 families are involved in drought responses, and that different changes in gene expression of SnRK2 genes could occur in different plant organs.

INTRODUCTION

Brazil is main world coffee producer, and occurrence of drought in Brazil is considered the major factor affecting its international price.

The Brazilian coffee genome project gene bank is characterized by a significant number of incomplete sequences. This fact brings problems to genome annotation, and problem becomes worse in the case of low abundance transcript, as the case of the majority of protein kinases, and expected to be case of SnRK family. This brings problems to contig assemble and confidence achieved is not enough to sequence analysis. .

Gene expression induced by water deficit could be dependent or independent of abscisic acid (ABA).. Reversible phosphorylation plays an important role in ABA signaling. However, the details of the specific role of several kinases and phosphatases involved are unknown. Several protein kinases were described as members of the osmotic stress response in plants. Some SnRK kinases (SNF1 related kinases), a family with 32 members in *Arabidospsis*, paly a role

drought signaling cascades, and could be involved in the activation of transcriptional factors important to ABA response (Mustili et al, 2002; Yoshida et al, 2002; Guo et al, 2002). This family could be divided in three groups, SnRK1, 2 and 3. SRK2E/OST1, a SnRK2 member, was identified as key regulator of stomatal closure in to ABA response. The C-terminal of Arabidospsis SRK2E/OST1 is required to ABA signal in response to osmotic stress, and could be divided in two domains (I and II). The domain II is required only for ABA activation of SRK2E/OST1 and has na important role in the control of stomata closing. In other hand, domain I is responsible for a kinase activation independent of ABA. The aim of this work was to identify all member of SnRK present in all coffee cDNA sequences available, and to begin the characterization of gene expression of them.

MATERIALS AND METHODS

The identification of SnRK members were done by extensive analysis of all sequences present in the "CBPD Banco do Genoma Café" and at "Sol Genomics Networks", using as a pray the 32 SnRK members of Arabidopsis.

Plantlets from *Coffea canephora* Pierre var. Conillon clone 120 (drought tolerant) and clone 109 (drought sensible), were cultivated in pots with 12 L of substrate, in greenhouse. After six months of transplanting, half of the plants were subjeted to water withholding whereas the other half remained receiving normal irrigation. Plants were subjected to drought stress until leaf water potential reached –3,0 MPa.

Total RNA was isolated using the Plant RNA Reagent (Invitrogen). First strand synthesis using Superscript II cDNA synthesis Kit (Invitrogen). Sequence alignment and phylogenetic analysis were performed using the program Clustal W, using sequences from Arabidopsis and other plant species.

Primers for Real Time PCR were designed using the Primer Express software (ABI), using 3' untranslated regions or regions of low homology of the contig 6533 (SnRK3-23), 8877 (SnRK2.1) and 14513 (SnRK1-16), regarding *A. thaliana* heterologs. Specificity of primer pairs were checked by Blast analysis. Normalization of the expression was performed using the gene Actin II from *Arabidopsis.*

RESULTS AND DISCUSSION

The gene prospection performed has allowed identifying 16 members of SnRK in coffee. The contigs 6533, 8877 and 14513 delimitate the subgroups SnRK3, SnRK2 and SnRK1 branches, respectively. Sol database did not include new members as those found in the CBPDC bank, reason why all sequence characters belongs to the last database. The fact that in Arabidopsis was identified 32 genes, suggest that the sum of genes present in Sol and CBPDC gene banks do not cover 50% of the genes of this family.

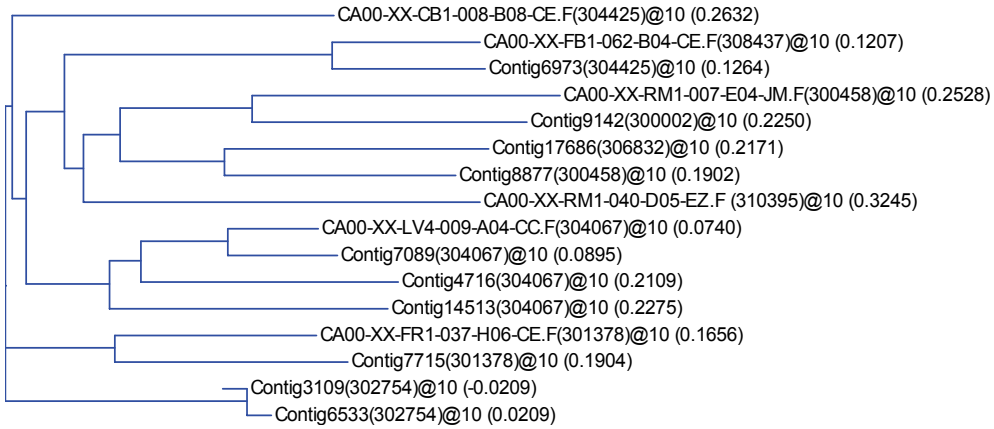


Figure 1. Phylogram of the 16 SnRK members found in coffee. The number in brackets refers to the genetic distances between the genes. Sequences are designed by the contig number or single read codes of CBPDC gene bank.

Initial characterization of gene expression was performed with 3 genes, one of each subgroup. Independent of water stress, the SnRK genes analyzed is more expressed in leaves than in roots.

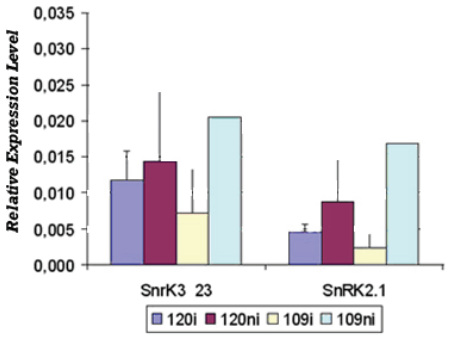


Figure 2. Transcript levels *in leaves* of two SnRK members under water stress (NI; -3Mpa), or absense of water stress (I, irrigated).

In leaf, no increase in the expression of SnRK 3 and SnRK2 genes was observed in tolerant clone (120), opposed that was observed for the drought sensible clone (109). In roots, decrease or no change in transcript levels were observed for SnRK3 and SnRK2 genes, whereas and increase in SnRK1 transcript could be observed only for the clone 109.

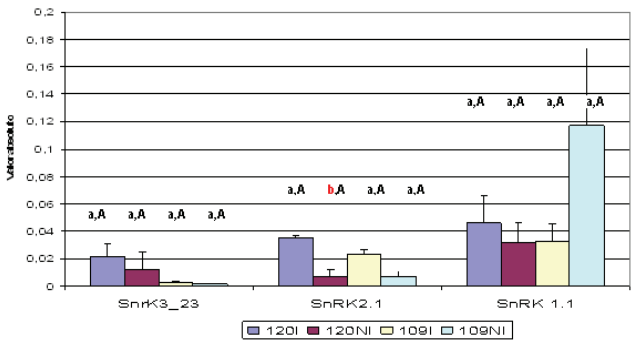


Figure 2. Transcript levels of three SnRK members in *roots* under water stress (NI; water stress(-3Mpa)), or absence of water stress (I, irrigated).

Two interpretations are possible from the data above. The first is that changes in transcript levels of any of this three genes is associated with the higher tolerance present in clone 120, and that other genes of this family could play a more important role in tolerance. The fact that specific increase in the transcript level was observed in the sensible clone (SnRK2.1 in leaves, and SnRK1.1, in roots) suggests that this change in gene expression could be associated with acclimation and not to tolerance responses to stress conditions. The second alternative interpretation is that post-trancriptional changes in gene expression of this genes is that are more likely to be involved in tolerance mechanisms. In fact, SnRK is phosphorylated and changes in its phosphorylation are associated with drought responses. Further studies are in progress do address this two possible hypothesis.

CONCLUSIONS

Detailed data-mining from two different coffee gene banks allow identification of only 16 SnRK gene members, wich probably represent only 50% of the members of this family. Increase in transcript levels was observed only for the sensible clone, and probably represent a role in acclimation but not tolerance to drought stress.

REFERENCES

Guo Y., Xiong L., Song C.P., Gong D., Halfter U & Zhu J.K. (2002). A calcium sensor and its interacting protein kinase are global regulators of abscisic acid signalling in Arabidopsis. Developmental Cell 3, 233-244.

Mustilli A.C., Merlot S., Vavasseur A., Fenzi F. & Giraudat J. (2002). Arabidopsis OST1 protein kinase mediates the regulation of stomatal aperture by abscisic acid and acts upstream of reactive oxygen species production. Plant Cell 14, 3089-3099.

Wang W.X., Vinocur B. & Altman A. (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218, 1-14.

Yoshida R., Hobo T., Ichimura K., Mizoguchi T., Takahashi F., Alonso J., Ecker J.R., Shinozaki K. (2002). ABA-activated SnRK2 protein kinase is required for dehydration stress signaling in Arabidopsis. Plant Cell Physiol 43: 1473-1483.

Identification and Characterization of Gene Expression of Three Coffee NCED Members under Drought

R.M.C. PAIVA, R.B. FREITAS, F.J.F. LOPES, M.E. LOUREIRO

Departamento de Biologia Vegetal, Universidade Federal de Viçosa, Viçosa, MG, Brazil.
E-mail: mehlers@ufv.br

SUMMARY

Several evidences support the importance of the increase in ABA synthesis in drought tolerance response. A rate-limiting step in abscisic acid (ABA) biosynthesis in plants is catalyzed by 9-cis-epoxycarotenoid dioxygenase (NCED). Only a restrict number of members of this NCED gene family is involved in ABA biosynthesis. The basic model is based in observations that show that occurs an increase in ABA synthesis in roots, even before any change in leaf water potential could be detected in leaves. Afterwards, this ABA is transported to leaves, where there promotes the stomata closure. However, other data are available that also provides support to the role of leaf ABA biosynthesis in drought tolerance and to mobilization of ABA conjugated forms, or different cell sensibility to this hormone. The objective of this work was to identify members of NCED family in coffee and identify a possible candidate that contributes to drought tolerance mechanisms. Analysis in coffee cDNA databases have resulted in identification two NCED gene. Degenerate primers were design, and sequencing of PCR products derived from the use of these primers have allowed the identification of one additional NCED gene. Real Time PCR was used to analyze differences in gene expression for these genes. Whereas no changes in mRNA levels in leaves was observed for genes CcNCED1 and 2 in both clones under water stress, the gene CcNCED3 show an increase only in the susceptible clone (109). In other hand, CcNCED4 has higher expression in drought tolerant clone (120) under absence of stress in leaves, with no changes in expression under stress, opposed as observed for clone 109, which show increase under drought. In roots, any change in expression for all genes was observed for clone 120, whereas only a increase in expression for the gene CcNCED4 was observed for clone 109. These results exclude a role for CcNCED 1 and 2 in ABA biosynthesis in response to drought stress. Additionally, the fact that significant similar increases in ABA in leaves under stress were observed for both clones and that increases in roots under this condition were higher for the clone 120, suggest that increases in ABA biosynthesis could not be attributed to changes in gene expression of the NCED genes here characterized. Since the CcNCED 3 is the clone highly homologous to AtNCED3, the main gene responsible for drought induced increase in ABA biosynthesis, our results suggest that post-transcriptional events could play an important role in modulating ABA biosynthesis under drought in coffee.

INTRODUCTION

Between the physiological mechanisms characterized in several plants, ABA signaling plays a central role for ABA in drought tolerance Several ABA deficient mutants show higher wilting and increased stomata conductance and a restriction in root development (Thompson et al., 2004). These phenotypes could be attenuated by exogenous ABA application (Schwartz et al., 2003). Additionally, increased drought tolerances was observed in tobacco and Arabidopsis

plants that over expressed NCED, the key enzyme controlling ABA biosynthesis (Qin e Zeevaart., 2002, Iuchi et al., 2001).

The isolation and gene expression characterization of genes important for ABA biosynthesis could supply important information to understand the function of changes in ABA biosynthesis in the coffee drought tolerance. The objective of this work was to isolate from Coffea canephora different complete cDNAs of the NCED family and to caracterize the effect of drought stress in gene expression of this genes in roots and leaves, using genotypes contrasting in drought tolerance.

MATERIAL AND METHODS

Plantlets from *Coffea canephora* Pierre var. Conillon clone 120 (drought tolerant) and clone 109 (drought sensible), were cultivated in pots with 12 L of substrate, in greenhouse. After six months of transplanting, half of the plants were subjeted to water withholding whereas the other half remained receiving normal irrigation. Plants were subjected to drought stress until leaf water potential reached –3,0 MPa.

Total RNA was isolated using the Plant RNA Reagent (Invitrogen), and used for first strand synthesis using Superscript II cDNA synthesis Kit (Invitrogen). Degenerate primers were designed using the the program CODEHOP (The amplified products were cloned in the vector pCR II and sequenced. Sequence alignment and phylogenetic analysis were performed using the program Clustal W, using sequences from Arabidopsis and other plant species.

Primers for Real Time PCR were designed using the Primer Express software (ABI), using 3' untranslated regions or regions of low homology. Specificity of primer pairs were checked by Blast analysis. Normalization of the expression was performed using the gene Actin II from Arabidopsis.

RESULTS AND DISCUSSION

The cloning performed allow us to identify 3 members of NCED family in coffee (Table 1), that are all full lenght and denominated CnNCED1, 3 and 4, regarding the homology shared with Arabidopsis heterologs. Only two of these genes presents chloroplast import signal.

Table 1. Comparison between the NCEDs genes isolated from *Coffea canephora.*

	CnNCED3	CnNCED1	CnNCED4
Gene size (base pairs)	1908	1957	1329
Total aminoacids	636	548	443
Chloroplast signal	Yes	No	Yes

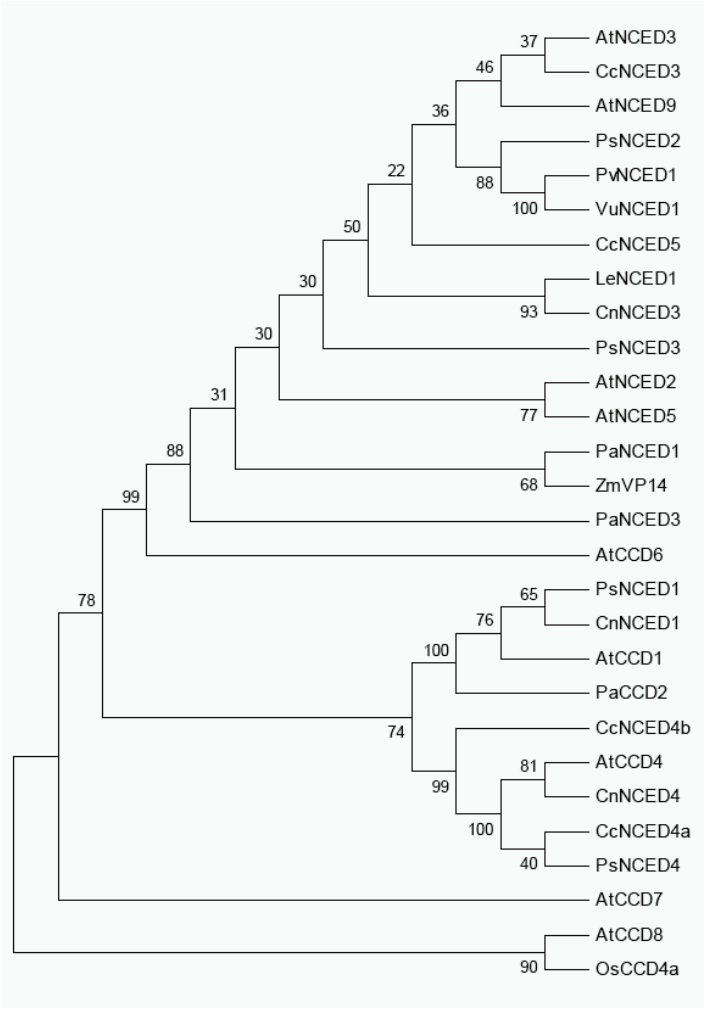


Figure 1. Phylogenetic tree of aminoacid sequence of NCED genes of *Coffea canephora* and other plant species (At: *Arabidopsis thaliana*; Cc: *C. clementina*; Cn: *Coffea canephora*; Le: tomate, *Lycopersicon esculentum*; Os: *Oryza sativa*; Pa: *Persea americana*; Ps: *Pisum sativum*; Pv: *Phaseolus vulgaris*; Vu: *Vigna unguiculata*; Zm, *Zea mays)*.

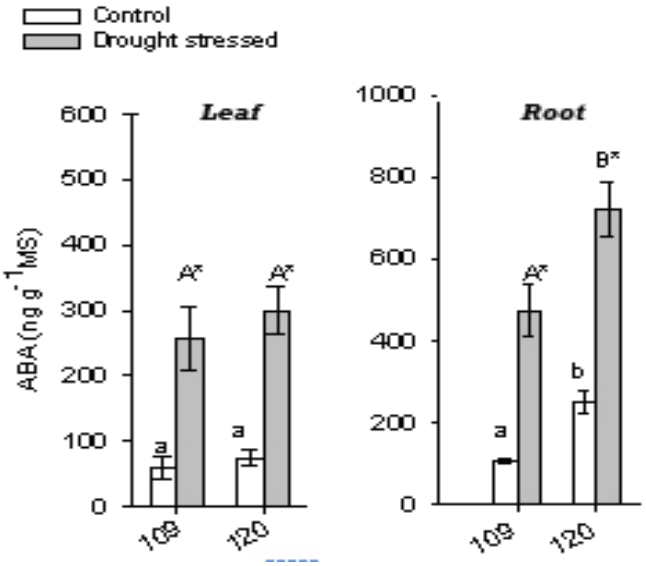


Figure 2. ABA levels in leaf and roots of plants under drought stress (ψ_w = -3,0 Mpa)

Increase in ABA levels in leaves under drought occurs in the same magnitude in both clones, whereas in roots, the ABA levels are increased more in the tolerant Clone (120). In the absence of water stress, the level of ABA is already lower in the sensible clone (109).

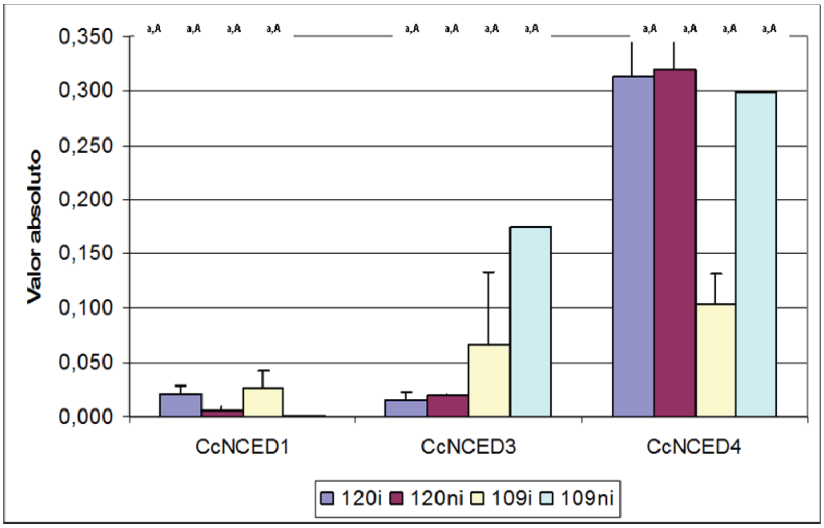


Figura 3. Drought effect in CnNCED1, CnNCED3 e CnNCED4 transcript levels in leaves. Vertical bars indicate the standard error. Different capital letters indicate significant differences between clones under the same water conditions (P < 0,05), whereas small letters indicate differences between the water treatments (i, irrigated; ni, water stress) for the same clone (P < 0,05).

In leaves, water stress was associated with an increase in NCED 3 and 4 transcript levels only FOR clone 9 (P = 0,10 and 0,14 respectively). No significant changes could be detected for NCED1. The increase in ABA in leaves of clone 120 was not associated with any change in the transcript levels of any NCED gene analyzed.

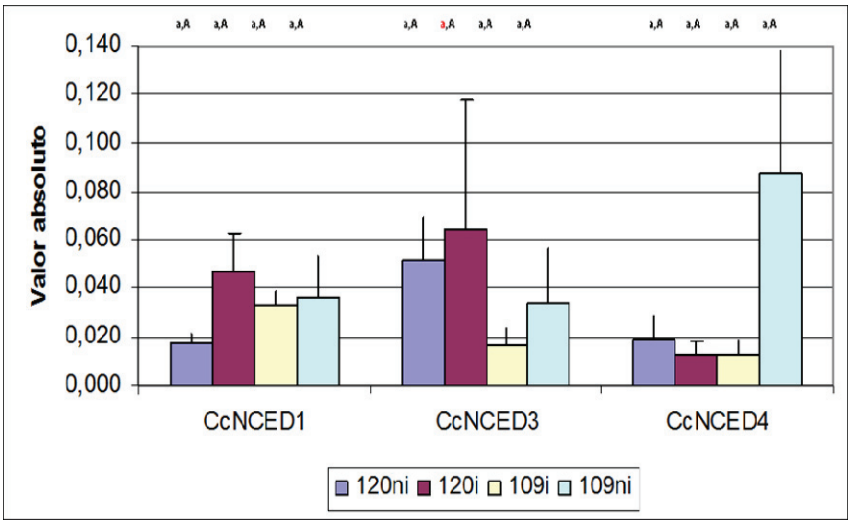


Figure 4. Drought effect in CnNCED1, CnNCED3 e CnNCED4 transcript levels *in roots.* Vertical bars indicate the standard error. Different capital letters indicate significant differences between clones under the same water conditions (P < 0,05). , whereas small letters indicate differences between the water treatments (i, irrigated; ni, water stress) for the same clone (P < 0,05).

In roots, drought was associated with a decrease in NCED1 transcript levels under drought in the tolerant clone 120, and an increase in NCED4 for clone sensible. This results indicates that the higher levels of ABA in the roots of the tolerant clone could not be explained by changes in the transcript levels of any NCED gene studied here.

This work was able to show us that any change in transcript levels of any NCED gene isolated could explain the higher tolerance of the clone 120 to drought. This fact suggest that the contribution of ABA metabolism to drought tolerance in coffee could by due to post-transcriptional changes in the modulation of ABA biosynthesis, or/and to changes in the mobilization of conjugated forms of this hormone.

REFERENCES

Iuchi S., Kobayashi M., Taji T., Naramoto M., Seki M., Kato T., Tabata S., Kakubari Y., Yamaguchi-Shinozaki K., Shinozaki K. Regulation of drought tolerance by gene manipulation of 9-cis-epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in Arabidopsis. The Plant Journal 27, 325-333, 2001.

Qin X., Zeevaart J.A.D. Overexpression of a 9-cis-Epoxycarotenoid Dioxygenase Gene in Nicotiana plumbaginifolia Increases Abscisic Acid and Phaseic Acid Levels and Enhances Drought Tolerance. Plant Physiology. 128, 544-551, 2002.

Schwartz S.H., Qin X., Zeevaart J.A.D. Elucidation of the Indirect Pathway of Abscisic Acid Biosynthesis by Mutants, Genes, and Enzymes. Plant Physiology. 131, 1591-1601, 2003.

Thompson A.J., Thorne E.T., Burbidge A., Jackson A.C., Sharp R.E., Taylor I.B. Complementation of notabilis, an abscisic acid-deficient mutant of tomato: importance of sequence context and utility of partial complementation. Plant Cell and Environment, 1-13, 2004.

Transcription Analysis of a Pectin Methylesterase Isoform in *Coffea arabica* L.

T.F. LEITE[1], I.G.F. BUDZINSKI[1], S.M.B. CAÇÃO[1], L.F.P. PEREIRA[2], L.G.E. VIEIRA[1]

[1]Plant Biotechnology Laboratory, Agronomic Institute of Paraná (IAPAR), Londrina, PR, BR
[2]Embrapa – Café, Brasília, DF, BR

SUMMARY

The coffee quality has been directly related to the ripening stages of fruits during harvesting. Non uniform maturation of the coffee fruits, combined with inadequate harvest and post harvest practices, may negatively affect the final quality of the product. Pectin methylesterase (PME - EC 3.2.1.11) has an important role in fruit softening and ripening. This enzyme catalyzes the methyl esterification of esters from polygalacturonic acid, increasing the susceptibility of pectins for the activity of polygalacturonases during rippening. In order to study the changes occurring during the coffee fruit maturation in silico and in vivo studies on PME genes were initiated. Trought in silico analysis on Brazilian Coffee Genome Project database we identified 31 PME contigs, however only eight of them had ESTs from fruit libraries. One of those PME isoform, CaPME04 was selected for further characterization. CaPME04 (1946 nucleotides) has a multicopper oxidase domain and a pro-region that is cleaved when the protein is mature. The pro-region shares some homology with PME inhibitors and probably acts as an intracellular inhibitor of PME activity while the protein is not mature. To analyze CaPME04 transcription during coffee ripening, fruits were monthly collected, after flowering, from *Coffea arabica* cv IAPAR-59. Northern Blot analysis was performed from total RNA of pericarp, perisperm and endosperm tissues and from different tissues of coffee plant. Specific spatial transcription of CaPME04 was found in pericarp at 210 days after flowering (DAF). We also observed low levels of CaPME04 transcripts in endosperm at 210 DAF. These results suggest that this isoform acts specifically during the later stages of fruit ripening and probably contributes to the coffee fruit softening.

INTRODUCTION

The plant cell wall is an intricate structure involved in the determination of cell size and shape, growth and development, intercellular communication, and interaction with the environment. The primary cell wall is largely composed of polysaccharides (cellulose, hemicelluloses and pectins), enzymes and structural proteins (Micheli, 2001). Pectins are a highly heterogeneous group of polymers that are degradeted by pectinases as pectin methylesterase and polygalcaturonase. Pectin methylesterase catalyzes the methyl esterification of esters from polygalacturonic acid, increasing the susceptibility of pectins for the activity of polygalacturonases during ripening. PME activity was detected in fruits like apple, banana, cherry, citrus, grape, papaya, peach, pear, tomato, and strawberry (Pilnik and Voragen, 1970). The activity of PME increases as mature green tomatoes pass through different color stages to become full red. In plants PME was found to act in roots, shoots, leafs, fruits and is probably involved in fruit ripening and abcission. In coffee fruit there is a high percentage of pectins located in the seeds and pericarp (Carvalho, 1989). To study the whole changes that occurs during coffe fruit ripening some analyses in silico and in vivo of PME has been initiated.

MATERIALS AND METHODS

Through analyses on the Brazilian Coffee Genome Project database (http://www.lge.ibi.unicamp.br/cafe/), we identify different PMEs isoforms. These isoforms were analyzed through Sequencher 4.5 , BioEdit and Blast (BlastX and BlastP from NCBI home page) programs. ORF Finder (NCBI) and ClustalW programs were used to predict amino acid sequences and construct filogenetic trees, respectively. Contigs from HarvEST Coffee database, constructed with ESTs mainly from *C. canephora*, were also selected to construct filogenetic trees. Fruits at five different ripening stages were harvested from plants of *C. arabica* cv. IAPAR 59 cultivated under field conditions at Instituto Agronomico do Paraná (IAPAR). Fruit tissues (perisperm, endosperm, and pericarp) were separated and used independently to total RNA extraction according to Chang et al (1993). For Northern blot analysis, 15 μg of total RNA was denatured and resolved by electrophoresis. The RNA was transferred to nylon membranes and hybridized with *CaPME04* cDNA labeled with $dCTP^{32}$-α using a random primer method. Hybridization with Ultrahyb buffer (Ambion) and washing steps were performed according to the manufacturer's recommendations.

RESULTS AND DISCUSSION

In silico analysis

Based on Brazilian Coffee Genome databases 162 sequences from PME were identified. After local clusterization, sequences were aligned using Sequencher 4.5, generating 31 contigs. The sequences were analyzed and we observed that just eight contigs (named contig01 to contig08) were formed by sequences from fruits libraries. Each contig were analyzed through BlastP program to identify domains characteristic of PME. We were able to verify fours different domains, two of them related to PME (Pfam01095 and COG4677), one related do PME inhibition (pfam04043) and the last one related to Cu-Oxidize (table 1). Some PME proteins have a pro-region which is out in mature PME, this region must be degraded before the protein be secreted to cell apoplasm. This fact can explain the presence of the inhibitory domain in *CaPME04*.

Table 1. PME contigs characterization.

CONTIGS	*Lenght (pb)*	*Lengh (aminoacid)*	*Total of sequences*	*Sequences from fruit libraries*	*Domains*	*E-value (Domains)*
Contig 01	2382	261	20	04	pfam01095 COG4677	1e-122 3e-22
Contig 02	1946	583	14	06	pfam01095 COG4677 pfam04043	1e-134 8e-29 7e-23
Contig 03	1029	225	11	01	pfam04043	2e-28
Contig 04	1712	550	4	01	pfam01095 COG4677 pfam04043	3e-134 2e-36 5e-27
Contig 05	2193	448	17	02	pfam00394	3e-33
Contig 06	1847	541	10	03	pfam00394	3e-33
Contig 07	2146	546	19	04	pfam00394	2e-35
Contig 08	1085	274	03	01	pfam01095 COG4677	8e-60 4e-18

To predict the temporal expression, the selected contigs were compared with the sequences on the HarvEST coffee database, where fruit ESTs libraries were separated by developing stages Five contigs showed high expression during the final steps of fruit ripening and three others showed constitutive expression during fruit development (Figure 2)

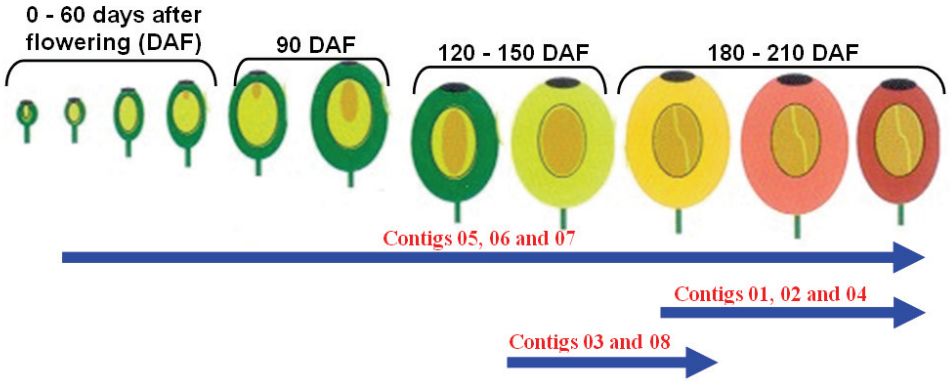


Figure 2. Stages of coffee fruit development and ripening and the probably expression of the selected contigs in silico (adapted from Marraccini and Castro 2006).

Contig04, named *CaPME04*, was chosen to initial molecular analysis. According to HarvEST plataform this isoform presents specific expression at the final stages of fruit ripening. Specific spatial transcription of *CaPME04* (Figure 3) was found in pericarp at 210 days after flowering (DAF). The highest levels of mRNA accumulation was observed in cherry fruit. We also observed low levels of *CaPME04* transcripts in endosperm at 210 DAF. These results suggest that this isoform acts specifically during the later stages of fruit ripening and probably contributes to the coffee fruit softening. Thus, *CaPME04* probably causes the degradation of pectics compounds present in the fruit pericarp. Similar PME action has being reported during fruit maturation and ripening (AHRENS; HUBER, 1990). Those results found with molecular analysis will contribute to detailed studies about the whole participation of *CaPME04* in coffee fruit ripening.

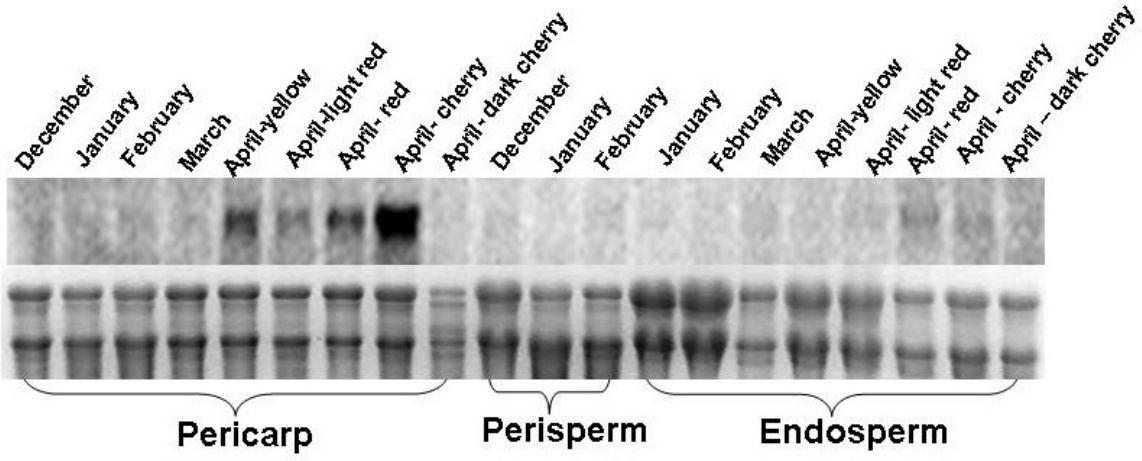


Figure 3. Northern blot of *CaPME04* during fruit development. Total RNA from the pericarp, perisperm and endosperm of *C. arabica* cv. IAPAR-59 in different stages of fruit development and ripening. Fruits were monthly collected: December to April. Different stages of fruit ripening were also collected during April according to fruit colours.

REFERENCES

Ahrens M.J., Huber D.J. Physiology and firmness determination of ripening tomato fruit. Physiologia Plantarum, V. 78, p.8-14, 1990.

Carvalho V.D., Chalfoun S.M., Chagas S.J. de R. Relação entre classificação do café pela bebida e composição físico química, química e microflora do grão beneficiado. In: Congresso brasileiro de pesquisas cafeeiras, 15., 1989, Maringá. Resumos... Rio de Janeiro: MIC/IBC, p.25-26, 1989.

De Castro R.D., Marraccini P.R. Cytology, Biochemestry and molecualr changes during coffee fruit development, Braz. J. Plant Physiol (2006), v.18, p. 175-199.

Micheli F. Pectin methylesterases: cell wall enzymes with important roles in plant physiology, Trends in Plant Science (2001), p. 414-419.

Pilnik W., Voragen A.G.J. Pectin substances and other uronides. In: A.C. Hulme, Editor, The Biochemistry of fruits and their products Academic Press, New York (1970), v.1, p. 53-87.

CoffEST is the Complete Resource for *Coffea* spp. EST Analysis

A.V. BARBOSA[1,2], R.M.O.B. SALES[1,2], F.R. DA SILVA[1]

[1]Embrapa Recursos Genéticos e Biotecnologia, CxP 02372, cep70.849-970, Brasília, DF, Brazil
[2]Universidade de Brasilia, Brasília, DF, Brazil
https://alanine.cenargen.embrapa.br/CoffEST
Supported by Embrapa, FINEP and CNPq

SUMMARY

EST Genome Projects are a relatively inexpensive way to describe genes. Effectively finding genes of interest, however, is complicated by the overwhelming amount of partial and redundant sequence data generated in such projects. We present here the Web interface to the EST sequence database maintained at Embrapa Recursos Genéticos e Biotecnologia. The database and the interface were originally developed to support the Brazilian Coffee Genome EST project , and latter incorporated *Coffea canephora* EST data contributed by Cornell University (59,718 raw EST sequences, Lin *et al.*, 2005) and Institut de Recherche pour le Développement – IRD (8,782 raw EST sequences, Poncet *et al.*, 2006). It is constantly updated as more data becomes publically available and can be freely accessed at https://alanine.cenargen.embrapa.br/CoffEST.

MATERIALS AND METHODS

CoffEST runs on a client-server architecture developed to support EST sequencing genome projects, loosely based on the one described by Telles *et al.* (2001), that we call WeBEST. The present work was done on a Sun V890 (4 UltraSPARC IV+ 1.5 GHz processors, 24 GB RAM, 500 GB HD) running Solaris 10. Apache is used as the front end, PHP scripts connect to the PostgreSQL database. The Fisher's Exact Test was implemented using the Python script language.

The CoffEST is built by processing the raw chromatogram data and assembling highly similar sequences on UniGenes, as described by Telles and da Silva (2001). UniGene sequences are then compared with all the nucleotide and protein sequences available at GenBank. All data are stored on a PostgreSQL relational database.

Chromatograms were kindly provided by their original authors. Lin et al. (2005) contributed with 59,718 chromatograms of clones from seven different libraries. Poncet et al. (2006) supplied 8,782 chromatograms of clones from one single library.

RESULTS

The 68,500 raw ESTs generated 53,615 trimmed ESTs that were assembled in 16,439 UniGenes. Contig4187 is the UniGene with the largest number of reads (450 reads from 7 libraries) and codes for a 2S albumin homologue. 9,192 trimmed ESTs show no significant similarity to any other EST on this set, and are single-read UniGenes. The cause of exclusion

of a raw EST from assembly is depicted on Table 1. Detailed explanations of the used criteria for trimming and discarding are found on Telles and da Silva (2001).

Table 1. Breakdown of the 14.885 ESTs not used on the UniGene assembly.

Motive of exclusion	Number of discarded sequences
Ribosomal sequences	58
Slippage sequences	5,746
Insert size (< 100bp)	8,032
Quality (less than 50bp > 20)	1,049

Searches on the resulting database can be directly performed on https://alanine.cenargen.embrapa.br/CoffEST and are based on 2 main criteria: homology and origin.

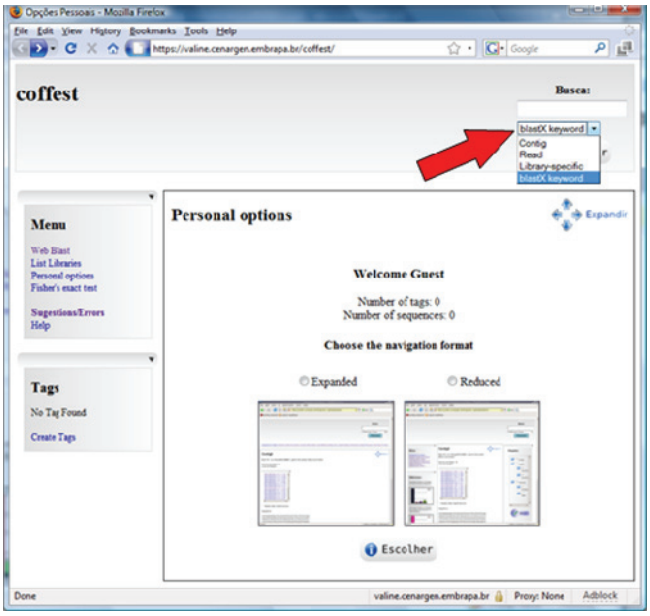


Figure 1. Initial CofEST page, showing the dropdown search menu.

There are three distinct homology based searches available. One can start from a nucleotide or peptide arbitrary sequence and find, using the Blast software, UniGenes, cleaned or raw sequences similar to it. Blast results link directly to the CoffEST database (Figure 2 and 3). It is also possible to input one or more keywords (including Boolean expressions) and find occurrence of those keywords on the pre-computed GenBank similarities. Finally, it is possible to browse the phylogenetic tree of similarities found on GenBank (Figure 4).

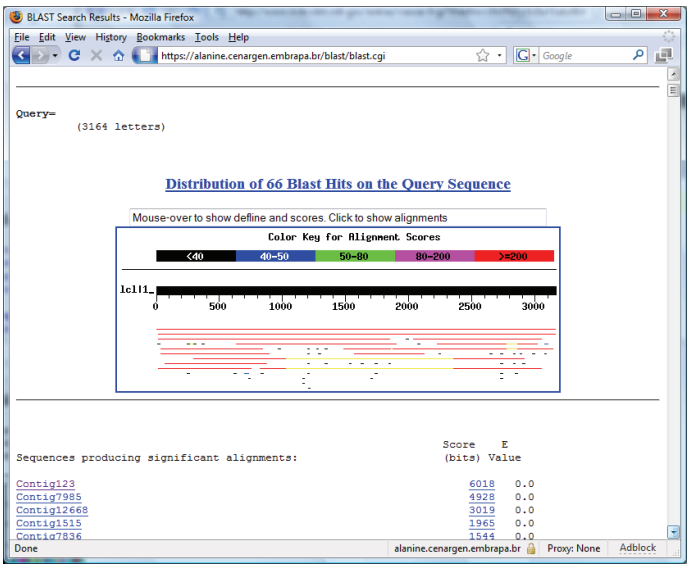


Figure 2. Blast results, directly linking to the CofEST results (Figure 3).

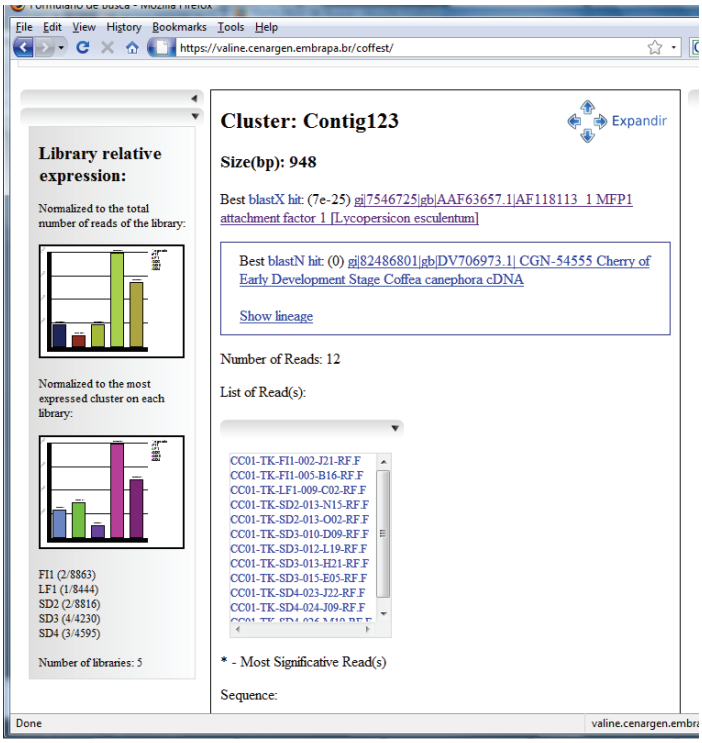


Figure 3. CofEST UniGene initial page, showing best hits found against nr (protein) and nt + est (nucleotide) NCBI databases, reads that form that UniGene and graphics depicting relative expression of the UniGene on the libraries.

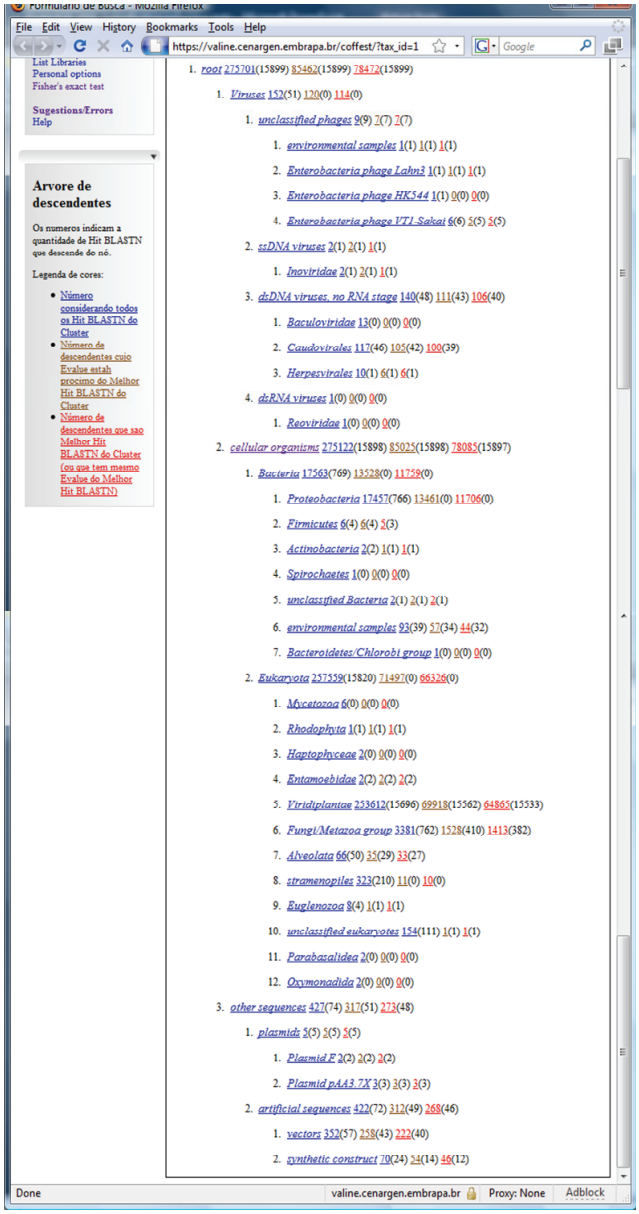


Figure 4. Phylogenetic tree of GenBank blastn hits found for all CoffEST UniGenes.

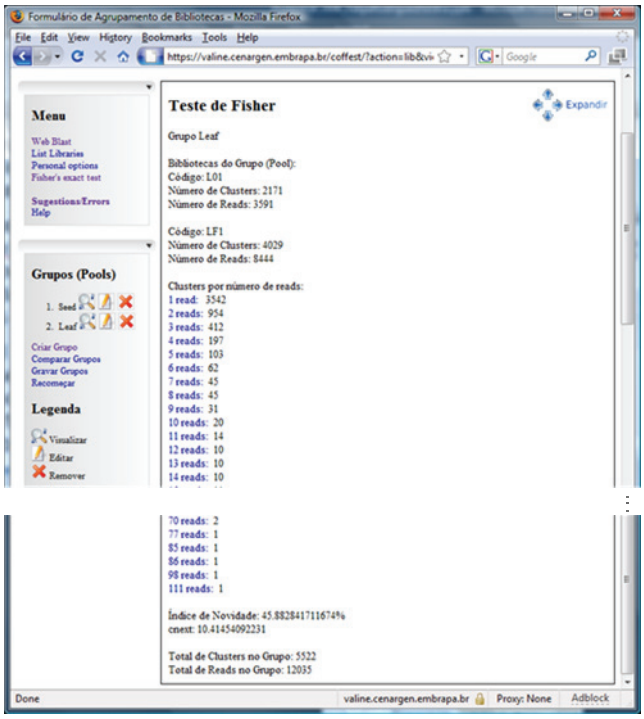


Figure 5. Visualization of a custom made set of libraries. Two sets of libraries can be compared using Fisher's Exact Test to find UniGenes over or under represented on one of the sets.

Origin searches can be as simple as retrieving a sequence by its name (read or UniGene) or the library it came from. More elaborated searches allow one to find UniGenes exclusively, preferentially or differentially expressed on one single library (or custom set of libraries – Figure 5).

Finally, CoffEST has all functionalities related to macro- and microarray analysis present on the Brazilian Coffee Genome EST project, including tools for building custom array designs and integration of data from diverse sources. As array data become publically available, it will also be available at CoffEST.

REFERENCES

Lin C., Mueller L.A., Carthy J.M., Crouzillat D., Pétiard V. and Tanksley S.D. (2005). Coffee and tomato share common gene repertoires as revealed by deep sequencing of seed and cherry transcripts. *Theoretical and Applied Genetics*, 112(1): 114-130.

Poncet V., Rondeau M., Tranchant C., Cayrel A., Hamon S., de Kochko A., Hamon P. (2006) SSR mining in coffee tree EST databases: potential use of EST-SSRs as markers for the Coffea genus. *Mol Genet Genomics* 276: 436-449

Telles G.P. and da Silva F.R. (2001). Trimming and clustering sugarcane ESTs. Genetics and Molecular Biology 24 (1-4): 17-23.

Telles G.P., Braga M.D.V., Dias, Z., Lin T.Z., Quitzau J.A.A., da Silva F.R. and Meidanis J. (2001). Bioinformatics of the sugarcane EST project. Genetics and Molecular Biology 24 (1-4): 8-15.

Optimization of Protein Coffee Leaf Quantification by Fluorescent Immunodetection Method

E.A. SOARES, S.H.C. ARAÚJO, C.Q.G. SOARES, A.M. ALMEIDA, M.E. LOUREIRO

Departamento de Biologia Vegetal, Universidade Federal de Viçosa, Viçosa – MG, Brazil.
E-mail: mehlers@ufv.br

SUMMARY

Protein immunodetection (Western blotting) is an important tool to dissect the function of a gene and physiological processes. In this work, we used a labeled secondary antibody with fluorophore (IgG – Cy5TM; goat – α – rabbit). The fluorescence was scanned by scanner (FLA-3000), and the image was analyzed by image quantitative analyzer software (Multi Gauge). Total coffee leaf protein from two *Coffea canephora* clones with contrasting drought tolerance, was extracted through phenol buffer method and separated by SDS-PAGE. The proteins were transferred to PVDF membranes using a semi-dry apparatus. Specific primary antibody against photosystem II D1 protein and Ribulose-1,5-biphosphate carboxylase-oxidase large subunit (RbcL) were used. These proteins play central role in key steps of photochemical and biochemical pathways of photosynthesis. Both proteins were detected as sharp bands emitting strong fluorescent signal in the imunoblottings, highest signal was obtained using chemioluminescent detection method (CDP-Star, GE), or colorimetric detection method (NBT + BCIP).

INTRODUCTION

Imunodetection is a technique broadly utilized for identification and quantification of proteins. It is substantiated in the specificity of adhesion of an antibody to its antigen, in case of a primary antibody linking to the protein of interest, and a secondary antibody coupled to a detection system that recognize the primary antibody. The commonly used approaches are laborious and of difficult diagnosis, since they are based in the addition of substrates for the enzymes connected to the secondary antibody. The direct detection by fluorescence is based in a secondary antibody linked to a fluorophore improving in this way the relation signal/background.

METHODOLOGY

Protein extraction was done through buffered pheno method in which soluble citosolic proteins extracted in 50 mM Tris-HCl pH 8.0 were precipitated in cetonic TCA 10% and subsequently precipitateda in 0,1 M metanolic amonium acetate (Guimarães, 2007). After protein quantification (Bradford, 1976), it was performed a SDS-PAGE 12% electrophoresis followed by semi-dry transferring to PVDF membrane. The membranes containing protein were blocked with 5% casein and incubated with 0,4 ng/ml anti RbcL and 0,75 ng/mL Anti D1 at different incubation times. Then the membranes were incubated with 0,75 ng/ml secondary antibody coupled to fluorophore Cy 5^{TM} (IgG-Cy5$^{TM;}$ goat-α-rabbit), dried or not before the fluorescence scanning in FLA-3000. The images were analysed in MultiGauge software *(FujiFilm Co, Japão)*.

RESULTS

Among the treatments used in the imunoblottings (Figure 1) 0,75 ng/mL secondary antibody solution incubated for 2h and anti-D1 for 12h without blocking with casein and dried for 1 h at 40 °C presented the best results for D1 protein detection. On the other hand, the most efficient condition for RbcL detection were obtained with 0,75 ng/mL secundary antibody incubated for 2h and 4 ng/mL Anti-RbcL for 12h without previous dry as can be seen in the fluorescence emission data generated by the imaging presented in Figure 2. The image analysis can be done visually as well (Fig. 1) with the best treatments lanes 4 and 15 for D1 e RbcL respectively or using the MultiGauge data (Figure 2) with the best treatments lanes 8 and 12 for D1 e RbcL respectively.

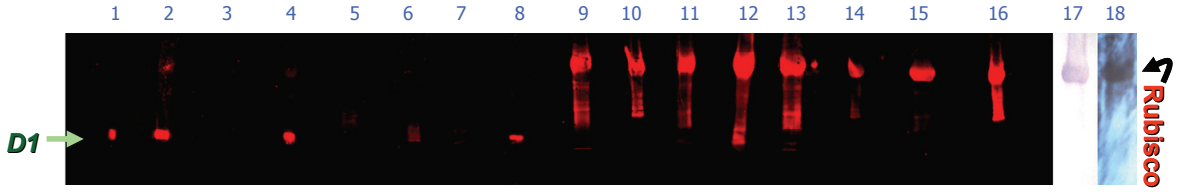


Figure 1. Imagens of coffee leaf protein Western blottings obtained with fluorescence canner de fluorescência (FLA-3000). 1- Anti-D1 (4h), Cy5TM + 5% casein, wet; 2- Anti-D1, (12h), Cy5TM + 5% casein, wet; 3- Anti-D1 (4h), Cy5TM , wet; 4- Anti-D1 (12h), Cy5TM , wet; 5- Anti-D1 (4h), Cy5TM + 5% casein, dry; 6- Anti-D1 (12h), Cy5TM + 5% casein, dry; 7- Anti-D1 (4h), Cy5TM, dry; 8- Anti-D1 (12h), Cy5TM, dry; 9- Anti-RbcL (4h), Cy5TM + 5% casein, wet; 10- Anti-RbcL (12h), Cy5TM + 5% casein, wet; 11- Anti-RbcL (4h), Cy5TM, wet; 12- Anti-RbcL (12h), Cy5TM, wet; 13- Anti-RbcL (4h), Cy5TM + 5% casein, dry; 14- Anti-RbcL (12h), Cy5TM + 5% casein, dry; 15- Anti-RbcL (4h), Cy5TM, dry; 16- Anti-RbcL (12h), Cy5TM, dry; 17- Colorimetrous detection through NBT/BCIP, 18- Chemiluminescent deteccion through CDP-Star®.

CONCLUSION

Visual analyses showed that fluorescence method was more efficient than the chemiluminescent (Figure 1, lane 17) and colorimetrous (Figure 1, lane 18) approaches. These methods can be used only for qualitative of detection of a protein. On the other hand fluorescent detection is both qualitative and quantitative.

Due to the availability of IgG-Cy3TM antibody and other labeled antibodies in the market we are able to carry out multiple detections in a same reaction, diminishing variability, improving resolution and saving resources.

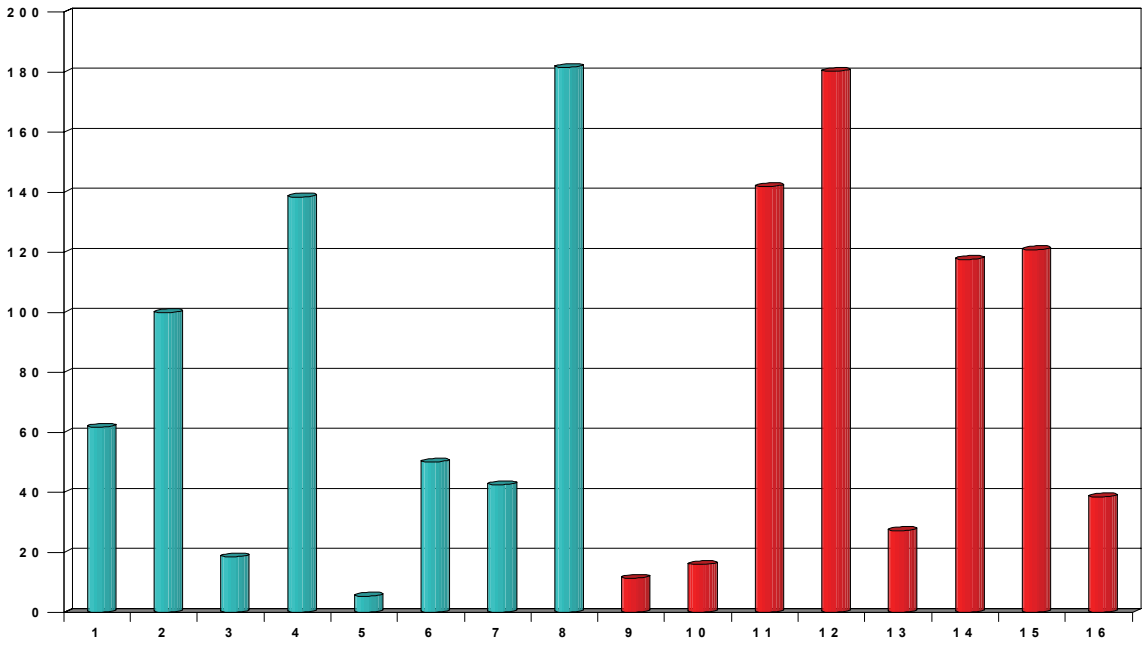


Figure 2. Fluorescence quantification obtained through Western blotting imaging analysis through MultiGauge (FujiFilm Co, Japão). Sample identifications are the same as figure 1.

Financial support: Brazilian consortium of coffee research and development.

REFERENCES

Bradford M.M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 284-254, 1976.

Guimarães B.L.S. Caracterização molecular da resposta adaptativa de clones de *Coffea canephora* em condições de déficit hídrico e otimização de metodologia para identificação de proteínas de folhas e raiz de café. Master thesis. UFV, 2007. 61pp.

Coffee Beans Contents of Soluble Solids and Chlorogenic Acids in Stock Plants of Robusta*

L.C. FAZUOLI[1], C.A. BRAGHINI[1], T.J.G. SALVA[1], M.T. BRAGHINI[1], M.B. SILVAROLLA[1], J.C. MISTRO[1], P.B. GALLO[2]

[1]Coffee Center 'Alcides Carvalho', Agronomic Institute, Campinas (IAC/APTA), SP, Brazil.
Email: café@iac.sp.gov.br
[2]Regional Center of Northeast Paulista, Mococa (APTA Regional), SP, Brazil
*Supported by the CBP&D and the CNPq.

SUMMARY

Since 1970, the Agronomic Institute, at Campinas, State of São Paulo, Brazil, has been selecting coffee plants of the *Coffea canephora* ex A. Froehner species, better known as robusta coffee. This study reports on the determination of the levels of soluble solids and chlorogenic acids in selected robusta coffee stock plants, which are characterized by high yield, high leaf-rust resistance, large beans with high medium-size sieve values and differentiated ripening patterns. The soluble solids contents of green beans of 125 coffee plants analyzed varied from 29.33% to 36.88%, while the level of chlorogenic acids ranged from 3.30% to 6.71%. The results obtained showed that those chemical constituents levels are subject to considerable variability. For this reason, it is possible to select clones of *C. canephora* with high levels of chlorogenic acids and soluble solids, which is of utmost importance to the soluble coffee industry.

INTRODUCTION

Nowadays, the species *Coffea canephora*, generically designated by robusta coffee, is responsible for 36% of the world's coffee production. This is a diploid and allogamous species, due to the occurrence of genetic incompatibility. Therefore, the crossed fecundation among plants of *C. canephora* originates coffee progenies with different genotypes. The clonal selection has been widely used by plant breeders, resulting that, potentially, each selected matrix may become a future clone. The development of coffee clones with higher soluble solids contents is fundamental to the soluble coffee industry. The chlorogenic acids are compounds that contribute to positive health effects, and besides, to better coffee beverage quality. In this context, research studies are going on at the Agronomic Institute, to select elite matrix plants of Robusta coffee in order to gather in an unique matrix, characters of high productivity and grains with high contents of soluble solids and chlorogenic acids.

MATERIALS AND METHODS

Seeds samples of *C. canephora* and derivates

In this study, seeds of 125 coffee plants were used from the germplasm bank of the Agency of Agricultural Research and Technology – APTA, located at Mococa county, State of São Paulo, Brazil, and coordinated by the Coffee Center 'Alcides Carvalho', Agronomic Institute, at Campinas, State of São Paulo, Brazil. The treatments are described as follows: 29 coffee plants from the EP 329 trial, corresponding to F1 hybrids of *C. canephora*; 69 coffee plants from the 'Lote Chácara', corresponding to several selections of *C. canephora*; and 27 coffee

plants from 'Lote 78', corresponding to 21 plants of cv. Apoatã IAC 2258 of *C. canephora* and six plants from the Bangelan population, (natural) native hybrids between *C. congensis* and *C. canephora*.

The coffee fruits were collected at the berry stage, the pulps were removed and approximately after 24 h they were washed to remove the mucilage layer. Then, beans were transferred to trays and sun-dried during one day; after that, beans were left to dry under shade during several days, until reaching 11% humidity. Afterwards, the bean skins were removed and seeds were sent to chemical analysis.

Chlorogenic acids quantifying

Chlorogenic acids extraction procedure: 100 mg of ground (green, unroasted) coffee seeds were transferred to 5 mL of 70% methanol solution for HPLC, at 60 °C, during one hour. This mixture was centrifuged and the supernatant was filtered through a 0.22 μm thick membrane. The compound was quantified by high-performance liquid chromatography (HPLC), according to procedure adapted from Casal et al. (2000). A constant-composition mobile phase (isocratic elution) was used, constituted of methanol: acetic acid: water (50:0.5:49.5, v:v:v), at a rate of 1 mL/min, at room temperature. The chlorogenic acid concentrations were determined through daily obtained standard curves using standard solutions of 5-caffeoylquinic acid (Sigma). Analysis were run in duplicates.

Soluble solids quantifying

The soluble solids concentrations were determined in duplicates according to the following procedure: 5 g of ground green coffee seeds were mixed with 100 mL of hot water, filtered and the filtrate was dried at 105 °C until constant mass, according to procedure adapted from method # 15034 of AOAC (1997).

RESULTS AND DISCUSSION

Soluble solids

The data obtained showed a variation range of 30.60-36.88% among the F1 coffee hybrids from the EP 329 trial; of 29.33-34.67% among the robusta coffee plants; 30.77-35.60% among the cv. Apoatã; and of 31.21-35.49% among coffee hybrids from the *C. congensis* x *C. canephora* crossing (Table 1). Among the 125 coffee beans samples analyzed, the ten coffee samples with higher contents of soluble solids, presented also high chlorogenic acids contents (Table 2). It is important to point out that the high soluble solid contents found in the matrix plants of the present research work were superior to the levels found in other matrix plants of Robusta coffee analyzed by Aguiar et al. (2005) and Veneziano and Fazuoli (2000). As concerned to the Bangelan population (natural *C. congensis* x *C. canephora* hybrids), only one elite plant was found with high soluble solids contents (35.49) (Table 2).

Table 1. Variation range and means of soluble solids and chlorogenic acids concentrations found in coffee beans of 125 elite matrix plants selected from *C. canephora* of trials EP 329, 'Lote Chácara', cv. Apoatã IAC 2258 and Bangelan population of 'Lote 78' (L.78).

Elite matrix plants selected in the trials	Total # of analyzed plants	Variation range of chemical components concentrations		Means of chemical components concentrations	
		Soluble solids (%dm)	Chlorogenic acids (%dm)	Soluble solids (%dm)	Chlorogenic acids (%dm)
EP 329	29	30.60 – 36.88	3.30 – 6.71	33.84	5.25
Lote Chácara	69	29.33 – 34.67	3.87 – 5.93	31.64	4.95
L.78-cv. Apoatã IAC 2258	21	30.77 – 35.60	3.78 – 5.91	32.39	5.11
L.78- Bangelan pop.	6	31.21 – 35.49	5.17 – 5.67	33.32	5.36
TOTAL	125				

Table 2. The ten selected matrix plants presenting high soluble solids concentrations and the respective chlorogenic acid concentrations.

Selected coffee plants	Chemical components	
	Soluble Solids (%dm)	Chlorogenic acids (%dm)
Plant 42 – EP 329	36.88	5.40
Plant 10 – EP 329	36.25	3.30
Plant 44 – EP 329	36.01	5.78
Plant 50 – EP 329	35.74	4.97
Plant 28 – EP 329	35.73	6.18
Plant 332 – L.78-cv. Apoatã	35.60	5.54
Plant 37 – EP 329	35.59	4.90
Plant 75 – L.78- Bangelan pop.	35.49	5.48
Plant 2 – EP 329	35.32	5.50
Plant 7 – EP 329	35.09	5.01

Chlorogenic acids

These compound concentrations varied from 3.30 to 6.71% among hybrid coffee plants from EP 329 trial. The variation range among the robusta coffee plants was 3.78 to 5.93%; and, among the hybrid plants from the *C. congensis* x *C. canephora* crossing, was 5.17 to 5.67% (Table 1). Therefore, the data evidenced the feasibility of selecting elite plants of robusta type with high chlorogenic acids levels, which is desirable for a better robusta coffee beverage quality.

Table 3. The ten selected matrix plants presenting high chlorogenic acids concentrations and the respective soluble solids concentrations.

Selected coffee plants	Chemical components	
	Chlorogenic acids (%dm)	Soluble Solids (%dm)
Plant 53 – EP 329	6.71	34.80
Plant 28 – EP 329	6.18	35.73
Plant 25 – EP 329	5.97	30.60
Plant 23 – Lote Chácara	5.93	32.89
Plant 438 – L.78-cv. Apoatã	5.91	32.31
Plant 846 – Lote Chácara	5.79	33.85
Plant 323 – L.78-cv. Apoatã	5.79	31.72
Plant 44 – EP 329	5.78	36.01
Plant 1010 – Lote Chácara	5.76	32.00
Plant 60 – EP 329	5.72	34.22

The chlorogenic acids concentrations found in Robusta coffee beans were similar to the ones reported by Aguiar et al. (2005). It is interesting to point out that some of the ten selected plants with high levels of chlorogenic acids showed also high levels of soluble solids (Table 3).

CONCLUSIONS

1. The robusta coffee plants evaluated presented high variability for the soluble solids and chlorogenic acids concentrations.
2. It is feasible to select highly productive robusta clones with high bean levels of soluble solids and chlorogenic acids.

REFERENCES

Aguiar A.T.E., Fazuoli L.C., Salva T.J.G., Favarim J.L. Diversidade química de cafeeiros na espécie *Coffea canephora*. Bragantia, Campinas, v.64, n.4, p.577-582, 2005.

Casal S., Oliveira B., Ferreira M.A. HPLC/diode-array applied to the thermal degradation of trigonelline, nicotinic acid and caffeine in coffee. Food Chemistry, London, v.68, n.4, p.481-485, 2000.

Veneziano W., Fazuoli L.C. Avaliação de cultivares de cafeeiros robusta (*Coffea canephora*) em Rondônia. In: SIMPÓSIO DE PESQUISAS DOS CAFÉS DO BRASIL, 1, Poços de Caldas, 2000. Anais. Brasília, Embrapa Café/Minasplan, p. 459-461, 2000.

Use of Pichloram for Somatic Embryogenesis Induction in Coffee (*Coffea arabica* L.) Leaf Explants

M.R. DE A. FERREIRA, J.M. FERREIRA, J.B. TEIXEIRA

Embrapa-Recursos Genéticos e Biotecnologia, Parque Estação Biológica – PqEB, Av. W5 Norte (final) 70770-900 – Brasília, DF – Brazil

SUMMARY

A protocol for cloning adult coffee (*Coffea arabica* L.) plants using pichloram as the primary auxin source has been developed. Mature leaves taken from the second and third nodes of plagiotropic branches from 'Mundo Novo' greenhouse-grown adult plants were disinfected in 2.4% sodium hypochlorite solution for 15 minutes, and then washed three times in sterile distilled water. Leaf explants measuring 0.7 by 0.7 cm approximately were inoculated on modified C medium (Van Boxtel and Berthouly, 1996), supplemented with 2% sucrose and different concentrations of pichloram (2.5, 5, 10 and 20 μM) in the absence of 2,4-D. After one month the explants were transferred to fresh media with the respective pichloram concentrations. The cultures were kept in the dark under 25 ± 1 °C. After four to five months from the beginning of experiment, the percentage of embryogenic callus formation was evaluated. After subculturing the explants were maintained on the same Petri dish without renewing the media. Each treatment consisted of 10 Petri dishes with 6 explants each in a full randomized statistical design. The rate of contamination was lower than 5%, and consisted basically of fungi colonies. Primary callus formation became visible after two weeks of culture around de cut edge of explants at the position of secondary veins. After one month of culture the amount of primary callus was the maximum on those responsive explants. The rate of primary callus formation was always higher than 90%. After transferring to fresh medium primary calli stop growing and began to turn brown. At the moment of embryogenic callus evaluation 3 to 4 months later the primary calli were completely oxidized. Leaf explants first turned yellow at the end of around one month and then turned brown by the end of second month of culture. Embryogenic sectors began to form on the surface of primary oxidized calli after around three to four months from the beginning of experiment. First they were seen as tinny yellow spots when observed under stereomicroscope. At the end of four to five months embryogenic calli measuring 2 to 5 mm in diameter could easily be isolated and cultured on multiplication medium. In general one embryogenic sector formed on each explant although it was frequently found two or more embryogenic sectors per explant. The embryogenic callus formation appeared synchronously on different explants of the same Petri dish and on explants of different Petri dishes. Embriogenic sectors presented a light yellow color, were friable, and consisted of small (around 0.5 mm in diameter) cell aggregates. The rate of embryogenic callus formation was 16.7, 50.0, 40.0 and 33.3% respectively for 2.5, 5, 10 and 20 μM. When these calli were isolated and cultured in liquid multiplication medium the fresh weight doubling time was around two weeks. After transferring to regeneration medium high frequency of normal somatic embryos began to form.

INTRODUCTION

A protocol for cloning adult coffee (*Coffea arabica* L.) plants using pichloram as the primary auxin source has been developed.

MATERIALS AND METHODS

Mature leaves taken from the second and third nodes of plagiotropic branches from 'Mundo Novo' greenhouse-grown adult plants were disinfected in 2.4% sodium hypochlorite solution for 15 minutes, and then washed three times in sterile distilled water. Leaf explants measuring 0.7 by 0.7 cm approximately were inoculated on modified C medium (Van Boxtel and Berthouly , 1996), supplemented with 2% sucrose and different concentrations of pichloram (2.5, 5, 10 and 20 μM) in the absence of 2,4-D. After one month the explants were transferred to fresh media with the respective pichloram concentrations. The explants were maintained in the same Petri dish without renewing the media. The cultures were kept in the dark under 25±1 °C. After four to five months from the beginning of experiment, the percentage of embryogenic callus formation was evaluated. Each treatment consisted of 10 Petri dishes with 6 explants each in a full randomized statistical design.

RESULTS AND DISCUSSION

The rate of contamination was lower than 5%, and consisted basically of fungi colonies. Primary callus formation became visible after two weeks of culture around de cut edge of explants at the position of secondary veins. After one month of culture the amount of primary callus was the maximum on those responsive explants (Table 1; Figure 1A). After transferring to fresh medium primary calli stop growing and began to turn brown. Leaf explants first turned yellow at the end of first month and then turned brown by the end of second month of culture. At the moment of embryogenic callus evaluation 3 to 4 months later the primary calli were completely oxidized. Embryogenic sectors began to form on the surface of primary oxidized calli after around three to four months from the beginning of experiment. Initially they were seen as tinny yellow spots when observed under stereomicroscope. At the end of fourth to fifth months embryogenic calli measuring 2 to 5 mm in diameter could easily be isolated and cultured on multiplication medium (Figure 1B). In general one embryogenic sector formed on each explant although it was frequently found two or more embryogenic sectors per explant. The embryogenic callus formation appeared synchronously on different explants of the same Petri dish and on explants of different Petri dishes. Embriogenic sectors presented a light yellow color, were friable, and consisted of small (around 0.5 mm in diameter) cell aggregates (Figure 1C). The rate of embryogenic callus formation varied from 16.7 to 50.0 depending on pichloram concentration (Table 1). When these calli were isolated and cultured in liquid multiplication medium the fresh weight doubling time was around two weeks. After transferring to regeneration medium high frequency of normal somatic embryos have formed within 2 to 3 months (Figure 1D).

Table 1. Embryogenic callus induction in 'Mundo Novo' leaf explants as affected by the concentration of pichloram.

Pichloram concentration	Primary callus formation (%)	Embryogenic callus induction (%)
2.5 μM	100	16.7
5.0 μM	100	50.0
10.0 μM	100	40.0
20.0 μM	100	33.3

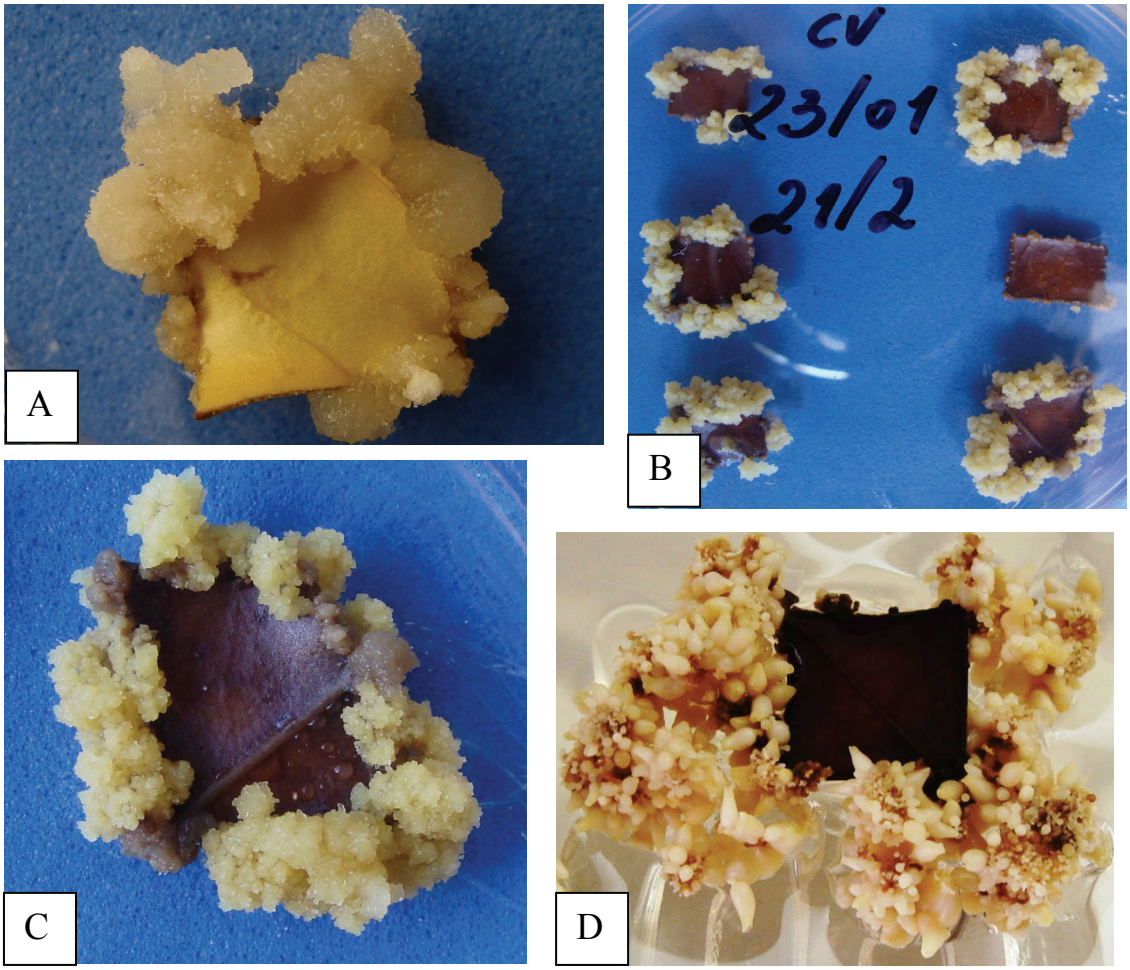


Figure 1. Somatic embryogenesis induction by pichloram. A. Primary callus formation after one moth of culture on primary medium; B. Explants showing embryogenic callus formation after 5 months of culture; C. Embryogenic sector formed around the original explant; D. Somatic embryos originated from embryogenic sector after 3 months on regeneration medium.

REFERENCE

Van Boxtel J. and Berthouly M. High frequency somatic embryogenesis from coffee leaves. Factors influencing embryogenesis, and subsequent proliferation and regeneration in liquid medium. Plant Cell, Tissue and Organ Culture 44:7-17, 1996.

Optimization of Somatic Embryogenesis in Coffee (*Coffea arabica* L.) Leaf Explants

J.B. TEIXEIRA, C.S. JUNQUEIRA, C.H.S. CARVALHO

Embrapa-Recursos Genéticos e Biotecnologia, Parque Estação Biológica – PqEB, Av. W5 Norte (final) 70770-900 – Brasília, DF – Brazil

SUMMARY

Cloning superior plants of coffee (*C. arabica* L.) requires a high efficient somatic embryogenesis protocol. Cloning via cutting or grafting is very inefficient. With the objective of optimizing somatic embryogenesis protocol, sucrose (1, 2, 3, 4 e 8%), and maltose (2, 4 e 8%) in presence of 1% sucrose were tested using the 'Catuaí Vermelho' variety. In another experiment 2,4-D (10, 20, 30 and 40 µM) in presence of 2% sucrose were tested for five different genotypes ('Mundo Novo', 'Icatu Amarelo', 'Acaiá Cerrado', 'Catuaí Vermelho' and 'Rubi'). Expanded leaves from greenhouse growing plants, approximately three years old were disinfected in alcohol 70% for 1-3 minutes, followed by sodium hypochlorite 2.4% for 20 minutes and washed three times with sterile distilled water. Explants measuring approximately 0.7 by 0.7 cm were inoculated on 20 ml basic C medium (Van Boxtel and Berthouly, 1996) in Petri dishes. After 30 days, explants were transferred to fresh medium, remaining there for 3 to 4 months until the somatic embryogenic sectors have formed. The cultures were kept in the dark under temperature of 25 ± 1 °C. The evaluation was done by calculating the percentage of explants presenting at least one embryogenic sector. Each treatment consisted of 10 plates with 6 explants each, under a full randomized statistical design. The embryogenic callus formation rate for 'Catuaí Vermelho' was 37.5%; 70.3%; 12.5%; 9.3% e 0.0%, respectively for 1, 2, 3, 4, and 8% sucrose. Regarding maltose, the rate was 51.9%; 12.5%, and 14.8%, respectively for 2; 4 and 8%. The auxin 2,4-D was evaluated for three coffee varieties, i.e., 'Catuaí Vermelho', 'Mundo Novo', and 'Rubi'. The rate of embryogenic callus formation varied from 0 to 55.6% according to 2,4-D concentration. The rate of 55.6% was observed for the concentrations of 10 and 20 µM 2,4-D for 'Catuaí Vermelho' and 51.7% for the concentration of 20 µM 2,4-D for 'Rubi'. On the other hand for 'Mundo Novo' the best result was observed for 10 µM 2,4-D. In another experiment embryogenic callus formation was evaluated for different varieties in the presence of 20 µM 2,4-D and 2% sucrose. The rates were 8.3%; 36.8%; 41.9%; 100%, and 84.3%, respectively for 'Mundo Novo', 'Icatu Amarelo'; 'Acaiá Cerrado'; 'Catuaí Vermelho', and 'Rubi'. Embryogenic callus formation was considerably lower for 'Mundo Novo'. We concluded that embryogenic callus formation depends on 2,4-D as well as type and concentration of sugar utilized. Besides, different genotypes behave differently regarding embryogenic response. . The embyogenic competence of embryogenic sector was demonstrated when they were cultivated in the regeneration liquid medium under agitation. Therefore, the Van Boxtel and Berthouly (1996) protocol can substantially be improved by increasing 2,4-D concentration in C medium as well as by replacing the secondary E medium by the modified C medium.

INTRODUCTION

Cloning superior plants of coffee (*C. arabica* L.) requires a high efficient somatic embryogenesis protocol. Cloning via cutting or grafting is very inefficient. Somatic embryogenesis of *Coffea* species is well documented (Staritsky, 1970; Söndahl and Sharp,

1977; Yasuda et al., 1985; Van Boxtel and Berthouly, 1996). Van Boxtel and Berthouly (1996) have described a somatic embryogenesis protocol for *Coffea arabica*, *Coffea canephora*, Arabusta and Congusta clones. According to these latter authors, using their new protocol was possible to obtain as much as 96.9% of explants with friable HFSE callus formation for *C. canephora* clones. On the other hand for *C. arabica* genotypes HFSE callus formation was at most 10%. According to several authors (Söndahl and Sharp, 1977; Van Boxtel and Berthouly, 1996) including a strong auxin like 2,4-D into the medium is very important to induce somatic embryogenesis in coffee leaves. With the objective of trying to improve the protocol for HFSE induction in coffee leaves several experiments were carried out involving a few genotypes of *Coffea arabica* and the basic C medium developed by Van Boxtel and Berthouly (1996).

MATERIALS AND METHODS

Expanded leaves from greenhouse growing plants, approximately three years old were disinfected in alcohol 70% for 1-3 minutes, followed by sodium hypochlorite 2.4% for 20 minutes and washed three times with sterile distilled water. Explants measuring approximately 0.7 by 0.7 cm were inoculated on 20 ml modified C medium (Van Boxtel and Berthouly, 1996) in Petri dishes. After 30 days, explants were transferred to the same C basic medium, remaining there for 3 to 4 months until somatic embryogenic sectors have formed. The cultures were kept in the dark under temperature of 25±1 °C. The evaluation was done by calculating the percentage of explants presenting at least one embryogenic sector. Each treatment consisted of 10 plates with 6 explants each, under a full randomized statistical design. Initially, 2,4-D (10, 20, 30 and 40 μM) in presence of 2% sucrose was tested for three different varieties (‘Rubi’, ‘Mundo Novo’, and ‘Catuaí Vermelho’). The respective 2,4-D concentration was maintained in the secondary medium. In another experiment, sucrose (1, 2, 3, 4 e 8%), and maltose (2, 4 e 8%) in presence of 1% sucrose were tested using 'Catuaí Vermelho', and C medium containing 20 μM 2,4-D. Finally, embryogenic callus formation was evaluated for five different varieties (‘Mundo Novo’, ‘Icatu Amarelo’; ‘Acaiá Cerrado’; ‘Catuaí Vermelho’, and ‘Rubi’) on C medium containing 20 μM 2,4-D and 2% sucrose.

RESULTS AND DISCUSSION

The rate of embryogenic callus formation varied from 0 to 55.6% according to 2,4-D concentration (Figure 1). The rate of 55.6% was observed for the concentrations of 10 and 20 μM 2,4-D for ‘Catuaí Vermelho’ and 51.7% for the concentration of 20 μM 2,4-D for 'Rubi'. On the other hand, for 'Mundo Novo' the best result was observed for 10 μM 2,4-D (Figure 1). The embryogenic callus formation rate varied from 0 to 70.3% according to sucrose or maltose concentration (Figure 2). The highest percentage of embryogenic callus formation (70,3%) was found for 2% sucrose. The second highest value was observed for 2% maltose in presence of 1% sucrose. Finally, embryogenic response varied quite substantially when a basic protocol was essayed for different genotypes. The rates varied from 8.3% for ‘Mundo Novo’ to 100% for ‘Catuaí Vermelho’ (Figure 3).

Therefore we have concluded that high frequency of embryogenic callus formation depends on high 2,4-D as well as type and concentration of sugar utilized. Besides, different genotypes behave differently regarding embryogenic response. The embyogenic competence of embryogenic sector was demonstrated when they were cultivated on the Van Boxtel and Berthouly (1996) regeneration liquid medium under agitation (Fig. 4A-F). Therefore, the Van Boxtel and Berthouly (1996) protocol can substantially be improved by increasing 2,4-D concentration in C medium as well as by replacing the secondary E medium by the modified C medium. Nevertheless evaluation of possible somaclonal variation needs to be carry out as

substantial increase on 2,4-D levels can lead to genetic or epigenetic variations on derived plantlets. Visual observations on plants growing in the field has shown that no phenotypic variations have been detected.

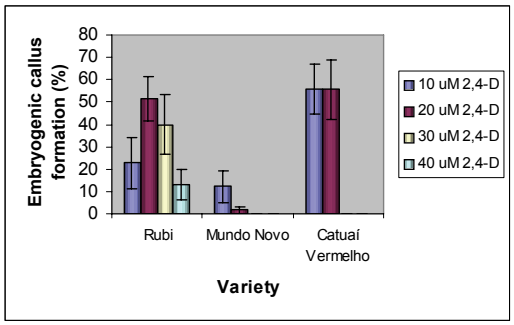


Figure 1. Embryogenic callus formation in leaf explants from three *C. arabica* L. genotypes in presence of different concentrations of 2,4-D, and 2% sucrose.

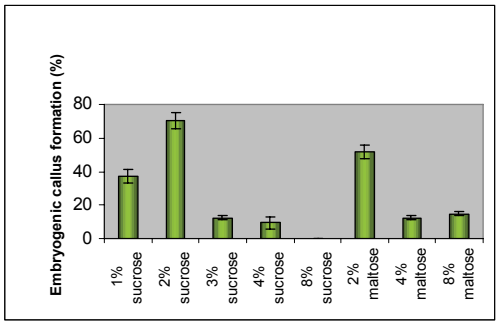


Figure 2. Embryogenic callus formation in leaf explants of *C. arabica* L. var. 'Catuaí Vermelho', under different concentrations of sucrose and maltose.

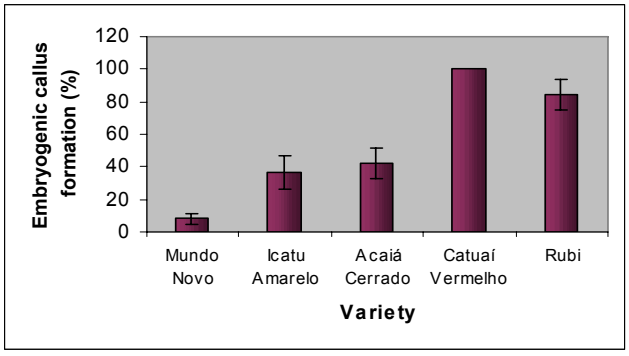


Figure 3. Embryogenic callus formation in leaf explants from five *C. arabica* L. genotypes on modified C (Van Boxtel and Berthouly, 1996) medium, 20 µM 2,4-D, and 2% sucrose.

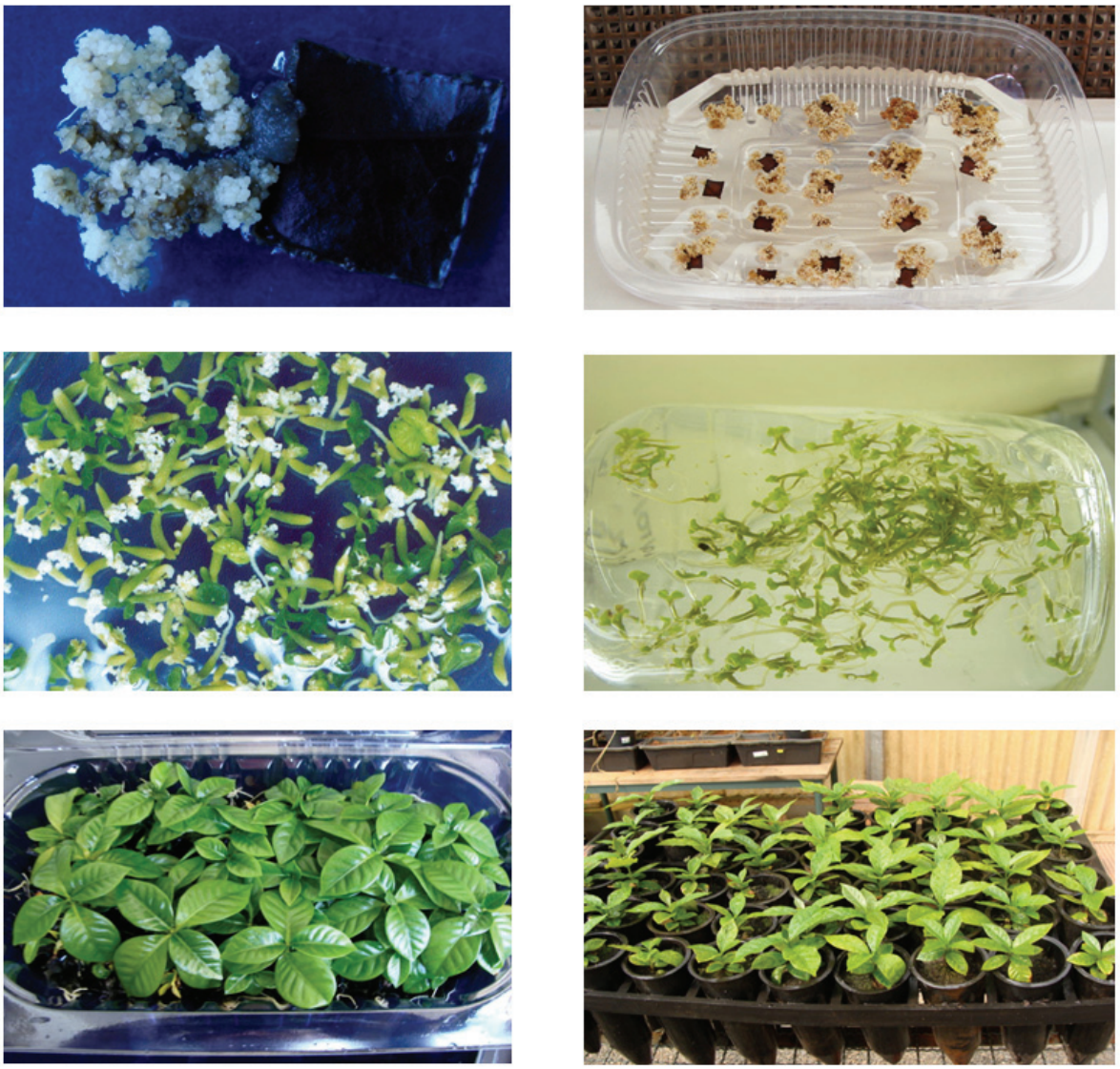


Figure 4. Somatic embryogenesis and plant recovery from coffee leaf explants. A. Embryogenic callus formation; B. Embryo differentiation; C. Embryo maturation; D. Embryo germination in temporary immersion bioreactor; E. Plant development; F. Plant acclimatization.

REFERENCES

Staritsky G. Embryoid formation in callus tissues of coffee. Acta Bot. Neerl. 10 (4):509-514, 1970.

Söndahl M.R. and Sharp W.R. High frequency induction of somatic embryos in cultured leaf explants of *Coffea arabica* L. Zeitschrift für Pflanzenphysiology 81:395-408, 1977.

Yasuda T., Fujii Y. and Yamaguchi T. Embryogenic callus induction from *Coffea arabica* leaf explants by benzyladenine. Plant Cell Physiology 26(3):595-597, 1985.

Van Boxtel J. and Berthouly M. High frequency somatic embryogenesis from coffee leaves. Factors influencing embryogenesis, and subsequent proliferation and regeneration in liquid medium. Plant Cell, Tissue and Organ Culture 44:7-17, 1996.

Effect of PEG on the Somatic Embryogenesis of *Coffea arabica* Genotypes*

J.A.S. ALMEIDA[1*], D.F.S.P. MACHADO[2], M.B. SILVAROLLA[1], E.C. MACHADO[3]

[1]Centro de Café 'Alcides Carvalho', Instituto Agronômico de Campinas, CP 28, 13.001-970. Campinas, SP, Brasil. *E.mail: julietasa@iac.sp.gov.br
[2]Student of Masters, Agricultura Tropical e Subtropical, Instituto Agronômico de Campinas, Brasil
[3]Centro de Pesquisa e Desenvolvimento de Ecofisiologia e Biofísica, Instituto Agronômico de Campinas, CP 28, 13.001-970, Campinas, SP, Brasil
**Supported by the Financiadora de Estudos e Projetos (FINEP) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

SUMMARY

In vitro cultivated tissues generally show similar behaviour to plants grown in the field when submitted to stress, although the pattern of morphological and/or physiological responses of the tissues may be modified. Thus the objective of the present study was to verify the effect of adding polyethylene glycol (PEG) on the somatic embryogenesis capacity of the *Coffea arabica* genotypes AC1 and Mundo Novo IAC 376-4 (MN). Rectangular foliar explants of these genotypes were inoculated into a semi-solid culture medium that consisted of ½ MS salts added of 30 μM 6-benzyladenine and 0, 5 or 7% PEG 6000, and maintained at 25 °C in the absence of light. The treatments were evaluated with respect to the percentage of explants showing initiation of the formation of embryogenic structures, the number of sides of the explant showing the formation of embryogenic structures, the coloration and an estimate of the size of these structures and the number of embryos. In addition the osmotic potentials of the culture medium and of the explants inoculated into it were determined. It was verified that amongst the genotypes, AC1 presented greater efficiency with respect to the somatic embryogenesis capacity. An alteration in the osmotic potential was also observed both in the culture medium and in the tissue, which became more negative with the occurrence of the somatic embryogenesis process.

INTRODUCTION

In Brazil, coffee bean production contributes meaningfully for the country economy, however, its production efficiency can be influenced in a negative way by the drought caused by climate changes due global warming.

The water stress condition can be induced in vegetal tissues *in vitro* by polyethylene glycol (PEG) addition in culture medium, in a similar way to the verified plant cells under water restriction (Hsiao, 1973).

The PEG has a high molecular weight, is inert, water soluble, non ionic and non toxic (Ahmad et al., 2007), it does not penetrates in vegetal cells and cause reduction in the medium osmotic potential (Bressan et al., 1981). In literature, it is verified that water stress induction *in vitro*, through PEG, has caused development changes in different species among which *Lycopersicon esculetum* (Bressan et al., 1982); *Glicine max* (Lucca-Braccini et al., 1996);

Phaseolus acutifolius (Mohamed and Tawfik, 2006); *Lycopersicon esculetum* (Kulkarni and Deshpande, 2007) and *Brassica napus* (Ferrie and Keller, 2007).

The *Coffea* has been broadly cultivated *in vitro* through somatic embryogenesis process, which can occur by indirect means, from calluses or direct means, whose embryos are formed directly from explants edge cells (Emons, 1994). Thus the objective of the present study was to verify the effect of adding PEG on the somatic embryogenesis capacity of the *Coffea arabica* genotypes AC1 and Mundo Novo IAC 376-4.

MATERIAL AND METHODS

Leaves were collected from AC1 *Coffea arabica* and the Mundo Novo IAC 376-4 (MN) cultivar plants as control, grown under greenhouse conditions at the IAC. Square-shaped explants of 2 cm^2 from these leaves were inoculated in flasks in modified medium of Yasuda et al. (1985) containing half-strength MS (Murashige and Skoog, 1962), enriched with 6-BA (benzyladenine) at concentration of 30 μM/L. Besides, with the addition of PEG 6000 at concentrations of 0, 5 and 7 %. It was too determinated the osmotic potentials of the culture medium and of the explants inoculated by Psicrometro C58 (Wescor).The treatments comprised 10 flasks with three explants in each one, which were maintained in absence of light, at 25 ^{o}C. The following parameters were evaluated in the treatments: explant with structures formation, number of explant sides with structure formation, estimated structure size and the number of embryos formed.

RESULTS AND DISCUSSION

Leaf explants of *Coffea arabica* genotypes AC1 and MN had somatic embryogenesis responses under PEG effect in all used concentrations (0, 5 and 7 %) (Figure 1). Nevertheless, the answer was different among the genotypes and also related to PEG concentrations. It can be noticed that the AC1 genotype have shown most explants with a higher percentage of embryogenic structures (Figure 1A) and number of explant sides with structure formation than MN in all PEG concentrations (Figure 1B). Moreover, these structures formed by explants from AC1 genotype, also, reached a bigger size than the ones formed by MN genotype (Figure 1C). It can be observed yet, that these structures presenting oxidation at the end of the experiment.

The embryos formation had started, for both genotypes, at 120 days from the beginning of the experiment (Figure 1D). However, the genotype AC1 formed a larger embryos number than MN genotype. Thus, the use of PEG has favored the embryos production through the AC1 genotype compared to the treatment control, mainly, for the 7% concentration (Figure 2).

The Table 1 presents evaluation values from the culture medium osmotic potential and from the explants tissues, with or without PEG addition. At the 30 day, it is noticed an osmotic potential reduction comparing to the beginning of the experiment (0 day) both for culture medium as explants. It is observed that the MN genotype had presented a higher osmotic potential reduction than AC1 genotype. These results suggest that for the AC1 genotype, its somatic embryogenesis capacity can be influenced by through PEG addition in the culture medium.

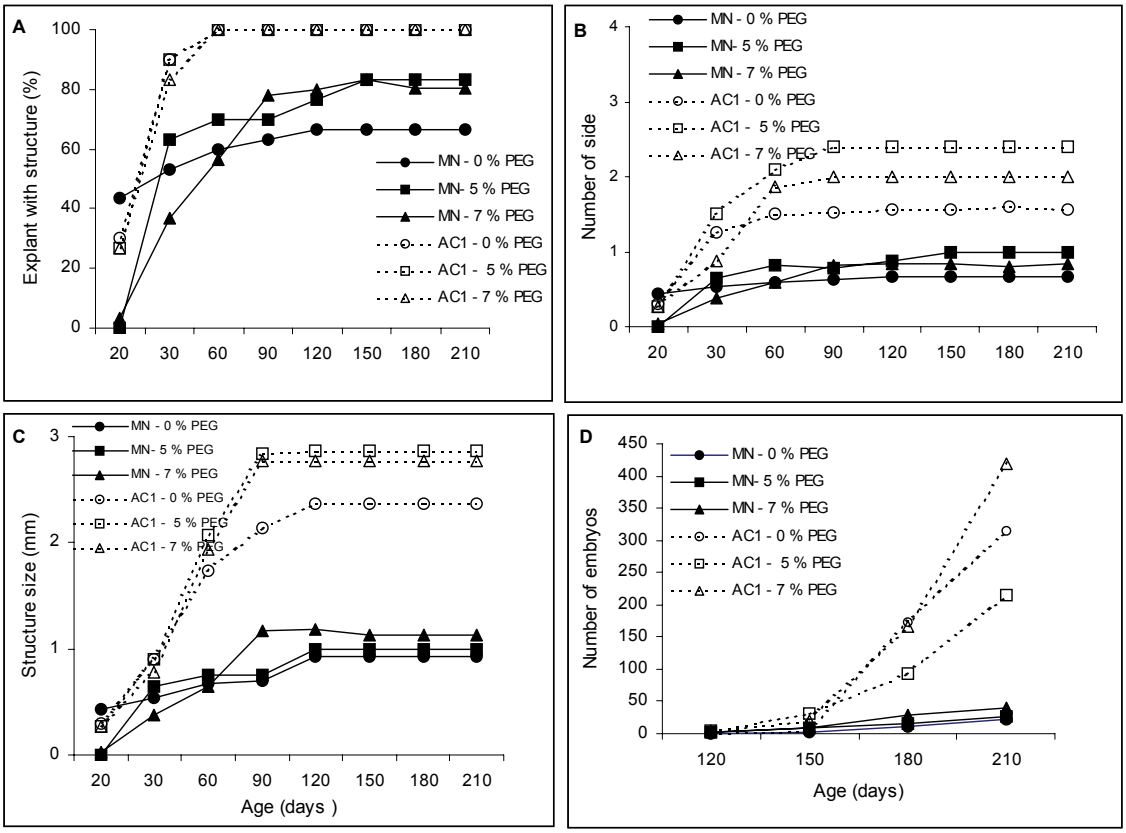


Figure 1. Somatic embryogenesis on leaf explants of genotypes *C. arabica* in medium with addition of 0, 5, 7% PEG 6000. *A*. Presence of embryogenic structures; *B*. Number of explant side with structure formation; *C*. Structure size and *D*. Number of embryos.

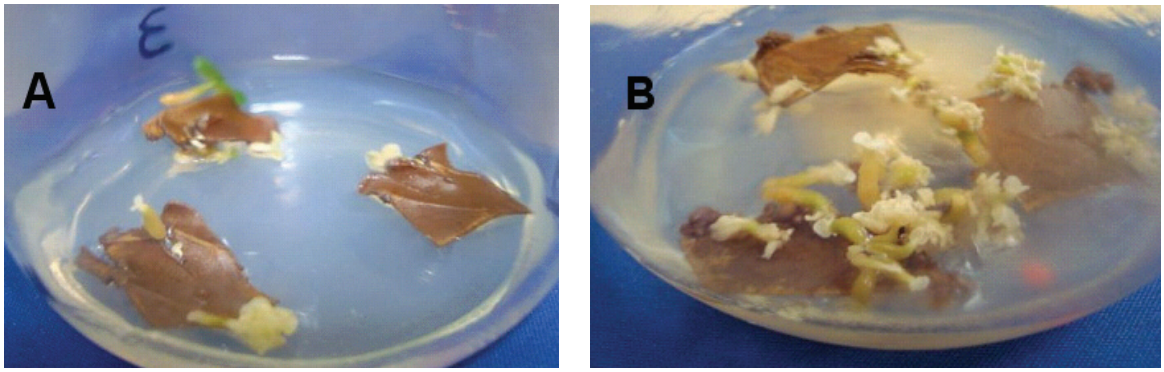


Figure 2. Explants of genotypes *Coffea* with formation of somatic embryos from explants inoculated in medium with addition of PEG 6000 (7%). A. Cultivar Mundo Novo; B. Genotype AC1.

Table 1. Determination of the osmotic potential (MPa) from the medium culture and the genotypes *Coffea arabica* explants in medium with addition of PEG 6000.

	CULTURE MEDIUM			EXPLANT			
AGE*	0	30		0		30	
PEG (%)	MN/AC1	MN	AC1	MN	AC1	MN	AC1
0	-0,35	-0,63	-0,54	-1,45	-1,58	-2,93	-4,06
5	-0,56	-0,76	-0,94			-2,99	-1,81
7	-0,61	-0,72	-0,89			-2,31	-1,79

REFERENCES

Ahmad M.S.A., Javed F., Ashraf M. 2007. Iso-osmotic effect of NaCl and PEG on growth, cations and free proline accumulation in callus tissue of two indica rice (*Oryza sativa* L.) genotypes. Plant Growth Regulation 53:53-63.

Almeida J.A.S., Simioni K.C.M., Fazuoli L.C., Ramos L.C.S. 2001. Obtenção de embriões a partir de calos de genótipos de *Coffea.* In II Simpósio de Pesquisa dos Cafés do Brasil. Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café, Vitória. pp. 1: 34-35.

Bressan R.A., Hasegawa P.M., Handa A.K. 1981. Resistance of cultured higher plant cells to polyethylene glycol-induced water stress. Plant Sci Lett 21:23-30.

Bressan R.A., Handa A.K., Handa S., Hasegawa P.M. 1982. Growth and water relations of cultured tomato cells after adjustment to low external water potential´s. Plant Physiology 70:1303-1309.

Emons A.M.C. 1994. Somatic embryogenesis: cell biological aspects. Acta Botanica Neerlandica, 43:1-14.

Ferrie A.M.R., Keller W.A. 2007. Optimization of methods for using polyethylene glycol as a non-permeating osmoticum for the induction of microspore embryogenesis in the *Brassicacea.* In Vitro Cellular & Developmental Biology-Plant 43(4):348-355.

Hsiao T.C. 1973. Plant responses to water stress. Ann Rev. Plant Physiol. 24:519-70.

Kulkarni M., Deshpande U. 2007. *In vitro* screening of tomato genotypes for drougth resistence using polyethylene glycol. African Journal of Biotechnology 6 (6): 691-696.

Lucca-Braccini L.A., Ruiz H.A., Braccini M.C.L., Reis M.S. 1996. Germinação e vigor de sementes de soja sob estresse hídrico induzido por soluções de cloreto de sódio, manitol e polietileno glicol. Revista Brasileira de Sementes, 18(2):10-16.

Mohamed F.M., Tawfik A.A. 2006. Dehydration-induced alterations in growth and osmotic potential of callus from six terapy bean lines varying in drought resistance. Plant Cell Tissue Organ Culture 87:255-262.

Murashige T., Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, v.15, p.473-497.

Ramos L.C.S., Yokoo E.Y., Gonçalves W. 1993. Direct somatic embryogenesis is genotype specific in coffee. ASIC, 15º Colloque, pp. 763-766.

Yasuda T., Fujii Y., Yamaguchi T. 1985. Embryogenic callus induction from *Coffea arabica* leaf explants by benzyladenine. Plant Cell Physiol**.** 26(3):595-597.

Comparative Proteomic Analysis of *Coffea arabica* Somatic Embryos in the Different Stages of Embryogenesis

J.H. SATO[1], J.B. TEIXEIRA[2], M.T.S. EIRA[3], O.L. FRANCO[4], A. MEHTA[2]

[1]Universidade de Brasília, Brasília, Distrito Federal, Brasil
[2]Embrapa Recursos Genéticos e Biotecnologia, Brasília, Distrito Federal, Brasil
[3]Embrapa Café, Brasília, DF, Brasil
[4]Universidade Católica de Brasília, Brasília, Distrito Federal, Brasil

SUMMARY

Coffee is one of the most important cultivated agricultural crops and is of great importance for Brazilian economy. Studies focusing on somatic embryogenesis have been widely performed aiming at the comprehension of the mechanisms involved. Somatic embryogenesis is an important process that helps *in vitro* micropropagation, which is performed to obtain a high number of plants with high economic value, genetically uniform and free from diseases. The objective of this study was to analyze the protein profile of *Coffea arabica* cv. Catuaí Vermelho somatic embryos in the stages of torpedo and cotyledonary by two-dimensional electrophoresis (2-DE). Samples were collected and macerated in liquid nitrogen. Protein extraction was performed by using phenol and extraction buffer. The proteins obtained were quantified, analyzed by 2-DE and stained with silver nitrate. The 2D gels revealed proteins varying in size from 10 to 160 kDa and in pI from 3 to 10. The analysis of the protein profiles showed a higher amount of proteins in the cotyledonary phase, especially in the area of pH 3 to 5,5. A total of 45 differentially expressed proteins were observed when comparing both profiles, including 8 proteins observed only in the cotyledonary phase and 4 exclusive to the torpedo phase. Theses proteins will be further identified by mass spectrometry in order to better understand the expression changes during somatic embryogenesis in coffee.

INTRODUCTION

Coffee is one of the most important cultivated agricultural crops and is of great importance for Brazilian economy (Vieira et al., 2006). Studies focusing on somatic embryogenesis have been widely performed aiming at the comprehension of the mechanisms involved. Somatic embryogenesis is an important process that helps *in vitro* micropropagation, which is performed to obtain a high number of plants with high economic value, genetically uniform and free from diseases (Carvalho et al., 2006).

The objective of this study was to analyze the protein profile of *Coffea arabica* cv. Catuaí Vermelho somatic embryos in the torpedo and cotyledonary stages by two-dimensional electrophoresis (2-DE).

MATERIALS AND METHODS

Catuaí Vermelho leaves were collected from plants maintained in a Greenhouse. Leaves were disinfected, cut and inoculated in Petri dishes with culture media C (Boxtel and Berthouly, 1996) with a modification in the 2,4-D concentration.

Embryos in the Torpedo and Cotyledonary stages were collected and stored at -80 °C until use. Samples were macerated in liquid nitrogen and protein extraction was performed by using phenol and extraction buffer, according to de Mot & Vanderleyden (1989). The proteins obtained were quantified, analyzed by 2-DE and stained with silver nitrate or Coomassie blue.

The differentially expressed protein spots were excised from the gels and digested with trypsin according to Shevchenko et al. (1996). The peptides were analyzed in a MALDI TOF-TOF Ultra Flex II (Bruker Daltonics) and the proteins were identified using the Mascot program.

RESULTS AND DISCUSSION

The 2D gels revealed proteins varying in size from 10 to 160 kDa and in pI from 3 to 10 (Figure 1). The analysis of the protein profiles showed a higher amount of proteins in the cotyledonary phase, especially in the area of pH 3 to 5,5. A total of 45 differentially expressed proteins were observed when comparing both profiles, including 8 proteins observed only in the cotyledonary phase and 4 exclusive to the torpedo phase (Figure 1).

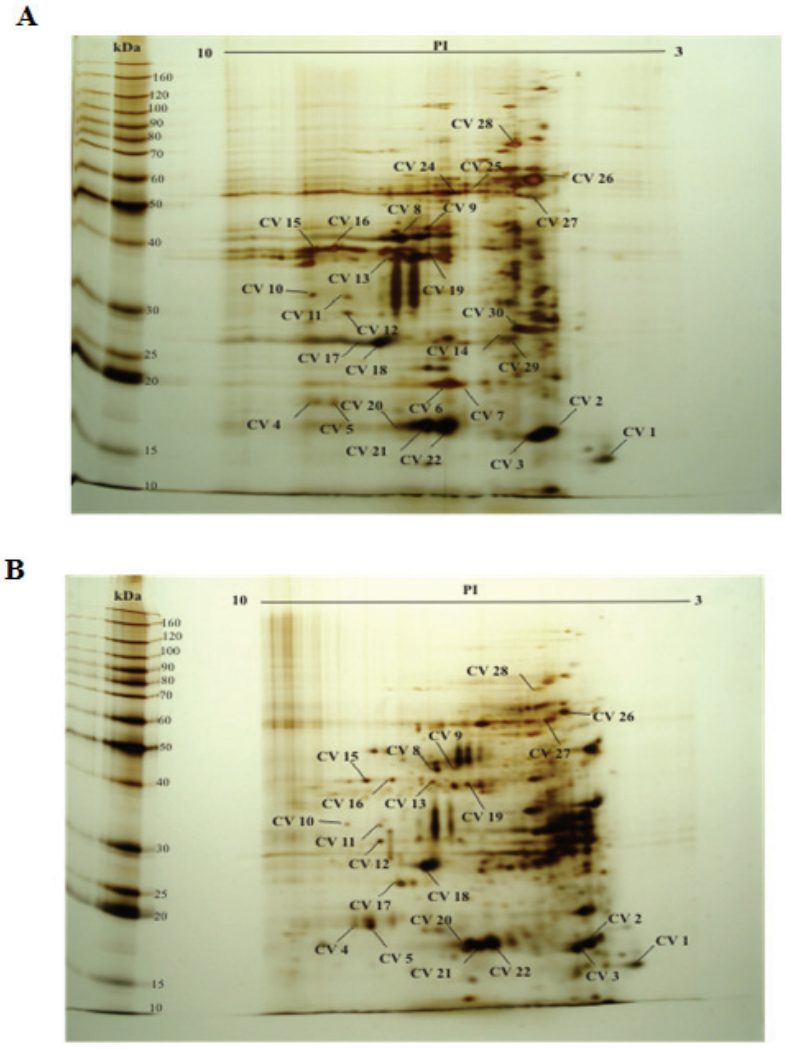


Figure 1. 2-DE protein profile of somatic embryos in the torpedo (A) and cotiledonar (B) stages.

Two protein spots, observed only in the cotyledonary stage, were identified as the same protein cyclophilin (Table 1). These proteins are characterized by their enzymatic activity related to the structure and maintenance of chloroplast protein complexes (Romano et al., 2005). The presence of these proteins in abundance in the thylakoid lumen is associated to the maintenance of photosystems I and II (Fu et al., 2007).

Another protein identified in this study was the 11S storage globulin, which was up-regulated in the torpedo stage. Globulins are the major protein reserve sources in seeds of dicotyledonous plants (Shewry et al., 1995).

Other proteins are being analyzed by mass spectrometry and the identification of these proteins may help better understand the expression changes during somatic embryogenesis in coffee.

Table 1. Differentially expressed proteins identified by mass spectrometry.

Spot	Accession #	Organism	Protein identification	Cal pI	Cal mass	Gel pI	Gel mass
CV4	gi|38708272	*Thellungiella halophila*	cyclophilin	8,65	18,37	9,0	19,3
CV5	gi|38708272	*Thellungiella halophila*	cyclophilin	8,65	18,37	8,7	19,0
CV6	gi|2979526	*Coffea arabica*	11S storage globulin	6,5	54,10	6,0	25,5
CV7	gi|2979526	*Coffea arabica*	11S storage globulin	6,5	54,10	5,6	26,0

REFERENCES

Boxtel, J. and Berthouly M. (1996). High frequency somatic embryogenesis from coffee leaves. Factors influencing embryogenesis, and subsequent proliferation and regeneration in liquid medium. Plant Cell, Tissue and Organ Culture 44:7-17.

Carvalho, J.M.F.C., Lima M.M de A., Aires P.S.R., Vidal M.S., Pimentel N.W. (2006). Embriogênese somática. Boletim de Pesquisa e Desenvolvimento - Embrapa Algodão. ISSN 0103-0205.

Fu A., He Z., Cho H.S., Lima A., Buchanan B.B., Luan S. (2007). A chloroplast cyclophilin functions in the assembly and maintenance of photosystem II in *Arabidopsis thaliana.* PNAS. 104 (40):15947-15952.

Mot R., Vanderleyden J .(1989). Application of two dimensional protein analysis for strain fingerprinting and mutant analysis of *Azospirillum* species. Can J Microbiol, 35:960-967.

Romano P., Gray J., Horton P., Luan S. (2005). Plant immunophilins: functional versatility beyond protein maturation. New Phytologist, 166: 753–769

Shevchenko A., Wilm M., Vorm O., Mann M. (1996). Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. Anal Chem. 68:850-858.

Shewry P.R., Napier J.A., Tatham A.S. (1995). Seed storage proteins: structure and biosynthesis. Plant Cell 7:945–956

Vieira L., Andrade A., Colombo C., Moraes A., Mehta A. et al. (2006) Brazilian coffee genome project: an EST-based genomic resource. Braz. J. Plant Physiol, 18(1):95-108.

Zeng F., Zhang X., Zhu L., Tu L., Guo X., Nie Y. (2006). Isolation and characterization of genes associated to cotton somatic embryogenesis by suppression subtractive hybridization and macroarray. Plant Molecular Biology. 60:167-183.

Exsudate Coloring Test to Evaluate the Physiological Quality of Coffee Seeds

P.C. HILST*, D.C.F.S. DIAS, E. MANTOVANI-ALVARENGA, L.A.S. DIAS

Department of Plant Science, Federal University of Viçosa, Viçosa, MG, Brazil.
*E:mail: pchilst@yahoo.com.br

SUMMARY

This work was carried out to obtain a fast and efficient method to evaluate seed lot quality, relating exsudate color and physiological quality of coffee seeds. Five seed lots were submitted to the following tests: standard germination, first germination count, electrical conductivity, accelerated aging and percentage of seedling emergence. In order to conduct the exudate coloring test, seeds with different moisture content, having the parchment previously removed, in four replications of ten, were distributed over three moistened paper towels placed on a wire mesh screen and suspended over 40 ml of distilled water inside a plastic gremination box. The boxes were held in a incubator at 25 °C, for five days. Every 24 hour period, was made a visual evaluation of the exsudate color intensity (strong, medium, weak and absence) underneath each seed, attributing scores for each intensity. The moisture content and the period of evaluation influenced the efficiency of the exsudate coloring test. Seeds with 12% moisture content, and periods of imbibition between 72 and 120 hours on the exudate coloring test, can be used to estimate the physiological quality of coffee seeds.

INTRODUCTION

Though the standard germination test is conducted in optimum conditions, it lasts at least 30 days, the physiological quality of the seed could not be the same as in the beginning of the test, at time the results are issued, due to the fast lost of viability during storage.

The cell membrane permeability is one of the first alterations to occur deriving of seed deterioration (Delouche and Baskin, 1973), and culminate in the lost of seed germination capability (Delouche, 1975).

According to Bewley and Black (1985), the ability to reorganize the membrane in the initial stage of imbibition will determine the quantity of seed exudate.

Low quality seeds exudates brownish colors, in different intensities when imbibed in water and disposed over paper towels on a petri dish (Sera and Miglioranza, 2006), serving as a seed quality indicator.

OBJECTIVE

The objective of this research was to validate the efficiency of the exudate coloring test to evaluate the physiological quality of coffee seeds.

MATERIAL AND METHODS

This research was carried out in the Seed Routine Laboratory of the Plant Science Department, of the Federal University of Viçosa, in Viçosa, MG, Brazil.

Five lots of coffee (*Coffea arabica* L.) seeds were used after had been processed and dried until 12% of moisture content.

After removing the parchment, the seeds were imbibed until moisture contents of 20 and 30%, over screens, inside a closed container, with water in the bottom.

For each moisture content were made the following tests:

- **Moisture Content:** as established by the RAS (Brasil, 1992).
- **Standard Germination:** four replications of 50 seeds were disposed over paper towels, moistened with water equivalent to 2.5 times the dry paper weight. The rolls were kept inside germinator adjusted at 30 °C (86 °F), as recommended by RAS (Brasil, 1992). Countings were performed 21 and 30 days after seeding and the results were expressed in mean percentage of normal seedlings.
- **First Germination Count:** the mean percentage of normal seedlings on the first count of the standard germination test.
- **Accelerated Aging:** 200 seeds were distributed over a screen, inside a gerbox, with 40 ml (1.35 oz) of distilled water in the bottom. The gerbox were kept inside a BOD, at 45 °C (113 °F) for 72 hours. After this period, four replications of 50 seeds were submitted to germination test, and the mean percentage of normal seedlings were counted at the 21st day.
- **Electrical Conductivity:** eight replications of 50 seeds, previously weighted, were placed to imbibe in 75 ml (2.5 oz) of distilled water, inside germinator at 25 °C (77 °F) for 120 hours. Every each period of 24 hour, it was measured the electrical conductivity, and expressed in μS/cm/g of seed.
- **Seedling Emergence:** four replications of 50 seeds were put to germinate in commercial substrate, inside small plastic bags.
- **Exudate Coloring Test:** four replications of 10 seeds, previously removed the silver skin after imbibed in water for about 10 min., were distributed over 3 paper towels, moistened with water equivalent to 3 times the dry paper weight, over screens, inside a closed gerbox, with 40 ml (1.35 oz) of distilled water in the bottom. The gerbox were maintained inside germinator adjusted at 25 °C (77 °F) for 120 hours. Every each 24 hour, the seeds were moved to make the visual evaluation, attributing the scores 0, 3, 5 and 10, for seeds with no exudation, weak, medium and strong exudation, respectively, as in the following picture. The mean Vigor Index was calculated for each seed lot. The following equation was applied to each replication: VI = 100 - (3 x W) - (5 x M) - (10 x S), where W is the number of seeds with weak exudation, M, the number of seeds with medium exudation and S, the number of seeds with strong exudation, totaling 10 seeds per replication.

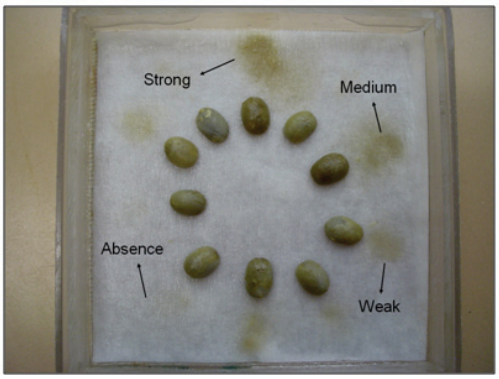
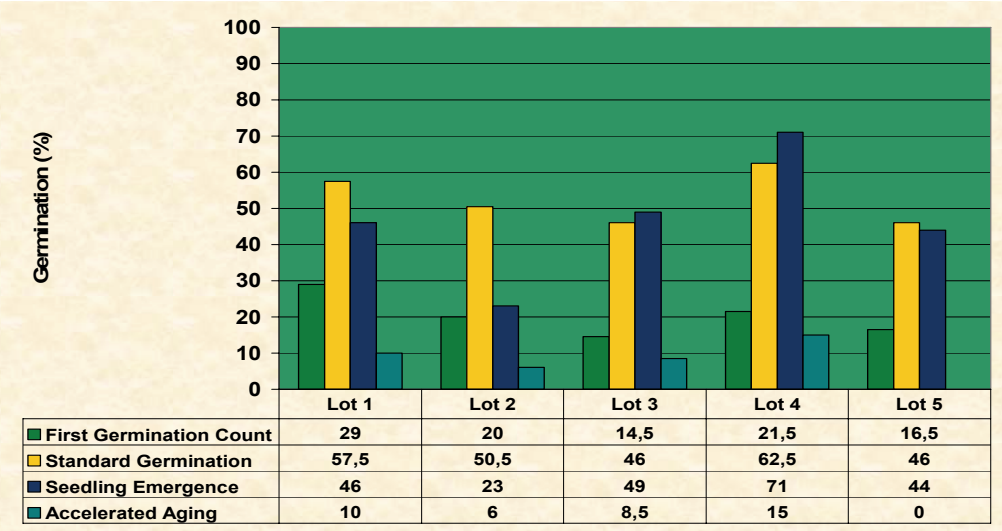


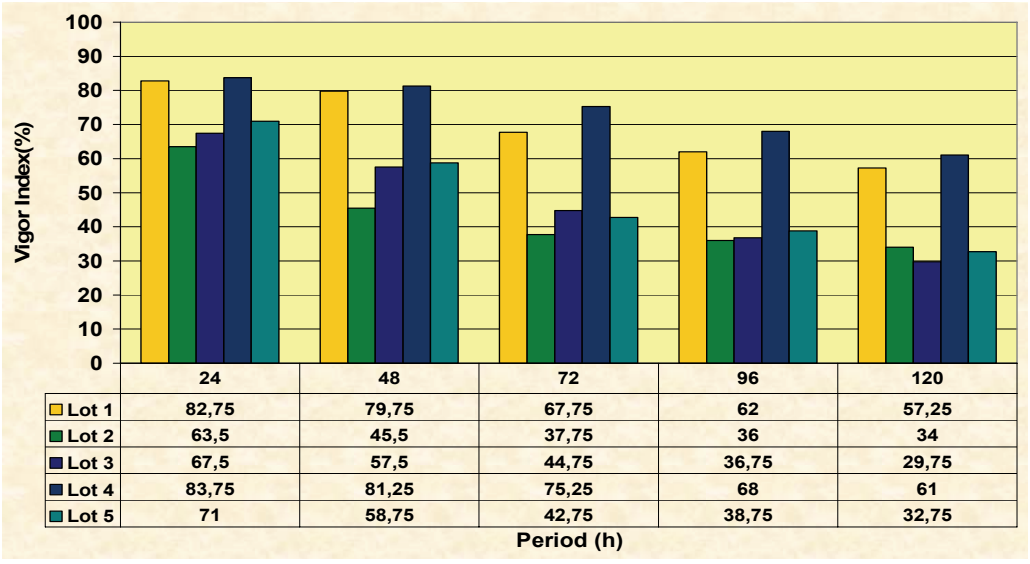
Figure 1.

RESULTS



	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5
First Germination Count	29	20	14,5	21,5	16,5
Standard Germination	57,5	50,5	46	62,5	46
Seedling Emergence	46	23	49	71	44
Accelerated Aging	10	6	8,5	15	0

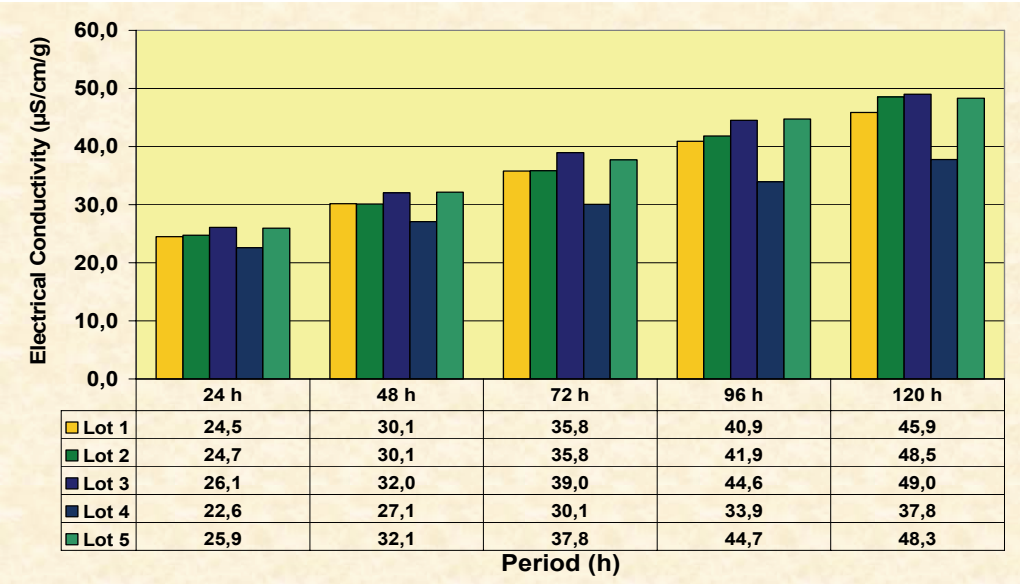
Figure 2. First Germination Count, Standard Germination, Seedling Emergence and Accelerated Aging percentage of the lots with 12% moisture content.



	24	48	72	96	120
Lot 1	82,75	79,75	67,75	62	57,25
Lot 2	63,5	45,5	37,75	36	34
Lot 3	67,5	57,5	44,75	36,75	29,75
Lot 4	83,75	81,25	75,25	68	61
Lot 5	71	58,75	42,75	38,75	32,75

Period (h)

Figure 3. Vigor Index means of the lots with 12% moisture content at each period of exudation.



	24 h	48 h	72 h	96 h	120 h
Lot 1	24,5	30,1	35,8	40,9	45,9
Lot 2	24,7	30,1	35,8	41,9	48,5
Lot 3	26,1	32,0	39,0	44,6	49,0
Lot 4	22,6	27,1	30,1	33,9	37,8
Lot 5	25,9	32,1	37,8	44,7	48,3

Figure 4. Electrical Conductivity of the seeds with 12% moisture content, at each period of imbibition.

CONCLUSION

The results indicated that seeds with 12% moisture content, and periods of imbibition between 72 and 120 hours on the exudate coloring test, can be used to estimate the physiological quality of coffee seeds.

BIBLIOGRAPHY

Bewley J.D., Black M. Seeds: physiology of development and germination. New York: London Plenum Press, 1994. 367p.

Brasil. Ministério da Agricultura. Secretaria Nacional de Defesa Agropecuária. Regras para análise de sementes. Brasília, DF: 1992. 365p.

Delouche J.C., Baskin C.C. Accelerated aging techniques for predicting the relative storability of seed lots. Seed Science & Technology, v.1, n.2, p.427-452, 1973.

Delouche J.C. Pesquisa em sementes no Brasil. Brasília, DF: Ministério da Agricultura, 1975. 69 p.

Sera G.H., Miglioranza E., Sera T. Avaliação da viabilidade de sementes de café por exsudatos. Semina: Ciências Agrárias, Londrina, v. 27, n. 1, p. 21-26, jan./mar. 2006.

Genetic Diversity of C*olletotrichum* Species Associated to Coffee in Colombia and Their Relationship to CBD Causing Isolates

A. RODRIGUEZ[1], A. LOUREIRO[2], V. VARZEA[2], A. GAITAN[1]

[1]Centro Nacional de Investigaciones de Café (CENICAFE), Chinchiná, Colombia
[2]Instituto de Investigacão Cientifica Tropical – Centro de Investigacão das Ferrugens do Cafeeiro (CIFC), Oeiras, Portugal

SUMMARY

Colletotrichum species are commonly found worldwide associated to phyllosphere and berry flora in coffee plantations. However, under appropriate conditions, some isolates can be pathogenic, the most severe being the Coffee Berry Disease (CBD), caused by the species *C. kahawae*, currently present only in Africa, and responsible for severe loses. In order to characterize the population of *Colletotrichum* spp, associated to Colombian coffee plantations and to determine their genetic variability at the inter- and intra-specific levels, 25 *Colletotrichum* isolates were collected in the central coffee zone of Colombia. DNA samples from African isolates of *C. kahawae* originated from Zimbabwe, Cameroon, Kenya and Malawi, as well as from the isolate of *C. gloeosporioides* Mal1, were included in the study in order to find relationships with CBD causing isolates. Fragments from the rDNA (ITS1 and 2) and β-tubulin sequences were amplified and sequenced. Gene annotation was performed through BLASTn analysis against reported sequences in Genbank, and isolates were grouped using Clustal W to verify homologies. AFLPs markers from genomic DNA were used to determine intra-specific variation, amplifying the primer combinations E-AC/M-A and E-AC/M-C. Microscopic characterization did not provide clues for species discrimination, although high variability was observed macroscopically among mycelia growing on PDA plus streptomycin. ITS and β-tubulin homologies indicated that all the Colombian isolates corresponded to either *C. gloeosporioides* or *C. acutatum*. The Casein Hydrolysis test failed as an alternative method to easily discriminate these two species, since intra-specific variability in this trait was found. Sequence based clustering was able to separate at a preliminary level two groups, one for *C. acutatum* and the other that included *C. gloeosporioides* and *C. kahawae* isolates. AFLPs made evident the intra-specific diversity of *C. gloeosporioides*, whose isolates shared themselves only 10% of the markers (9 bands out of 87), followed by *C. acutatum* with 29% and finally *C. kahawae* with 84% of common markers. Clustering and PCOORDA analyses were able to resolve the three different *Colletotrichum* groups, being the African *C. gloeosporioides* Mal1 isolate the intermediate organism between the *C. kahawae* and the *C. gloeosporioides* groups. In order to use the markers in early detection and diagnosis methods based on qRT-PCR, primers for SCAR amplifications were designed after sequencing inter-specific AFLP polymorphic markers. Melting curves from the amplification with primers G10ScarF and G20ScarR resulted in two common amplicons for the three species, with Tm of 74 °C and 78 °C, with a third amplicon at 84.5 °C present only in members of the *C. gloeosporioides* group, that indicates the potential of these tests for epidemiological and quarantine purposes once local populations and their diversities have been characterized.

INTRODUCTION

Three *Colleotrichum* spp. species have been isolated from coffee berries, leaves and flowers, *C. gloeosporioides* Penz, *C. acutatum* Simmond and *C.kahawae* Waller & Bridge. *C. gloeosporioides* and *C.acutatum* have been associated with anthracnose except on green berries, where *C. kahawae*, the only parasitic species, causes the Coffee Berry Disease (CBD), resulting in losses ranging between 20 and 80%. Nowadays, coffee growing countries around the world must implement measures to avoid the dispersion of the disease. In plant protection programs there is a clear need for guidelines to differentiate non-CBD-causing *Colletotrichum* genotypes from *C. kahawae*, which requires the use of reliable and fast diagnosis methods. At present time, identification criteria in the *Colletotrichum* genus are based on the size and shape of conidia and apresoria, formation of acervuli, setae or sclerotia, and expression if symptoms in the host. Morphologically, however, the reduced number of characteristics, the large intraspecific variation, and the influence of environmental conditions such as temperature, substrate and pH, result in difficult diagnostic analysis. Although enzymatic activity has also been used for species differentiation, with tests such as tartrate or citrate assimilation as carbon sources on solid medium, or protease activity (Martin and Garcia-Figueres, 1999). use of molecular techniques is a priority to identify and characterize this complex group of species

METHODS

Coffee flowers, berries and leaves with symptoms of Antrachonose were collected from the central coffee zone municipalities of La Plata (Huila) and Chinchina (Caldas). Tissue processing and fungi isolation was carried out and isolates were grown on Potato-Dextrose Agar (PDA) amended with Streptomycin 0.2 g/L and incubated at 26 °C for four days. Microscopic characterization was accomplished by means of a fungal dying with Lactophenol Blue. Characterized isolates as *Colletotrichum* spp. were preserved on sterile distilled water and stored at 4 °C (Bueno and Gallardo, 1998). DNA isolation was performed in 25 *Colletotrichum* isolates (Wendland et al., 1996) and DNA samples from *C. kahawae* were sent to Colombia by CIFC.

For both Colombian and African isolates, rDNA (ITS1 and 2) and β-tubulin amplification and sequencing were accomplished. Gene annotation was performed through BLASTn analysis against reported sequences in Genbank, and isolates were grouped using Clustal W to verify homologies. AFLPs markers from genomic DNA of both populations were used to determine intra-specific variation amplifying the primer combinations E-AC/M-A and E-AC/M-C. From the AFLPs band patterns, a binary matrix was obtained, which was used in Clustering and Principal Coordinates (PCOORDA) analysis. With the aim of distinguishing species with a biochemical test, the ability to hydrolyze casein was determined by their ability to grow on Skimmed Milk Agar. To use AFLP markers in early detection and diagnosis methods based on qRT-PCR, primers for SCAR amplifications were designed after sequencing inter-specific AFLP polymorphic markers.

RESULTS AND DISCUSSION

There is a wide diversity in the aerial mycelia between the isolates (Figure 1), from abundant to poor and from cotton;like to velvet-like appearance. Colony colors ranged form gray to white. Even when used together, ITS and b-tubulin sequences could not discriminate *C. gloeosporioides* from *C. kahawae* after clustering. AFLP markers provided the best species separation with significant bootstrap values (Figure 2), and indicated the presence of intermediate or connecting isolates between species.

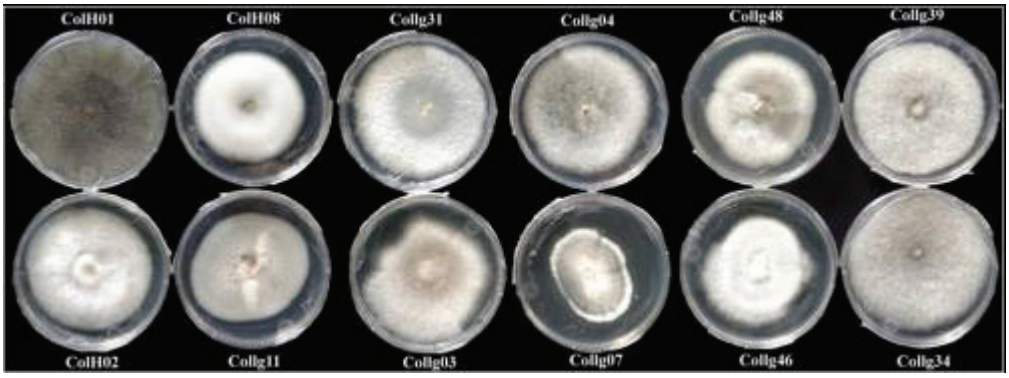


Figure 1. *Colletotrichum* macroscopic variability on PDA Agar + Streptomycin plates.

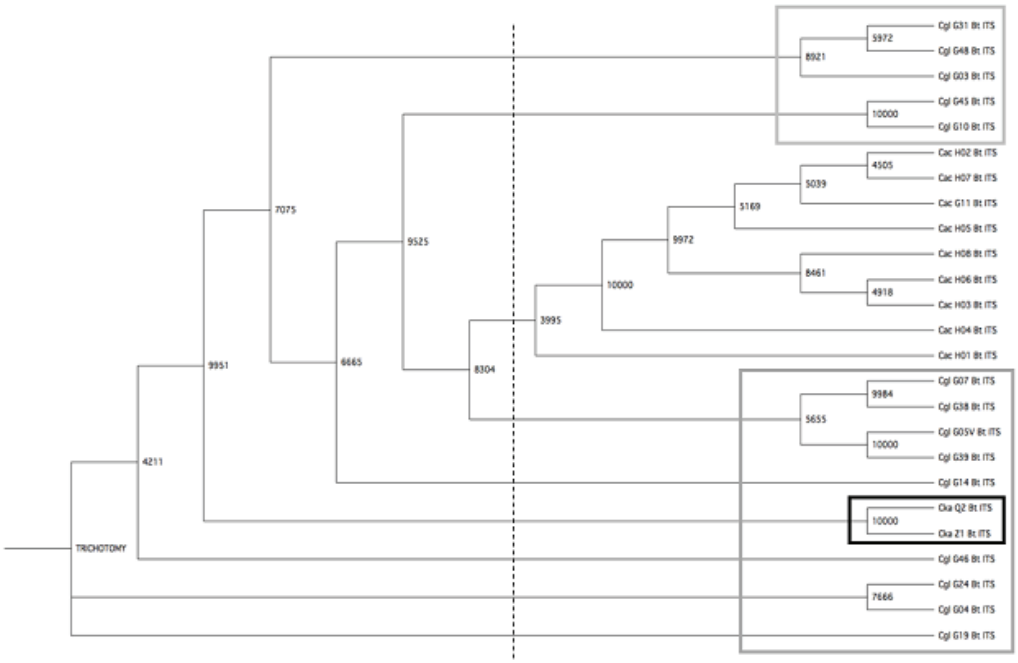


Figure 2. β-tubulin and rDNA dendrogram showing the inter-specific characterization of *Colletotrichum* spp. Gray boxes hold *C. gloeosporioides* and black box contains *C. kahawae*

β-tubulin was the gene that provided the most phylogenetic information form the genes evaluated in the inter-specific characterization of *Colletotrichum* spp. when compared to ITS. Its significance could be related to the amplicon length and its biological activity. The dendrogram built from the data provided by molecular markers, exhibited two clusters; one formed with the species *C. kahawae* and *C. gloeosporioides*, and other with the *C. acutatum* isolates. Although the African isolate Mal1 was previously identified as *C. gloeosporioides*, it was set in the dendrogram as the farthest organism form the Colombian isolates of *C. gloeosporioides*. In a similar way, *C. gloeosporioides* Collg05V was found to be the farthest Colombian isolate, which was near the *C. kahawae* cluster, as proved by the PCOORDA analysis.

By using the designed SCARS primers, it was possible to differentiate between most isolates of *C. gloeosporioides* and *C. kahawae*, and *C. gloeosporioides*. However, only two of the *C. gloeosporioides* isolates had the same profile shown by *C. kahawae* and *C. acutatum* (Figure 3).

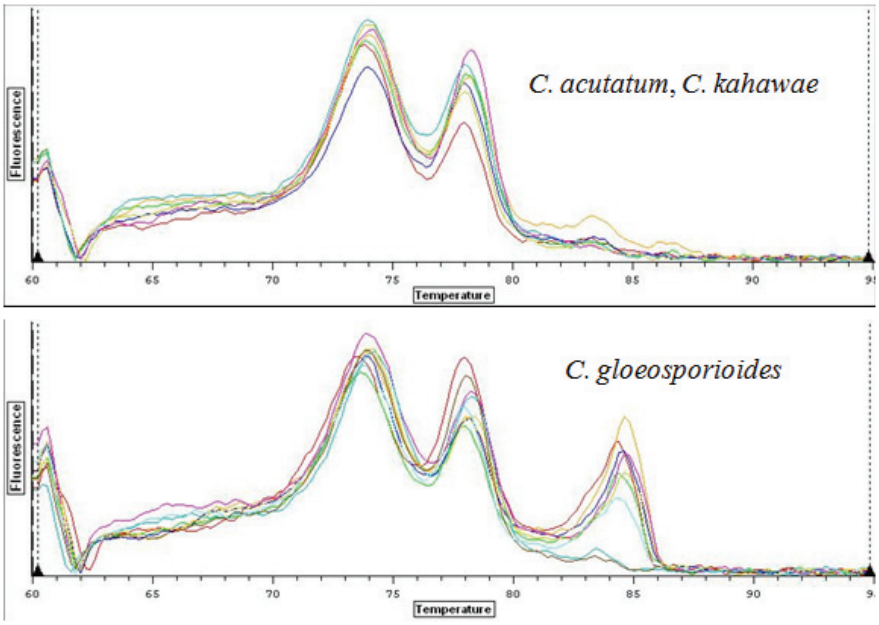


Figure 3. Melting curve showing two amplicons (74 and 78 °C) in *C. acutatum* and *C. kahawae* (A), and a third amplicon (84.5 °C) in *C. gloeosporioides* (B). RT-PCR amplification was carried out by using the primers G10ScarF and G20ScarR There is a potential to design diagnostic tools to identify the species using these markers

REFERENCES

Bueno L. and Gallardo R. Preservación de hongos filamentosos en agua destilada estéril. Revista Iberoamericana de Micología. 15:166-168. 1998

Martin M., Garcia-Figueres F. *Colletotrichum acutatum* and *C. gloeosporioides* cause anthracnose on olives. European Journal of Plant Pathology 105: 733-741. 1999

Wendland J., Lengeler K.B. and Kothe E. An instant preparation method for nucleic acids of filamentous fungi. En: Fungal Genetic. 43: 554-555. 1996

Differential Induction of Superoxide Dismutase in *Coffea arabica* - *Hemileia vastatrix* Interactions

L. GUERRA-GUIMARÃES[1], S. CARDOSO[1], I. MARTINS[1], A. LOUREIRO[1], A. BERNARDES DA SILVA[2], V.M.P. VÁRZEA[1], M.D.C.SILVA[1]

[1]Instituto de Investigação Científica Tropical (IICT) - Centro de Investigação das Ferrugens do Cafeeiro (CIFC), Oeiras, Portugal. E-mail: leonorguima@gmail.com
[2]Centro de Engenharia Biológica, Departamento de Biologia Vegetal, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal

SUMMARY

Coffee – rust interactions are governed by the gene-for-gene relationship, the resistance of coffee plants characterized by rapid plant cell death (hypersensitive reaction – HR). Previous cytological studies using scavengers of active oxygen species, showed that the superoxide dismutase (SOD) significantly inhibited the cell death, suggesting the involvement of the anion radical O_2^- in the HR. This work aims to understand the role of SOD in coffee resistance. Differential induction of superoxide dismutase (SOD) was observed in compatible and incompatible *Coffea arabica*- *Hemileia vastatrix* interactions. In the incompatible interaction a peak of SOD activity was detected around 17-24 hours after inoculation, prior to cell death observation. On the contrary, in the compatible interaction no significant changes in SOD activity was observed at this early stage of the infection process. Native PAGE analysis showed at least six isoenzymes of SOD, tree of which were CuZn-SOD, one was Mn-SOD and two Fe-SOD. The isoenzyme pattern of SOD obtained by IEF showed at least three groups of SOD isoenzymes with a pI of 5.4, 4.9 and 4.7 respectively. The involvement of SOD in coffee-rust interactions will be discussed.

INTRODUCTION

The coffee resistance to *H. vastatrix* is usually post-haustorial being associated with hypersensitive cell death (HR), haustoria encasement and early accumulation of phenolic-like compounds (Silva et al., 2002). The stimulation of phenylalanine ammonia lyase, β-1,3-glucanases and chitinases and the involvement of oxidative enzymes such as NADPH oxidases, lipoxygenase and peroxidase have also been associated with the expression of resistance in some coffee cultivars (Silva et al., 2002; Maxemiuc-Naccache et al., 1992; Rojas et al., 1993; Silva et al., 2008; Guerra-Guimarães et al., 2008).

Superoxide dismutases (SODs; EC 1.15.11) play a crucial role in the defence against reactive oxygen species catalyzing the dismutation of superoxide anion (O_2^-) to molecular oxygen and hydrogen peroxide. These enzymes which are characterized by their particular individual cofactor CuZn, or Mn or Fe are present in all aerobic organisms (Sheng et al., 2004).

This work aims to understand the role of SOD in coffee resistance to *H. vastatrix*.

MATERIAL AND METHODS

Biological Material

Coffee plants (*C. arabica* L.) of the genotype S_H4S_H5 (S4 Agaro) grown in greenhouse conditions were inoculated with fresh urediospores of *H. vastatrix,* races II (v_5) and XV ($v_{4,5}$) (D'Oliveira, 1954-1957) establishing an incompatible and a compatible interaction, respectively.

SOD extraction and assay

Inoculated and non-inoculated fresh leaf tissues were ground in liquid N_2, homogenised in a Tris-HCl buffer solution and centrifuged at 28000*g* during 15 min at 4 °C. Supernatant was concentrated on a Centricon microconcentrator (Amicon 10) and stored at –80 °C. Protein content was determined using the commercial Bio-Rad protein assay kit. Total SOD activity was measured spectrophotometrically by following the reduction of nitroblue tetrazolium by xanthine-xanthine oxidase system at 560 nm (Beauchamp and Fridovich, 1971). Polyacrylamide gels electrophoresis (PAGE) and isoelectric focusing (IEF) was performed as previously described (Laemmli, 1970; Robertson et al., 1987). After electrophoresis separation, SOD activity appears as colorless bands on the purple-stained gel (Azevedo et al., 1998). To assess the effects of inhibitors, the staining was carried out in the presence of 1 mM sodium cyanide (inactivates Cu/ZnSOD) or 3 mM hydrogen peroxide (inactivates both Cu/ZnSOD and FeSOD). Intensity of SOD bands on IEF zymograms was evaluated using the ImageQuant™ TL image analysis software (GE LifeSciences).

RESULTS AND DISCUSSION

In the incompatible interaction a peak of total SOD activity was detected around 17-20 hours after inoculation, prior to cell death observation. On the contrary, in the compatible interaction no significant changes in total SOD activity was observed at this early stage of the infection process (Figure 1).

Native PAGE analysis showed at least six bands with SOD activity. According to their different sensitivity to inhibitors (KCN, H_2O_2) they were identified as three CuZn-SOD, one Mn-SOD and two Fe-SOD isoenzymes (Figure 2). The different types of SOD isoenzymes had already been identified in other coffee cultivars such as Caturra and Colombia (Daza et al., 1989).

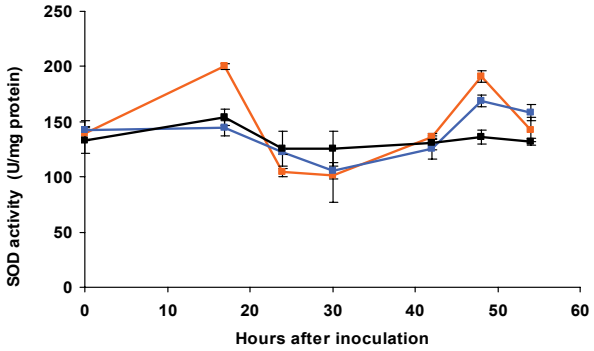


Figure 1. Total SOD activity in healthy (■) leaves and in incompatible (■) and compatible (■) interactions at 0, 17, 24, 30, 42, 48 e 54 hours after inoculation. SOD activity was determined at 560nm based on the ability of SOD to inhibit the reduction of NBT by O_2^- generated by xanthine oxidase.

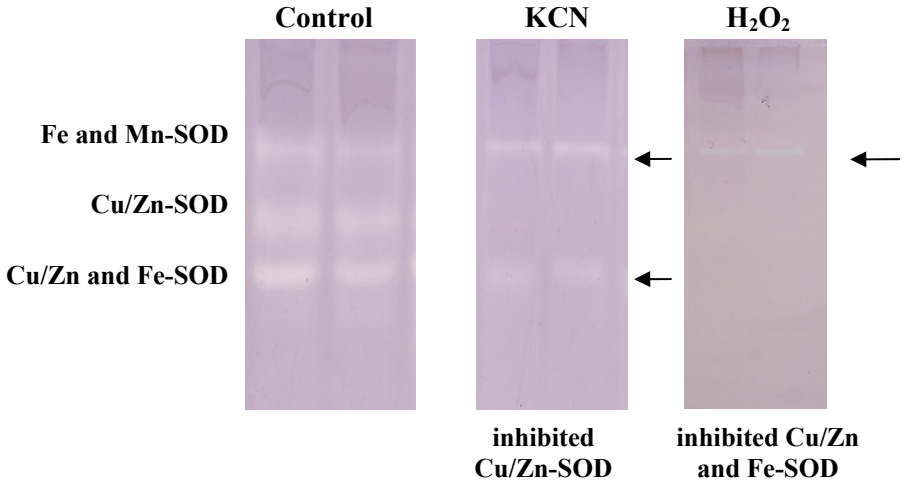


Figure 2. Identification of SOD isoenzymes using native PAGE electrophoresis. Isoenzymes (Fe, Mn and Cu/Zn-SOD) were differentiated based on their sensitivity to inhibitors: KCN (1 mM) and H_2O_2 (3 mM).

The isoenzymes pattern of SOD obtained by IEF showed at least three groups of SOD with a pI around 5.4, 4.9 and 4.7 (Figure 3). When comparing the densitometric volumes of the referred isoenzymes, we found in the incompatible interaction a higher intensity than in the compatible interaction and healthy leaves of the bands with pI 5.4 and 4.7, respectively at 17 and 24 hours after inoculation (data not shown).

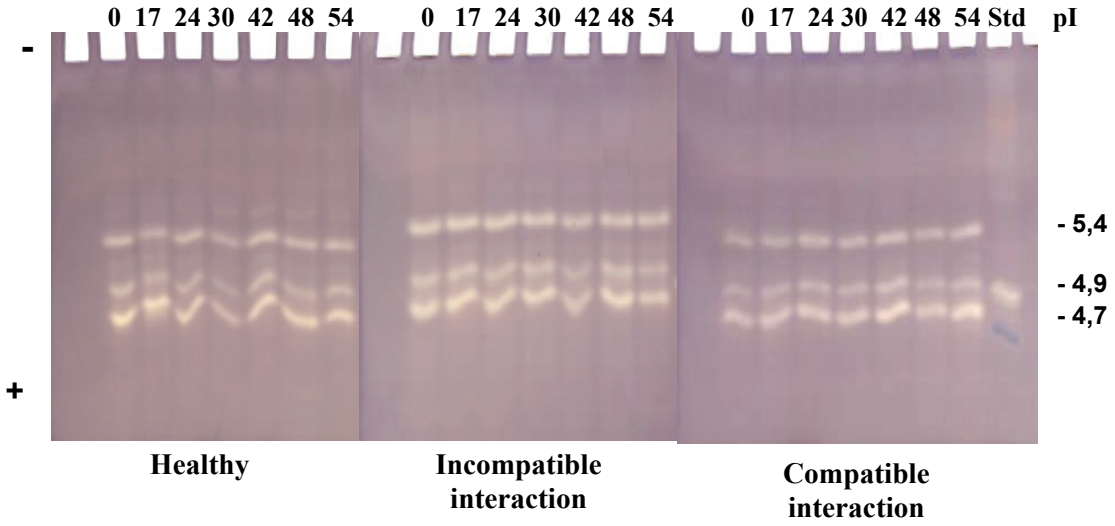


Fig. 3. IEF electrophoresis analysis of SOD activity of proteins from healthy leaves, incompatible and compatible interactions at 0, 17, 24, 30, 42, 48 e 54 hours after inoculation. Gels with pH 3-10 were stained with a solution of NBT, TEMED and Riboflavin. +, anode; -, catode; Std, IEF standard from BIO-RAD (broad range pI 4,45-9,6); pI, isoelectric points.

The increases in SOD activity associated with a selective stimulation of specific isoenzymes was also observed in resistant cabbage varieties during infection with *Xanthomonas campestris* and as a defence response of *Pennisetum glaucum* to *Sclerospora graminicola* infection (Gay PA, Tuzun , 2000; Babitha et al., 2002).

These results suggests that the early increase of SOD activity, in particularly the isoenzymes with a pI 5.4 and 4.7, my play a role in the hypersensitive response observed in the incompatible coffee-orange rust interaction. Molecular characterization of these isoenzymes is currently under study.

REFERENCES

Azevedo R.A., Alas R.M., Smith R.J., Lea P.J. (1998). Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots of wild-type and a catalase-deficient mutant of barley. Physiologia Plantarum 104:280-292.

Babitha M.P., Bhat S.G., Prakash H.S., Shetty H.S. (2002). Differential induction of superoxide dismutase in downy mildew-resistant and -susceptible genotypes of pearl millet. Plant Pathol. 51:480-486.

Beauchamp C.H., Fridovich I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44:276-287.

Daza M.C., Sandalio L.M., Del Río L.A., Quijano-Rico M. (1989). Superoxide dismutase in coffee plants. Presence of an iron-containing isoenzyme. In: ASIC, 13º Colloque, Paipa, Colombia. Pp.343-350.

D'Oliveira B. (1954-1957). As ferrugens do cafeeiro. Café Português 1(4):5-13; 2(5):5-12; 2(6):5-15; 2(7):9-18; 2(8): 5-22; 4(16):5-15.

Gay P.A., Tuzun S. (2000). Temporal and spatial assessment of defense responses in resistant and susceptible cabbage varieties during infection with *Xanthomonas campestris* pv. *campestris*. Physiol. Mol. Plant Pathol. 57:201-210

Guerra-Guimarães L., Silva M.C., Struck C., Loureiro A., Nicole M., Rodrigues Jr C.J., Ricardo C.P.P. (2008). Chitinases of *Coffea arabica* genotypes resistant to orange rust *Hemileia vastatrix.* Biologia Plantarum (in press).

Laemmli U.K. (1970). Cleavage of structural proteins during the essembly of the head of bacteriophage T4. Nature 227:680-685.

Maxemiuc-Naccache V., Braga M.R., Dietrich S.M.C. (1992). Chitinase and β-1,3-glucanase in compatible and incompatible combinations between coffee leaf disks and coffee rust (*Hemileia vas*tatrix). Revista Brasileira de Botânica 15:145-150

Robertson E.F., Dannely H.K., Malloy P.J., Reeves H.C.(1987). Rapid isoelectric focusing in a vertical polyacrylamide mini-gel system. Anal. Biochem.167:290-294.

Rojas M.L., Montes de Gómez V., Ocampo C.A. (1993). Stimulation of lipoxygenase activity in cotyledonary leaves of coffee reacting hypersensitively to the coffee leaf rust. Physiol. Mol. Plant Pathol. 43:209-219.

Sheng L., Zheng X., Tong H., Liu S., Du J., Liu Q. (2004). Purification and characterization of cytosolic isoenzyme III of Cu,Zn-superoxide dismutase from tobacco leaves. Plant Science 167:1235-1241.

Silva M.C., Nicole M., Guerra-Guimarães L., Rodrigues Jr. C.J. (2002). Hypersensitive cell death and post-haustorial defence responses arrest the orange rust (*Hemileia vastatrix*) growth in resistant coffee leaves. Physiol. Mol. Plant Pathol. 60:169-183,.

Silva M.C., Guerra-Guimarães L., Loureiro A., Nicole M.R. (2008). Involvement of peroxidases in the coffee resistance to orange rust *(Hemileia vastatrix)*. Physiol. Mol. Plant Pathol. 72:29-38.

Histological and Ultrastructural Characterization of Coffee Resistance to *Colletotrichum kahawae*

A. LOUREIRO[1], M.D.C. SILVA[1], V. VÁRZEA[1], P. MONCADA[2], B. BERTRAND[3], M. NICOLE[3]

[1]Instituto de Investigação Científica Tropical (IICT) - Centro de Investigação das Ferrugens do Cafeeiro (CIFC), Oeiras, Portugal
[2]Cenicafe – Chinchiná, Caldas, Colombia
[3]Unité Mixte de Recherche IRD-CIRAD-UM2, Montpellier, France

SUMMARY

The growth of *Colletotrichum kahawae* Waller and Bridge and the early responses it induced in hypocotyls with different levels of resistance were investigated by light and transmission electron microscopy. Fungal penetration occurred through melanized appressoria directly into the epidermal cell walls with formation of an infection vesicle. Plant susceptibility involved the intra- and intercellular ramification of the infection vesicle in the living host cells. This brief period of biotrophy was followed by the necrotrophic fungal growth and the production of symptoms (dark sunken lesions with sporulation). In the necrotrophic phase, colonization of host cells by the fungus was associated with severe walls alterations and death of the host protoplasts. The more resistant genotypes were characterized by a restricted fungal growth associated with hypersensitive-like host cell death, modifications in the cell walls (thickness and autofluorescence) and early accumulation of phenolic compounds, such as flavonoides and hydroxycinnamic acid derivatives.

INTRODUCTION

Coffee berry disease, caused by the fungus *Colletotrichum kahawae* Waller & Bridge is a major threat to the production of *Coffea arabica* in Africa. Selection of resistant coffee is an alternative to the use of chemical control since evidences has been obtained that Hibrido de Timor (HDT) derivatives may represent a good source of resistance (Silva et al., 2006).

Host resistance to *Colletotrichum* spp. have been usually associated to host cell wall modifications, namely the increase in the levels of hydroxyproline-rich glycoproteins (HRGPs), to an early accumulation of phenolic compounds in the infected host cells and in some cases with the rapid hypersensitive death of host infected cells characterized by a rapid loss of host cells membrane integrity and the accumulation of phenolic oxidation products (Esquerré-Tugayé et al., 1992; Goodman and Novacky, 1994; Skipp et al., 1995; Torregrosa et al., 2004).

The growth of a *C. kahawae* isolate from Malawi (CIFC - Mal2) and the sequence of responses it induced in resistant hypocotyls of HDT derivatives as well as on the susceptible cultivar Caturra were investigated by light and transmission electron microscopy.

MATERIAL AND METHODS

Hypocotyls inoculation

Resistant hypocotyls of HDT derivatives as well as susceptible hypocotyls of the cultivar Caturra were inoculated with the isolate Mal2 of *C. kahawae*, from Malawi. Hypocotyls at the soldier stage were inoculated according to the technique described by Van der Vossen et al. (1976) with slight modifications. The hypocotyls were placed on plastic trays lying down on a wet nylon sponge and then inoculated with a 5µl drop of a conidia suspension (2 x 10^6/ml). Covered trays were placed in a Phytotron 750 E at 22 ºC incubated the first 24h in the dark and then kept with a photoperiod of 12 hours.

Light microscope (LM) observation of fresh tissues

Cross sections of infected leaf fragments made with a freezing microtome were stained and mounted in blue lactophenol (Silva et al., 1999) to evaluate fungal post-penetration growth stages. Hyphal length inside hypocotyl tissues were estimated using a micrometric eyepiece. To detect autofluorescent cells, cross sections of infected hypocotyl fragments were placed in 0.07 M, pH 8.9 phosphate solution (K_2HPO_4), for 5 min and mounted in the same solution (Silva et al., 2002). Autofluorescence under blue light indicated accumulation of phenolic-like compounds. To detect callose deposition, cross sections of infected tissues were placed in 0.07 M, pH 8.9 phosphate solution (K_2HPO_4), for 10 min, and then transferred into a 0.01% solution of aniline blue in the phosphate solution, for 10 min before being mounted in the same solution (Silva et al., 2002). Callose deposition was identified by bright yellow fluorescence (Eschrich and Currier, 1964). To detect flavonoids, gallic and hydroxycinnamic derivatives the Neu's Reagent (Neu, 1956) was used. Cross sections of infected hypocotyls were placed in 1% of 2-amino-ethyldiphenyl-borinate diluted in absolute methanol for 5 min and mounted in glycerine-water. Observations were made with a microscope Leitz Dialux 20 equipped with a mercury bulb HB 100W, u.v. light (excitation filter BP 340-380; barrier filter LP 430) and blue light (excitation filter BP 450-490; barrier filter LP 515).

Transmission electron microscope (TEM) observations

Hypocotyl strips cut from healthy and infected tissues were fixed in glutaraldehyde and osmium tetroxide, embedded and polymerized (70 ºC, overnight) in Spurr´s resin (Sigma, Germany), as previously described (Rijo and Sargent, 1974). Semi-thin sections (2 µm) of the polymerized blocks were stained with 0.5% aqueous toluidine blue solution and observed using light microscopy. Ultrathin sections (80-90 nm) of the same selected blocks, collected on Formvar-coated nickel grids (200 mesh), were stained with uranyl acetate and Reynold´s lead citrate. The observations were made with a FEI transmission electron microscope operating at 70 kv.

RESULTS AND DISCUSSION

The early stages of *C. kahawae* development were essentially the same on the surface of resistant and susceptible coffee hypocotyls. Fungal conidia adhered to the cuticle and germinated producing germ tubes and melanized apressoria which penetrated the cuticle directly into the epidermal cell walls with the formation of infection vesicles that grew intra- and intercellularly (Figure 1).

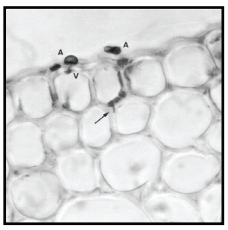
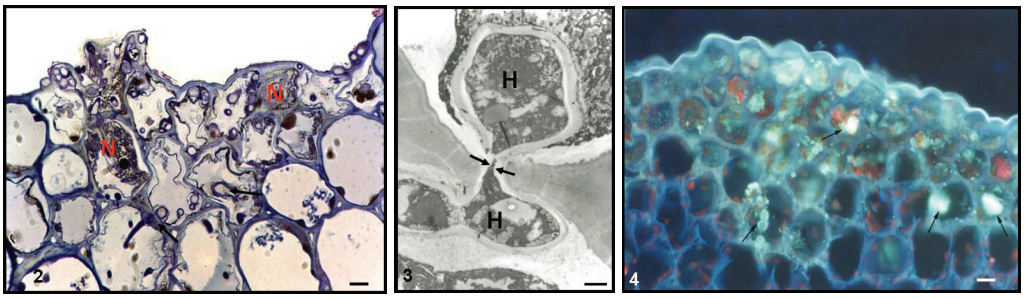


Figure 1. First stages of *C. kahawae* infection process on coffee hypocotyls. LM observations, blue lactophenol staining. Two infection sites showing melanized appressoria (A) and a infection vesicle (v) in one case and further intra- and intercellular growth (arrow) in the other, 41 h after inoculation (Scale bar = 11µm)

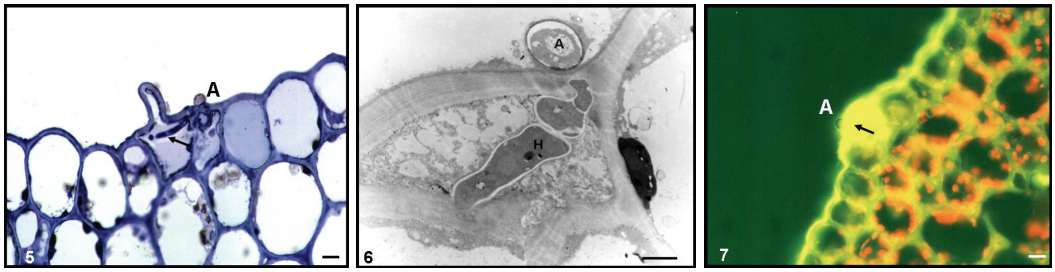
In the susceptible hypocotyls, the fungus pursued its growth on the living host cells. After a brief period of biotrophy, that lasted around 72 h, a necrotrophic fungal growth began culminating in the appearance of symptoms - dark sunken lesions with sporulation. The biotrophic phase was repeated as the fungus started the colonisation of new host cells. Consequently, it was possible to observe hyphal growth simultaneously in dead and living host cells. As shown by light and electron microscopic observations, the necrotrophic phase was associated with severe wall alterations and death of the host protoplasts (Figure 2 A and B) and the deposition of callose around some intracellular hyphae (Figure 2 C). These host responses in the susceptible tissues seemed to occur too late to prevent fungal growth and sporulation.



A **B** **C**

Figure 2. Necrotrophic growth of *C. kahawae* in susceptible hypocotyls, 7 days after inoculation. A - LM observation, toluidine blue staining. Fungal hyphae (arrows) in living and in necrotized (N) host cells (Scale bar = 9µm). B - TEM observation. Hypha (H) penetrating the cell wall between two cortex cells. Note the constriction of the hypha as it passes through the wall (arrows) and the disorganization of the cytoplasmic content of the invaded host cells and calose deposition around intracellular hyphae (arrow head) (Scale bar = 1µm). C - LM observation. Callose around intracellular hyphae (arrows) (Scale bar = 11µm).

In the resistant hypocotyls, the fungal hyphae were confined to epidermal cells or to the first layers of the cortex cells (Figure 3 A and B). As shown by light and electron microscopic observations this restricted fungal growth was associated with hypersensitive-like host cell death (membrane breakdown at the level of plasma membrane and in different organelles namely chloroplasts and mitochondria, change in the appearance of choroplasts and coagulation of cytoplasm), modifications in the cell walls (thickness and autofluorescence) and early accumulation of phenolic compounds, such as flavonoides and hydroxycinnamic acid derivatives (Figure 3 C). The hydroxycinnamic acid derivatives accumulation occurred mainly in the plant cell walls while flavonoids accumulation occurred in the cytoplasmic contents.



A B C

Figure 3. Fungal growth inside resistant hypocotyls and host responses. A - LM observation, toluidine blue staining. Infection site showing a melanized appressorium (A) and an intracellular hypha (arrow) confined to the epidermal plant cell, 7 days after inoculation (Scale bar = 8 µm). B - TEM observation, 7 days after inoculation. Melanized appressorium (A), intracellular hypha (H) with cytoplasmic content totally disorganised and invaded host cell with thicker walls (Scale bar = 2 µm). C - LM observation. Infection site showing autofluorescence of the epidermal cell wall and cytoplasmic contents (arrow), 60h after inoculation. (Scale bar = 12.5 µm).

The host responses observed in the incompatible interaction between *C. kahawae* and *Coffea* were similar to other *Colletotrichum*-plant interactions such as *C. lindemuthianum* with *Phaseolus vulgaris* and *C. trifolii* with *Medicago truncatula* (Esquerré-Tugayé et al., 1992; Skipp et al., 1995; Torregrosa et al., 2004).

Cytochemical tests are currently under study to investigate the composition of the fungal - plant interface and also the involvement of cell wall-degrading enzymes in different stages of the infection.

REFERENCES

Eschrich W. and Currier H.B. (1964). Stain Technology 39:303-304.

Esquerré-Tugayé M.-T., Mazau D., Barthe J.-P., Lafitte and Touzé A. (1992). In: *Colletotrichum.* Biology, Pathology and Control. Edited by Bailey JA & Jeger MJ. CAB International, Wallingford UK, pp 121-133.

Goodman R.N. and Novacky A.J. (1994). The hypersensitive reaction in plant to pathogen: a resistance phenomenon. APS Press, St. Paul, Minnesota.

Neu R. (1956). Naturwissenschaften 43:82.

Rijo L., Sargent J.A. (1974). Canadian Journal of Botany 52: 1363-1367.

Silva M.C., Várzea V., Guerra-Guimarães L., Azinheira H.G., Fernandez D., Petitot A.-S., Bertrand B., Lashermes P., Nicole M. (2006). Brazilian Journal of Plant Physiology 18: 119-147.

Silva M.C., Nicole M., Rijo L., Geiger J.P., Rodrigues Jr. C.J. (1999). International Journal of Plant Science 160 (1): 79-91.

Silva M.C., Nicole M., Guerra-Guimarães L., Rodrigues Jr. C.J. 2002. Physiological and Molecular Plant Pathology 60 (4): 169-183.

Skipp R.A., Beever R.E., Sharrock K.R., Rikkerink E.H.A. and Templeton M.D. (1995). In: Pathogenesis and Host Specificity in Plant Diseases. Histopathological, Biochemical

and Molecular Bases. Volume II Eukaryotes. Edited by Kohmoto K, Singh US & Singh RP. Elsevier Science Ltd (Pergamon), Oxford (UK), pp 357-374.

Torregrosa C., Cluzet S., Fournier J., Thierry H., Gamas P., Prospéri J.-M., Ésquerré-Tugayé M.-T., Dumas B. and Jacquet C. (2004). Molecular Plant-Microbe Interaction 8:909-920.

Van der Vossen H.A.M., Cook R.T.A. and Marakaru G.N.W. (1976). Euphytica 25: 733-745.

Defense-Related Gene Expression in Response to Leaf-Miner Infection

M. SILVESTRINI, D.A. RAMIRO, O. GUERREIRO-FILHO, M.P. MALUF

Coffee Center "Alcides Carvalho", Agronomic Institute (IAC), Campinas, SP, Brazil
Embrapa, Brasília, DF, Brazil

SUMMARY

In Brazilian coffee plantations, the leaf-miner (*Leucoptera coffeella*) represents a major threat. Insect control is mainly based on the use of pesticides, as resistant cultivars are not yet available. However, the coffee breeding program of IAC has already selected promising genotypes bearing resistance to the leaf-miner, which was transferred from the diploid species *Coffea racemosa.* In order to characterize molecular aspects of defense mechanisms associated to the resistance response, expression of key genes was evaluated in coffee leaves, from both resistant and susceptible plants, in response to leaf-miner infection. Infected leaves were collected at different time-intervals during insect development. Defense-related genes were identified in the Coffee Genome Database, through Blast searches. Gene specific primers were used to amplify corresponding transcripts on sampled leaves using a quantitative RT-PCR approach. Results indicated that there are no significant differences in the expression patterns of evaluated genes when comparing resistant and susceptible infected leaves. Major differences were observed for *lipoxygenase*, *glutathione transferase, protein-kinase receptor* and *glucanase*. However, these differences are mainly associated with expression timing along insect infection rather than with gene regulation, suggesting that resistance to leaf-miner in coffee may be associated with a previously built basal defense-response.

INTRODUCTION

Plants have developed diversified defense mechanisms in order to evolved and protect themselves from pathogen attacks. These mechanisms are regulated by a range of induced signaling pathways, triggered by pathogen recognition, which leads to regulation of several genes, and synthesis of defense compounds such as phytoalexins, antibiotics, proteinase inhibitors, and others. According to Ryan (2000) genes that are knowingly activated upon pathogen attack are classified in: defense-related genes, which encode resistance proteins and enzymes from secondary metabolism; signaling genes, such as transcription factors and others that act in the signal transduction cascade and metabolism reprogramming; and genes responsible for the synthesis of compounds that will act in the systemic defense.

In this study, the expression of defense-related genes is evaluated in coffee plants in response to leaf-miner infestation. Leaf-miner is an herbivore and represents a major threat of coffee plantations. Besides direct damage, leaf-miner field infestation leads to plant deterioration, as a result of leaves decay, loss of dry-matter from stem and roots, and reduction of photosynthetic activity (Koronnova and Vega, 1985). The use of leaf-miner resistant cultivars is considered the most effective solution against this plague.

At IAC, a breeding program aiming the selection of resistant cultivars is currently under way. Resistant genes were identified in *C. racemosa* and were transferred to *C. arabica* lines. Genetic analyses indicated that resistance is conditioned by two genes (Guerreiro-Filho et al.,

1999). So far, little information is available regarding molecular aspects of leaf-miner resistance in coffee plants. Previous biochemical analyses suggest that activity of defense-related enzymes such as peroxidase and poliphenol oxidase is not significantly different between susceptible and resistant leaves upon leaf-miner infestation (Ramiro et al., 2004).

In this context, the main objective of this study was to evaluate the expression of major defense-related genes, in both susceptible and resistant coffee plants, in response to leaf-miner attack.

MATERIALS AND METHODS

Plant material

The segregating population used in this study represents an advanced generation of an inter-specific hybrid between the susceptible species *C. arabica* and the resistant species *C. racemosa*. The population consists of 136 plants, derived from open pollination of the accession H14954-46 C1351 EP473. This accession is resistant to leaf-miner and corresponds to the F_2RC_5 generation.

Seedlings from both susceptible and resistant plants were infested with leaf-miner, according to methodology described before (Guerreiro-Filho et al., 1992). Leaves were collected at different times during insect development, and corresponding to: T0, non-inoculated; T1, oviposition; T2, larvae eclosion; T3, initial lesion; T4, late lesion. After collection, leaves were immediately frozen and kept at -80 ◦C until used.

Gene selection

Genes were selected based on information available in the literature describing their roles during plant defense responses. Complete transcript sequences, identified in the GeneBank, were used for directed Blast searches in the Coffee Genome Database (Vieira et al., 2006). The following genes were evaluated here: *glucanase*, *glutathione S- transferase*, *super oxide dismutase* (3 isoforms), *MAP Kinase WIPK* (2 genes), *lipoxygenase*, protein-kinase receptors *LRK1a, LRK1b and SIPKb*, bZIP transcription factors *RAR1a* and *RAR1b*, resistance genes *NDR1, NPR1a, NPR1b* and *PR1*.

Quantification of gene expression

Total RNA was extracted from collected leaves using a Trizol-based protocol (Invitrogen). Gene expression was evaluated by quantitative RT-PCR, using an ABI3700 Platform (Applied Biosysytems). Reactions were performed using a commercial kit containing both Syber Green and ROX fluorescences. Quantification analyses were performed using defined threshold value, baseline and Ct parameters (Iskandar et al., 2004). For these analyses the GAPDH gene was used as the endogenous control. For all relative quantifications, transcript levels of untreated leaves were used as normalizing values. Exceptions were untreated resistant leaves, where levels of expression from untreated susceptible leaves were used as normalizing values.

RESULTS AND DISCUSSION

The species *C. arabica* is highly susceptible to pathogens and, therefore, the development of new resistant cultivars by breeding programs relies on the transfer of genes through interspecific crosses. This is the case for development of a leaf-miner resistant cultivar, which is being selected from progenies derived of the intercross between *C. arabica* and *C.*

racemosa. One limitation for an efficient breeding is the lack of knowledge of the molecular aspects controlling this resistance response.

Molecular aspects of resistance to herbivore have been reported for several plant species (Reviewed by Kessler and Baldwin, 2002). Based on these reports, a group of genes can be indicated as potentially associated with defense, either acting in a signaling pathway or as antibiotic agents. Here expression of those genes was evaluated in coffee leaves, aiming to identify key genes that would indicate which response pathway is being activated in resistant plants.

Overall analysis of expression by quantitative PCR indicated that all genes evaluated are regulated in both resistant and susceptible coffee plants, upon leaf-miner infestation. However, for most genes regulation patterns are not significantly different between resistant and susceptible leaves. Among these genes, *lipoxygenase (LOX)*, *glutathione S-transferase* (GST), *glucanase* (GLU) and protein kinase receptor *LRK1* can be highlighted. Results from transcripts relative quantification from those genes are shown on Figure 1.

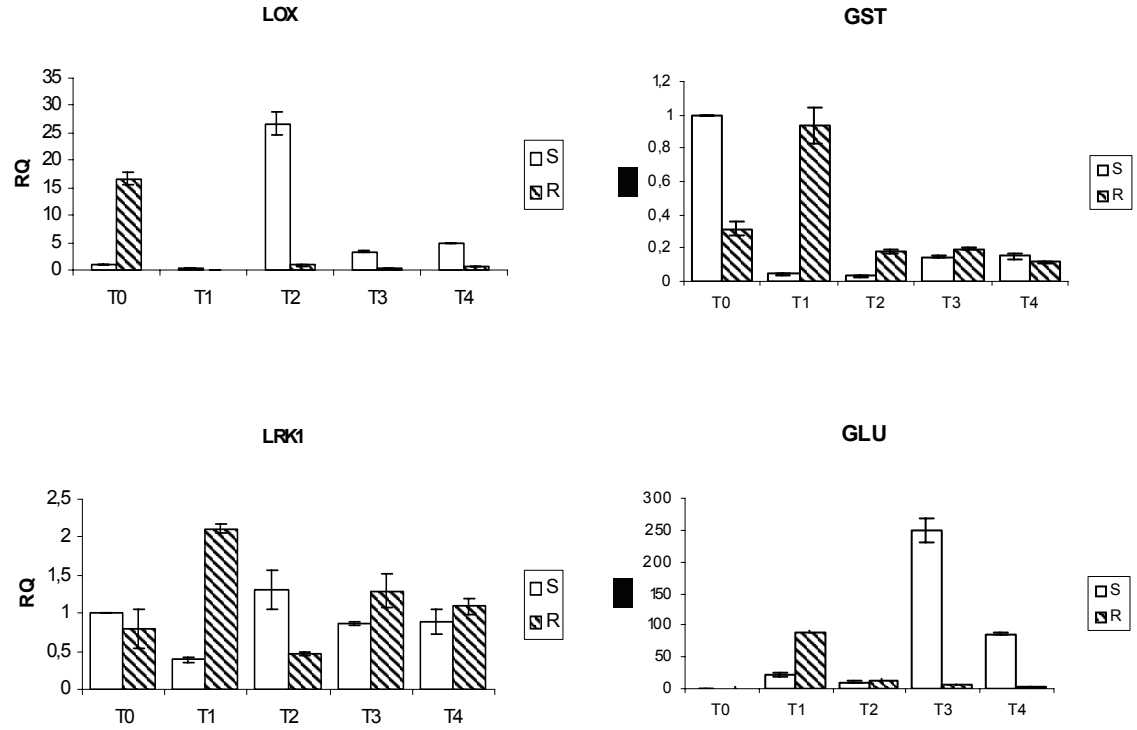


Figure 1. Transcript accumulation of defense- related genes in susceptible (S) and resistant (R) leaves. T0 to T4 refer to time-course infestation stages (see Material and Methods for details).

Significant differences can be observed in the expression patterns, which are mainly related with the timing of regulation during leaf-miner life cycle. For instance, LOX transcript levels are higher in control resistant leaves than in susceptible ones, and after oviposition (T1) those transcript levels are reduced. On the other hand, LOX transcript levels increase after larvae eclosion (T2) in susceptible leaves. Similar expression pattern was observed for GLU transcripts, as resistant leaves accumulated expressive transcript levels at oviposition (T1), while in susceptible leaves only during larvae development (T3) transcript levels reached a maximum point. Although expression levels of LRK1 and GST were not highly different between susceptible and resistant leaves, the accumulation pattern is interesting: for both

genes in resistant control leaves transcription levels are lower than in susceptible ones, but are higher upon leaf-miner oviposition (T1).

LOX is the first enzyme of the Methyl-jasmonate biosynthetic pathway, an important elicitor molecule associated with defense to wounding and herbivory (Reviewed by Kesller and Baldwin, 2002). Also, GLU is an important defense-related protein, activated in response to wound. Here we observed that LOX and GLU transcripts accumulated rapidly at early stages of leaf-miner infection in resistant leaves, and at later stages in susceptible ones. Also, transcripts from GST, an anti-oxidant protein, and LRK1, a kinase-receptor, were rapidly accumulated during the first infestation stage in resistant leaves. These results suggest that resistant leaves display a basal defense state, which can be rapidly activated in response to leaf-miner attack. On the other hand, despite their ability to trigger a defense response, susceptible leaves are not able to activate this response at the required timing. Further studies are under progress in order to confirm this defense pattern, and also to establish which genes are responsible for triggering the resistance response.

REFERENCES

Guerreiro-Filho O., Medina-Filho H.P., Carvalho A. (1992) Método para seleção precoce de cafeeiros resistentes ao bicho mineiro *Perileucoptera coffeella*. Turrialba 42:348-358.

Guerreiro-Filho O., Silvarolla M.B., Eskes A.B. (1999) Expression and mode of inheritance of resistance of leaf miner. Euphytica 105:7-15.

Iskandar H.M., Simpson R.S., Casu R.E., Bonnett G.D., Maclean D.J., Manners J.M. (2004) Comparison of reference genes for quantitative real-time polymerase chain reaction analysis of gene expression in sugarcane. Plant Mol Biol Report 22:325-337.

Kessler A., Baldwin I.T. (2002) Plant responses to insect herbivory: The emerging molecular analysis. Annu Rev Plant Biol 53:299-328.

Konnorova E., Vega (1985) Nocividad de *Leucoptera coffeella* (Lepdoptera-Lyonetiidae) III. Características y dimensiones de las lesiones producidas a las hojas del cafeto. Cienc. Tec. Agric. Café y Cacao, Habana, 7: 25-40.

Ramiro D.A., Guerreiro-FilhoO., Queiroz-Voltan R.B., Matthiesen S. (2004) Caracterização anatômica de folhas de cafeeiros resistentes e suscetíveis ao bicho-mineiro. Bragantia 63:363-372.

Ryan C.A. (2000) The systemin signaling pathway: differential activation of plant defensive genes. Biochim. Biophys. Acta 1477:112–21.

Vieira L.E.G., Andrade A.C., Colombo C.A., Pereira G.A.G. (2006) Brazilian coffee genome project: an EST-based genomic resource. Brazil J Plant Physiol 18:95-108.

Biochemical Characterization of Resistant and Susceptible Coffee Plants to the Coffee Leaf Miner (*Leucoptera coffeella*)[**]

F.H.H. MARTARELLO[1*], O. GUERREIRO-FILHO[1], T.J.G. SALVA[1], M.P. MALUF[2]

[1]Instituto Agronômico, Centro de Café "Alcides Carvalho", Caixa Postal 28, 13012-970, Campinas, SP, Brazil. [*]E-mail: fabiopg07@iac.sp.gov.br
[2]EMBRAPA Café, 70770-901, Brasília, DF, Brazil
[**]Supported by Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café

SUMMARY

Coffee Leaf Miner (*Leucoptera coffeella)* is the most serious pest in Brazil. This caterpillar causes significant looses in coffee arabica production, and this specie is highly susceptible to the insect. However, different resistance levels are observed in segregating progenies from crosses between *Coffea arabica* and *Coffea racemosa*. So far defense mechanisms of these plants are unknown. In this study, possible biochemical differences between susceptible and resistant plants. Extraction and quantification of carbohydrates, proteins and chlorophyll were performed in segregates progenies in selected plants from advanced generations of breeding program seeking leaf miner resistance. Higher levels of sugars and proteins were observed in resistant plants, while susceptible plants had higher levels of chlorophyll. A larger number of plants and progenies will be evaluated to confirm these results.

INTRODUCTION

Brazil is the main world coffee producer and exporter, with approximately 6.2 billion of coffee trees, distributed in 2.3 million hectare (Conab, 2007). In 2006/2007 coffee production was 41.5 million bags, being almost 70% is *Coffea arabica* and 30% of *Coffea canephora* (CBP&D – Café, 2007).

Coffea productivity is influenced to several phytosanitary problems, in special the Coffee Leaf Miner, *Leucoptera coffeella* (Guérin-Mìneville) (Lepidoptera-Lyonetidae). This pest causes significant looses to the plant, as it feeds from the leaf mesophyll, reducing photosynthetic area, leading to premature leaves fall and reduced frut production (Reis e Souza, 1986,1998; Guerreiro Filho, 1999).

Chemical control is relatively efficient, but causes a great agrosystem unbalance due to simultaneous reduction of naturals predators, the use of resistants cultivars is the most appropriate solution in the fight against insect.

At IAC, *C. arabica* resistant progenies to insect are under selection. These resulted from an interespecific cross with *C. racemosa* resistant species.This resistence is conditioned by two dominants and complementaries genes, denominated *Lm1* and *Lm2* (Guerreiro-Filho, 1999).

Defense mechanisms are still inknow. In previous studies, several authors verified differences in primary metabolism compounds, like sugars and proteins, as well as in secondary metabolism, like terpenos, phenois among others substances(Caixeta et al., 2004; Magalhães, 2005). However, so far the results are not conclusive. This work aimed to investigate biochemicals differences between resistants and susceptibles coffee trees Leaf Miner.

MATERIAL AND METHODS

Susceptible (25) and resistant (25) plants from the segregating progenie H-14954-46 were evaluated in this work. Leaf samples were collected and weighed and grouped in 10 samples of 5 plants each, being 5 of resistant plants and 5 of susceptible.

For total soluble sugars quantification, fresh leaves were lyophilized, grinded and weighed. Extraction was made with 60 ml of 80% ethanol. The solution was incubated in water bath for 6 hours and later centrifuged at 12.000 rpm for 10 minutes. The supernatant was collected and one volume of clorophorm was added, resulting in concentrated extract. Total sugars were quantified according to Dubois et al. (1956).

For total proteins analysis, fresh material was weighed and triturated in phosphate buffer as described in Mazzafera and Robinson (2000). Protein content was quantified according to Bradford (1976).

For the chlorophyll analysis, fresh leaves were softened in 96% ethanol (v/v). The extract was centrifuged at 12.000 rpm for 10 minutes and collected. Chlorophyll A and B contents as well as total chlorophyll content were measured according to Witermans and Mots (1965).

The experimental design was completely randomized, with 5 repetitions. Data were submitted to variance analysis and all means were compared by the Tukey test using 5% of confidence level.

RESULTS AND DISCUSSION

Table 1. Total soluble sugars (mg/g dm), total proteins (mg/g fm) and chlorophyll (mg/l alcoholic extract) content in plants resistant and susceptible to Leaf Miner.

Leaves sample	Total soluble sugars	Total proteins	Chlorophyll
Susceptible	1,08 b	0,46 b	14,27 a
Resistant	1,32 a	1,62 a	12,60 b

Means with same letter do not differ significantly at 5% of confidence level.

Resistant plants have higher total soluble sugars content when compared with susceptible plants. These results are in accordance with the observations of Caixeta et al. (2004), where an increase of leaf sugar content results in a lower insect attack.

A hypothesis to explain this phenomenon, based on Taiz and Zeiger (2004) is among total sugars the presence of lignin, a polysaccharide that leads to herbivory resistance due to reduction of the leaf digestibility for the insects (Appel, 1998; Mattson e Scriber, 1987).

Higher contents of total protein were observed in resistant plants disagreeing the results observed by Caixeta et al. (2004). These authors described higher protein contents in plants exhibiting intense leaf miner attack. The results shown were could be explained by an increase of synthesis of defense substances, for example oxidative stress enzymes (Felton et al., 1989), as well as terpenoids, jasmonates and cumarins (Taiz e Zeiger, 2004).

Regarding chlorophyll content, resistant plants have lower content when compared to susceptible plants. This fact can be explained by insect posture and feeding habit, which usually occurs at the superior third, and at third and fourth leaves pair (Nantes e Parra, 1977).

This plant section normally have a great photosynthetic activity and consequently, larger chlorophyll content (Fahal et al.,1994; Kimmerer and Potter, 1987).

REFERENCES

Appel H.M. The chewing herbivore gut lumen: physicochemical conditions and their impact on plant nutrients, allelochemicals, and insect pathogens. In: BERNAYS, E. A. (Ed.). Insect plant interactions. v.5, p.202-223, 1998.

Bradford M.N. A rapide and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, Duluth, v.72, p.248-254, 1976.

Caixeta S.L., Martinez H.E.P., Picanço P.R. , Esposti M.D.D. , Amaral J.F.T. Nutrição e vigor de mudas de cafeeiro e infestação por bicho- mineiro. Ciência Rural, Santa Maria, v.34, n. p.1429-1435, 2004.

CNP&D-CAFÉ. Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café. Economia Cafeeira http://www.embrapa.br/cafe/consorcio/home_4.htm. (23 abril 2007)

CONAB, Companhia Brasileira de Abastecimento. http://www.conab.gov.br/conabweb/ download/ safra/ Boletim.pdf. (03 maio 2007).

Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A., Smith F. Colorimetric method for determination of sugars and related substances. Analytical. Chemistry., v.28, p.350-56, 1956.

Fahal J.I., Carelli M.L.C., Vega J., Magalhães A.C. Nitrogen and irradiance levels affecting net photosynthesis and growth of young coffee plants (*Coffea arabica* L.). Journal of Horticultural Science, v.69, n.1, p.161-169, 1994.

Felton G.W., Donato K., Del Vecchio R.J., Duffey S.S. Activation of plant polyphenol oxidases by insect feeding damage reduces nutritive quality of foliage for noctuid herbivores. Journal of Chemical Ecology, New York, v.15, n.12, p.2667-2694, 1989.

Guerreiro-Filho O. Melhoramento do cafeeiro visando resistência às pragas. Simpósio de Atualização em Genética e Melhoramento de Plantas, 3, Genética e Melhoramento do Cafeeiro. Lavras, Brasil, 1999. p.36-49.

Kimmerer T.W., Potter D.A. Nutritional quality of specific leaf tissues and selective feeding by a specialist leafminer. Oecologia. v.71, p.548-551, 1987.

Magalhães S.T.V. Fitoquímicos na interação de bicho-mineiro com cafeeiros.. 2005, 83p. Tese (Doutorado). Universidade Federal de Viçosa, Viçosa-MG.

Mattson W.J., Scriber J.M. Nutritional ecology of insect folivores of woody plants: nitrogen, water, fiber, and mineral considerations. In. F. Slansky and J.G. Rodriguez (eds.). Nutritional ecology of insects, mites, spiders and related invertebrates. John wiley, New York. p.105-146. 1987.

Mazzafera P., Robinson S.P. Characterization of polyphenol oxidase in coffee. Phytochemistry, Oxford, v.55, p.285-296, 2000.

Medina-Filho H.P., Carvalho A., Monaco L.C. Melhoramento do cafeeiro. XXXVII - Observações sobre a resistência do cafeeiro ao bicho mineiro. Bragantia, Campinas, v.36, p.131-137, 1977.

Nantes J.F.D., Parra J.R.P. Avaliação de danos causados por *Perileucoptera coffeella* (Guérin – Méneville, 1842) (Lepidoptera – Lyonetidae), em três variedades de café (*Coffea* spp.) O Solo, v.69, n. 2, p.26–29, 1977.

Reis P.R., Souza J.C. Pragas do Cafeeiro. Cultura do Cafeeiro – Fatores que afetam a produtividade. Piracicaba, Potafós, 447p.,1986.

Reis P.R., Souza J.C. Manejo integrado do bicho-mineiro do cafeeiro *Perileucoptera coffeella* (Guérin-Menéville) (Lepdóptera-Lyonetiidae), e seu reflexo na produção de café. Anais da Sociedade Entomológica do Brasil, v. 25, n. 1, p. 77-82, 1998.

Taiz L., Zeiger E. Metabólitos Secundários. In*:* Taiz L., Zeiger E. Fisiologia Vegetal. 3ª Edição Artmed Editora S/A. Porto Alegre. p. 309-332. 2004.

Wintermans J.F.G.M., Mots A. De. Spectrophohometric characteristics of clorophylls a and b and their pheophytins in ethanol. Biochimica Biophysica Acta, v.109, p.448-453, 1965.

Peroxidase Activity as a Biochemical Marker for Resistance to the Coffee Wilt Disease

S. NAKENDO[1,2*], G.W. LUBEGA[1], A.O. KANGIRE[2], AND P. MUSOLI[2]

[1]Faculty of Veterinary Medicine, Makerere University Kampala
[2]Coffee Research Center-NARO, P.O. Box 185, Mukono, Uganda.
[*]E-mail: nakendosaleh@yahoo.co.uk or snakendo3@vetmed.mak.ac.ug

SUMMARY

The coffee wilt disease (CWD) caused by *Fusarium xylarioides* Steyaert is the major production constraint of *C.canephora* in Uganda, and could reduce coffee exports by $21M should the disease spread to East, Central and West Africa. Use of resistant varieties is the only reliable means of controlling CWD. The potential of peroxidase activity as a biochemical marker for resistance to CWD was assessed. Peroxidase was extracted from leaf tissue and peroxidase activity determined using Jennings et al. (1969) protocols. Peroxidase activity was then related to response of a particular variety to CWD. Peroxidase activity and survival rate due to CWD showed positive correlation, R = 0.652. Variations in survival rate and mortality rate due to peroxidase activity was found to be statistically significant (P = 0.0062) at 95% confidence level. The study investigated the relationship between peroxidase activity and CWD resistance in coffee genotypes and explore the potential of using peroxidase activity for selecting resistant genotypes

INTRODUCTION

The coffee wilt disease caused by *Fusarium xylarioides Steyaert* is the major production constraint of Robusta coffee in Uganda. The disease is vascular and specific to Robusta coffee. Result of surveys have shown that if the disease spreads in East, Cameroon and Cote d'lvoire, Africa's coffee export revenue might be reduced by $ 21.58M yearly (Onzima, 2001). There has been no viable strategy for addressing the problem other than breeding for resistance. Currently, there are no resistant varieties with durable resistance. Although breeding efforts to produce resistant varieties have been underway, this has been hampered by lack of robust and reliable markers to hasten the process. In resistance breeding, an effective screening procedure is essential. Use of peroxidase activity could fasten the screening process.

The conventional method of breeding for resistance is time consuming and lacks durability (Lindhout, 2002; Lamberti et al., 1982). This prompted the researchers to establish whether peroxidase activity could be used as a marker for identifying resistant genotypes to coffee wilt disease in Robusta coffee. Research done elsewhere, showed that peroxidase activity is involved in the fight against phyto-pathogens (Lovrekovich et al., 1986), *F. xylarioides* inclusive. This enzyme is a phenol oxidase enzyme and oxidizes plant compounds to fungi-toxic substances that inhibit the spread of the infecting pathogen in the plant tissues (Lovrekovich et al., 1986). The objective of this study was to use peroxidase activity as a marker for resistance against coffee wilt disease in *C.canephora.*

MATERIALS AND METHODS

Eight cultivars of *C.canephora* viz. $1^s/2$, $1^s/3$, $1^s/6$ (Partially susceptible); H/4/1, E/3/2, $257^s/53$ (Highly susceptible); J/1/1 and Q/3/4 (Prospective resistant); were studied. Coffee samples were selected for the study basing on the data of their individual response to CWD under field conditions (Musoli P.C, unpublished work).

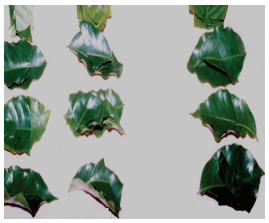
Table 1. Performance of *C.canephora* variants in CWD infested field.

Variety	No. of Marked Plants in Field	Surviving Plants	% Survival Rate (SR)	Dead Plants	% Mortality Rate (MR)
$1^s/3$	6	3	50	3	50
$1^s/2$	6	2	33.33	4	66.67
$1^s/6$	6	3	50	3	50
E/3/2	6	1	16.67	5	83.33
$257^s/53$	6	2	33.33	4	66.67
H/4/1	6	0	0	6	100
Q/3/4	6	6	100	0	0
J/1/1	6	6	100	0	0

Peroxidase was extracted from leaf tissues and peroxidase activity was determined using Jennings et al. (1969) protocols. Two grammes of leaf tissue were crushed in 1.5 ml of 0.05 M tris-hydroxymethyl amino methane-HCl Buffer (pH 7.5), using prechilled mortars and pestles. The crushed materials were centrifuged at 18000 g for 10 minutes at 4 °C, and all the peroxidases were assumed to be in the supernatant. Peroxidase activity was determined by placing 0.5 ml of 1:100 dilutions of the extracts into a spectrophotometer cuvette into which 0.5 ml of 1% guaiacol solution and 1.5 ml tris-HCl buffer (0.05m, pH 7.5), was added. The reaction was initiated by adding 0.5 ml of 1% H_2O_2 and optical density (OD) readings were taken at a wave length of 485 ηM. A blank consisting of 0.5 ml of diluted extract, 0.5 ml of 1% guaiacol and 2.0 ml tris-HCl buffer was used to set spectrophotometer at 100% transmittance. Changes in optical density of the reaction mixture were read at 15sec interval up to 4 minutes, after mixing all ingredients. Procedure was repeated 3× for each diluted extract and the mean readings calculated. A graphical representation was made of optical density versus time. The change in optical density was calculated from the straight path of the graph and the total peroxidase activity calculated as follows;
Peroxidase Activity = (Change in OD x 1/T x 1/0.5 ml x 100)


Diseased tree


Healthy tree


Leaf tissue for extraction


Extraction of peroxidase

Figure 1.

The incidence data was generally analyzed using Correlation analysis, ANOVA and Regression analysis under Intercooled STATA 8 statistical package.

RESULTS

The results of survival rate and peroxidase activity of *C.canephora* (Tables 1 and 2) and Figure 2; in relation to the resistance to *F.xylarioides* showed a positive correlation (R = 0.652). The variation in survival rate and mortality rate (MR) due to peroxidaes activity was significant (P = 0.0062) at 95% confidence level. Peroxidaes activity coefficient was also found significant (P = 0.006), but the constant was not significant (P = 0.282) at 95% confidence level, giving regression equation as SR = 95PA. This implied that min^{-1} ml^{-1} increase in peroxidase activity would result in 95 times increase in survival rate. Without peroxidase activity (PA = 0), the survival rate would be zero.

Table 2. Peroxidase activity for *C.canephora* variants.

Variety	Peroxidase activity /$min^{-1}ml^{-1}$ (PA)
$1^s/3$	0.200
$1^s/2$	0.204
$1^s/6$	0.204
E/3/2	0.204
$257^s/53$	0.544
H/4/1	0.204
Q/3/4	0.408
J/1/1	0.880

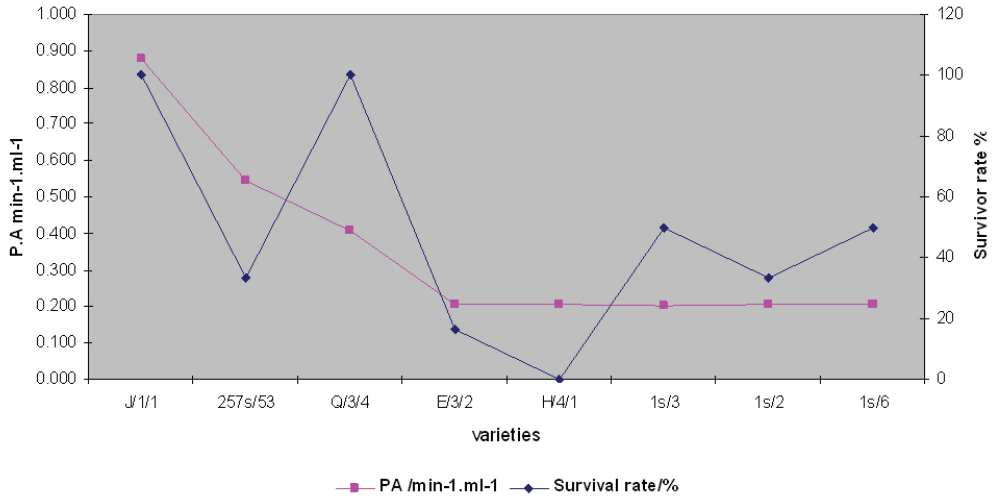


Figure 2. Peroxidase activity vs survival rate

DISCUSSION AND CONCLUSION

The results of PA and SR due to CWD correlated positively (R = 0.652). The PA of resistant varieties J/1/1 and Q/3/4 were higher with 0.880 min^{-1} ml^{-1} and 0.408 min^{-1} ml^{-1} respectively and statistically significant (P = 0.0062), indicating that PA has a contribution to resistance against CWD. These results are consistent with reports by Fehrmann and Diamond (1967) which found that resistance to *Phytophthora infestans* was positively correlated to PA in potato tissues. Similarly, there was increase in PA in highly susceptible varieties (Table 2). This corroborated with studies on peroxidase induction in response to wounding (Lagrimini and Rothstein, 1987). Such increase in PA in highly susceptible varieties most especially $257^s/53$ (Table 2), could have been due to infection by *F. xylarioides*. However, Rautela and Payne (1970) suggested that the failure of peroxidase to arrest the infection in susceptible

varieties was probably due to late increase or insufficient peroxidase. In conclusion, PA can be used as a biomarker for resistance to CWD through marker assisted selection and thus can be used to predict resistance against CWD in Robusta coffee.

Table 3. Comparative efficiency.

Parameter	Biotechnology	Conventional breeding
Experiment duration	16 weeks	580 weeks
Experiment cost	$1,500	$10,000
Labour	Personnel ≤5	Personnel ≥30
Reliability	75%	50%

GAP/FUTURE WORK

Peroxidase enzyme is known for its protective role against diseases and pests both in plants and animals, man inclusive (antiviral). However, at Coffee Research Center (COREC) in Uganda, success on coffee wilt disease, (1500 resistant variants/Robusta type), has been achieved through classical means (conventional breeding), thus conclusions based on morphological characteristics (Phenotypic relations). The resistance to wilt is determined by many factors; genetic variability being the main factor, but, could also be due to environmental interactions (susceptibility trait masking), due to coffee in relation to CWD. This implies that should there be environmental and climatic changes, the problem of CWD may resurface. This emergency may be appropriately managed with other combinations of state of art molecular and biochemical marker technology. Further development of this project/methods is the aim of further investigations, the need for an extension of the present database and recalibration will lead to more robust calibration and standardization of the results.

ACKNOWLEDGEMENTS

I would like to thank, Dr. Africano Kangire, Dr. Pascal Musoli and Dr. James Ogwang of Coffee Research Center, National Crops Resources Research Institute, NARO-Uganda; for the technical support and reviewing the various
drafts of this work.

I also thank, Dr. Prof. G.W. Lubega, Principal academic supervisor of this work, Makerere University Kampala. Dr. Jesca L Nakavuma and Dr. Eddie Wampande, Makerere University Kampala; for the technical support and reviewing of this work.

This work was funded by HON. A.B.Nakendo.

REFERENCES

Fehrmann H. and Diamond A.E. 1967. Peroxidase activity and *phytopthora* resistance in different organs of the potato plant. Phytopathology 57:69-72.

Jennings P.H., Brannaman B.L. and Zoheille F.P. Jr. 1969. Peroxidase and polyphenoloxidase activity associated with *Helminthosporium* leaf spot of maize. Phytopathology 59:963-967.

Lagrimini L.M. and Rothstein S. 1987. Tissue specificity of tobacco peroxidase isozymes and their induction by wounding and tobacco mosaic virus infection. Plant Physiology 84:438-442.

Lamberti F., Waller J. M. and Van der Graaff N.A. 1982. Durable resistance in crops. NATO Advanced science institutes series A, Life science; Vol.55.

Lindhout P. 2002. The perspectives of polygenic resistance in breeding for durable resistance. Euphytica 124:217-226.

Lovrekovich L., Lovrekovich H. and Stahman M.A. 1986. The importance of peroxidase in the wildfire disease. Phytopathology 58:193-198.

Onzima R. (2001). Coffee Wilt Disease (CWD), ACRN UPDATES. The African Coffee Research Network (ACRN). Vol. No.1 (June-Sept, 2001).

Rautela G.S. and Payne M .G. 1970. The relation of peroxidase and orthodiphenol oxidase to resistance of sugarbeet to Cercospora leaf spot. Phytopathology 60:238-245.

The Impact of Climatic Variability in Coffee Crop

M.B.P. CAMARGO*, B.P. MARCELO

Scientific Researcher (Agrometeorology, MS/PhD). Instituto Agronômico (IAC/APTA), Campinas, SP, Brazil. *E-mail: mcamargo@iac.sp.gov.br
Supported by PNP&D/Café and CNPq

SUMMARY

The climatic variability is the main factor responsible for the oscillations and frustrations of the coffee grain yield in the world. The relationships between the climatic parameters and the agricultural production are quite complex, because environmental factors affect the growth and the development of the plants under different forms during the growth stages of the coffee crop. Agrometeorological models related to the growth, development and productivity can supply information for the soil water monitoring and yield forecast, based on the water stress derived by a soil water balance during different growth stages of the coffee crop, quantifying the effect of the available soil water on the decrease of the final yield. Other climatic factors can reduce the productivity, such as adverse air temperatures happened during different growth stages. Solar radiation and relative humidity influence many physiological processes of coffee tree but are not generally thought to play an important role as thermal and rainfall conditions in defining potential yield or ecological limitations for this crop. According to the last report of the Intergovernmental Panel on Climate Change (IPCC), the global temperature is supposed to increase 1 °C to 5.8 °C and the rainfall 15% in the tropical areas of Brazil. Some Global warming as presented by IPCC will cause a strong decrease in the coffee production in Brazil. According to the literature besides the reduction of suitable areas for coffee production, the culture will tend to move South and uphill regions. This lecture analyze the effect that these possible scenarios would have in the agro-climatic coffee zoning in Brazil, and adaptive solutions, such as agronomic mitigations and development of cultivars adapted to high temperatures is considered.

CLIMATE AND COFFEE PRODUCTION

Among almost 100 species of the *Coffea* genus, *Coffea arabica* L. (arabica coffee) and *Coffea canephora* Pierre (robusta coffee) economically dominate the world coffee trade, being responsible for about 99% of world bean production. Arabica coffee accounts for about 70% of coffee consumed, and robusta coffee for the rest (Damatta and Ramalho, 2006).

Arabica coffee is native to the tropical forests of Ethiopia, Kenya, and Sudan, at altitudes of 1500-2800 m, between the latitudes of 4° N and 9° N. In this region, air temperature shows little seasonal fluctuation, with a mean annual air temperature between 18 and 22 °C. Rainfall is well distributed, varying from 1600 to more than 2000 mm, with a dry season lasting three to four months coinciding with the coolest period. In this environment, arabica coffee became established as an under-storey shrub (Sylvain, 1955). Arabica coffee is cultivated in more than 80% of the countries coffee grower, tends its largest diffusion in the American continent. In Asia, this species is almost extinguished, mainly due to the rust incidence (*Hemileia* sp.). Now Arabica coffee is in the high altitudes of India, where it is counted with resistant cultivars to the predominant races of this fungus, as well as in Philippines and in the Southeast region of Indonesia.

Arabica coffee vegetates and fructifies very well at tropical uplands, as in the Southeast area of Brazil. It is usually affected in their growth stages by the environmental conditions, especially by the photoperiodic variation and by the meteorological conditions, mainly, the rainfall distribution and air temperature that interfere in the crop phenology, and consequently in the coffee bean productivity and quality. According to Camargo (1985), for arabica coffee the optimum mean annual air temperature range from 18 to 23 °C. Above 23 °C, development and ripening of fruits are accelerated, often leading to loss of quality. Continuous exposure to daily temperatures as high as 30 °C could result in not only depressed growth but also in abnormalities such as yellowing of leaves (Damatta and Ramalho, 2006). A relatively high air temperature during blossoming, especially if associated with a prolonged dry season, may cause abortion of flowers. It should be noted, however, that selected cultivars under intensive management conditions have allowed arabica coffee plantations to be spread to marginal regions with mean annual air temperatures as high as 24-25 °C, with satisfactory yields, such as in the Northeast and North regions of Brazil (Fazuoli et al., 2007; Bergo et al., 2008). On the other hand, in regions with a mean annual air temperature below 18°C, growth is largely depressed. Occurrence of frosts, even if sporadic, may strongly limit the economic success of the crop.

Robusta coffee is native to the lowland forests of the Congo River basin; with extend up to Lake Victoria in Uganda. This species developed as a midstorey tree in a dense, equatorial rainforest. In that region, the annual mean temperature range from 23 to 26 °C, without large oscillations, with abundant rainfall superior to 2000 mm distributed over 9 to 10 month period. High temperatures can be harmful, especially if the air is dry (Coste, 1992). Robusta is much less adaptable to lower temperatures than arabica. Both leaves and fruits do not withstand temperatures below 6 °C or long periods at 15 °C. As altitude relates to temperature, robusta coffee can be growth between sea level and 800 m, whereas arabica coffee grows better at higher altitudes and is often grown in hilly areas, as in Colombia and Central America. Robusta coffee grows better in areas with annual mean temperature among 22 to 26 °C, as in the Republic of Congo, Angola, Madagascar, Ivory Coast, Vietnam, Indonesia and Uganda. In Brazil the main areas that cultivate the robusta are the lowlands areas of the Espirito Santo (Southeast) and Rondonia (North) states.

The climatic variability is the main factor responsible for the oscillations and frustrations of the coffee grain yield in the world. The relationships between the climatic parameters and the agricultural production are quite complex, because environmental factors affect the growth and the development of the plants under different forms during the phenological phases of the coffee crop. Agro-meteorological models related to the growth, development and productivity can supply information for the soil water monitoring and yield forecast based on the air temperature and water stress derived by a soil water balance during different crop growth stages, quantifying the effect of the available soil water on the decrease of the final yield. The processes of photosynthesis become limited when water stress occurs, due to closing of the stoma and reduction in other physiological activities in the plant. Other climatic factors can reduce the productivity, such as adverse air temperatures happened during different growth stages. An agro-meteorological study was conducted aiming to develop an agro-meteorological model (Camargo et al., 2006) that monitors and assesses the quantitative influence of climatic variables, such as air temperature and soil water balance on the coffee crop phenology and yield for different Brazilian regions. That kind of model could be an efficient tool to assess the environmental effects of new technologies, and future climate change scenarios.

On such background, this presentation covers some aspects of the coffee crop responses under current climatic conditions and analysis of the impacts of climate variability and future

climate scenarios issued from the IPCC would have in the agro-climatic zoning. Also, this lecture analyzes the effect of adaptive solutions, such as agronomic mitigations and development of cultivars adapted to high temperatures.

GLOBAL CLIMATE CHANGE AND CLIMATIC VARIABILITY

The increase of greenhouse gas emissions (GHG) in the atmosphere is causing wide changes in atmospheric events, influencing climate change and variability with critical impacts on vegetations. According to the Fourth Assessment Report of the WMO/UNEP Intergovernmental Panel on Climate Change (IPCC) released in 2007, semi-arid and sub-humid regions of Asia, Africa and Latin America are likely to warm during this century and freshwater availability is projected do decrease. Agricultural productivity in tropical Asia is sensitive not only to temperature increases, but also to changes in the nature and characteristics of monsoon. In the semi-arid tropics of Africa, which area already having difficulty coping with environmental stress, climate change resulting in increased frequencies of drought poses the greatest risk to agriculture. In Latin America, the air temperature is supposed to increase 1 °C to 5.8 °C and the rainfall 15% in the tropical areas of Brazil; and agriculture and water resources are most affected through the impact of extreme temperatures and changes in rainfall (Sivakumar & Stefanski, 2008). The report is obviously preoccupying, although the own reports contain a high uncertainty degree in the results of the long term forecast models.

According to the IPCC (2007) macroclimatic characteristics are showing changes, particularly in the last decade. The parameters more representative of these variations are air temperature. Really in the last 10 years, the agriculture has been suffering with high air temperatures, especially during 2002 and 2007 years. Periods with accentuated water deficits have also been frequent in those years, what would confirm that the meteorological adversities are happening in an atypical way, reaching the Brazilian coffee crop. However, these agro-meteorological adversities have happened in cyclical form during the XX century. For instance, the 1960's decade was marked by severe droughts, especially during the years of 1961 and 1963, which affected drastically the coffee production for the years of 1962 and 1964. Associated to these dry years, high air temperatures were observed, mainly during the months of August, September, and October, which were the highest of the XX century.

When we analyze the long term (118 years) meteorological data (1890/2007) collection from the weather station of Campinas, Sao Paulo State, Brazil, we can observe that agro-meteorological adversities (drought, mean, maximum and minimum air temperatures) happened in a cyclical way, with typical periods from 15 to 20 years of variability, but these annual temperatures show an increase up to 2.0 °C for annual mean, 1.3 °C for the annual maximum, and 2.6 °C for the annual minimum. For instance severe frosts, adversities that do not happen in the coffee crop of the Southeast region since 1994, are observed on average every 15-20 years. Examples are the severe frosts of the years of 1892, 1902, 1918, 1942, 1953, 1975, 1981, and 1994.

According to the IPCC, the global warming scenario, the maximum air temperatures would be higher, but the minimum air temperatures would be lower and frosts would be also more frequent (Orlandini et al., 2008). Air temperature increases can lead to several consequences: faster physiologic plant growth, and therefore smaller final production; greater risk of pathogenic attacks and greater request for irrigation. Global Circulation Models (GCMs) can reproduce climate features on large scales, but their accuracy decreases when proceeding from continental to regional and local scales because of the lack of resolution (Halenka, 2008). This

is especially true for surface fields, such as precipitation, surface air temperature and their extremes, which are critically affected by topography and land use.

The scientific community responsible for the forecast of long period scenarios for next decades does not present consensus on the actual warming of the climate of the Earth. Among the reports of IPCC, although all they contain warming scenarios, the fourth informed increases of 2 and 3 °C, while the first report informed warming scenarios up to 10 °C. Besides, a group of scientists are defending inverse scenario up to the year of 2050, when the Earth would arrive to a great cooling, as it happened in the middle of 1700, when several European rivers got to freeze during the winter seasons. Forecast of distant scenarios, such as for 20, 50, 100 years, would be more inconsistent for the conclusion of how the scenarios of the climate will be for the agricultural activities.

POSSIBLE CONSEQUENCES FOR THE COFFEE CROP

High temperatures are known to disturb plant metabolism. Coffee cultivation in the open is the usual practice in Brazil, and this provokes leaf exposure to high irradiance and the absorption of much more energy than that usable by photosynthesis. Such conditions may cause an energy overcharge and an overheating of leaves that, in extreme cases, can reach temperatures of 40 °C or even above, especially if stomata are closed, as occurs on sunny days in unshaded crops (Maestri et al., 2001).

A quality problem could arise, from the faster plant growth that will lead to lower coffee fruit quality. Besides, high maximum temperatures during summer months may cause an excessive fruit ripening, against fruit quality. Coffee trees are well resistant to high summer temperature and drought, but the increase of extreme conditions can be responsible of physiological stresses, such as the reduction of photosynthetic efficiency. Others critical phases are flowering and grain fill in relation with the anticipation of bud dormancy break. Moreover high temperature and dry conditions during the reproductive phase can be critical for the optimum coffee production and quality. The setting of adequate air temperature limits for coffee is decisive for the distribution and economic exploitation of the crop.

Taking into account the global warming phenomena, temperature may rise up to 5.8 °C in the tropical area up the end of the XXI century, as reported by the IPCC, and considering the actual genetic and physiological characteristics of the cultivated Brazilian cultivars of arabica coffee, severe reductions of adequate areas for growing the crop are to be expected (DaMatta and Ramalho, 2006). The forecast of global warming has been causing great concern for scientists and producers linked to the world coffee crop. Some global warming reports get to infer scenarios that these reductions might reach values as high as 95% in the Brazilian states of Goias, Minas Gerais and Sao Paulo, and 75% in Parana state (Assad et al., 2004).

More recently, a work was published (Pinto et al., 2008) concerning “Global Warming and the New Geography of the Agricultural Production in Brazil”. The evaluation of the impacts of the climatic changes in the coffee crop was made using the climatic model PRECIS (Providing Regional Climates for Impact Studies) which is a computer program developed by the Hadley Center (England). The program based on annual mean temperature and climatic risk zoning simulates the agricultural scenarios for the Brazilian coffee crop for the years of 2010, 2020, 2050 and 2070 in agreement with the forecasts of IPCC. The authors concluded that the warming up to 5.8 °C foreseen for 2070 would cause many climatic changes and it would make unfeasible the coffee crop in the Southeast region of Brazil (Minas Gerais and Sao Paulo). In 2070 the coffee crop will migrate for the South region (Parana, Santa Catarina and Rio Grande do Sul), where according to Pinto et al. (2008) frost risk will be much lower.

However, fundamental agroclimatic parameters were not considered such as photoperiodic variation, consistent frost risk and rainfall distribution for the Brazilian South region.

Drought and high and low temperatures are undoubtedly the major threats to agricultural crop production, and the possibility to develop projections of drought occurrence at the regional scale is a necessary step toward the definition of suitable adaptation strategies for the coffee sector. In deriving drought projections from regional climate scenarios, the capability of climate models to reproduce the key feature of the hydrological regime should be examined (Calanca, 2008). In addition, from a risk analysis standpoint there is a pressing need to quantify uncertainties in the projections and provide probabilistic assessments of the impacts of climate change, including frost.

Climate change can have a wide range of effects on agricultural systems and we must adapt to these changes to ensure that agricultural production is not only maintained but is increased to support a growing world population (Smith et al., 2008). Local adaptation practices and those practices introduced by national development, research and extension organizations need to be collected from the respective organizations and evaluated at different levels. Different agronomic, water and policy management adaptation strategies needed to be considered.

AGRONOMIC TECHNOLOGIES FOR MITIGATION AND ADAPTATION

Climate change mitigation strategies which include interventions to reduce the sources or enhanced the sinks of greenhouse gases have a marked management component aiming at conservation of natural resources such as improved fertilizer use, use of water harvesting and conservation techniques. These strategies are equally consistent with the concept of sustainability. Adaptation strategies include initiatives and measures to reduce the vulnerability of agro-ecosystems to projected climate change, such as changing varieties, altering the timing or location cropping activities, improving the effectiveness of pest, disease and weed management practices, making better use of seasonal climate forecast etc. It is essential to develop and integrate Agriculture Mitigation and Adaptation Frameworks for Climate Change into sustainable development planning at the national and regional levels to cope with the projected impacts of climate change (Sivakumar and Stefanski, 2008).

Thus, comprehensive agro-meteorological adaptation policy guidelines, focusing on preparedness, mitigation and adaptation measures to support sustainable agricultural development, are needed to cope with the impacts of climate change/variability (Motha, 2008). Adaptation strategies may range from a change in crop cultivars to accommodate drought or shifts in temperature to extreme measures such as a total change in land use away from agriculture production (Smith et al., 2008). Pertinent research from several sources is reviewed and examples are provided using IPCC climate change scenarios to demonstrate the effect that **agronomical mitigations (**production system and plant management) and **agronomical adaptation** (breeding programs) strategies may have on global warming in coffee crop areas of the world.

Under agronomical aspects some of the techniques that can be used that can attenuate the impact of unfavorable temperatures are:

Shading management system (Arborization)

Although native to shady environments, modern arabica coffee cultivars in Brazil grow well without shade and even may show higher productions than those of shaded trees, particularly in zones with adequate climate and soils (DaMatta, 2004). The coffee cultivation was adapted

and widespread for unshaded due to the highest latitudes (19-24° S) and lower altitudes (500-1300 m) than the origin area. Great part of the arabica coffee cultivation in traditional countries like Colombia, Costa Rica, Guatemala, El Salvador and Mexico feels under arborization system where the coffee plants are close to the microclimate of their natural habitat. However, in Brazil there is an increasing trend in expanding coffee cultivation to marginal lands where water shortages and unfavorable temperatures may significantly constrain crop production. Coffee plantations have been also expanded towards warmer regions with prolonged droughts. In these harsh environments, the use of shading management is highly advisable in order to allow economic yields (DaMatta and Rena, 2002) and make the environment more suitable for arabica coffee. The main effects of shading on the coffee crop provides associated with decreased of the air temperature fluctuations by as much as 3-4 °C (Camargo et al., 2008) and wind speeds, and increased air relative humidity. Shading has been adopted to avoid large reductions in night temperatures at high elevations, as in Kenya (Carr, 2001), or at high latitudes, as in Parana State (23-24° S), Southern Brazil (Caramori et al., 2003) in order to reduce frost damage. There are several possible tree species for use as arborization, such as grevílea robusta, cedrinho, macadamia, rubber tree, banana prata, avocado, dwarfish coconut among others. The technique of the arborization allows thinner shading, with a density of around 60 to 70 shading tree plants per hectare.

Planting at high densities

This agronomic practice is the latest trend in Brazilian coffee growth. New coffee cultivars, such as Tupi and Obatã, are of compact size and especially suitable for planting in smaller spacing among lines and among plants in the line (planting in row). So, this practice presents smaller productions per plant, but increasing the production per area. Besides, stressing less the coffee plant, allows maintaining it more grown leaves, providing a suitable microclimate, with lower air temperatures inside the plant, in relation to the external environment.

Vegetated soil

The good agricultural practice recommends maintaining during the rainy season vegetated soil with weed in the middle between lines handled with agriculture implements. Besides the good soil conservation practice, the maintenance vegetated soil reduces the soil and air temperatures and allows a better plant root system distribution because the superficial roots are affected by the high temperatures. This handling also increases the organic matter tenor and the soil water retention capacity making possible a more tolerant cultivation to the adverse climatic conditions.

Irrigation

This practice has been the main factor to allow the establishment of the coffee plant in Brazilian marginal areas of low altitude in that the mean air temperatures are high for the usual cultivation of the arabica coffee.

Genetic Breeding

The genetic improvement of arabica and robusta plants in the “Centro de Café Alcides Carvalho” (IAC) has always emphasized the development of material with high yields, quality, strength, and longevity. The cultivars developed at the IAC include Bourbon, Icatu, Mundo Novo, Acaiá, Catuaí, Obatã, Tupi, and Ouro Verde, which represent more than 90% of the arabica coffee trees currently in production in Brazil. The cultivar Obatã is resistant to coffee leaf rust, compact size, suitable for planting in rows or at high densities, and especially

good yield and quality. Bergo et al. (2008) evaluated 40 cultivars of arabica and robusta coffee from 1994 to 2004 in Rio Branco region, state of Acre, Brazil, where the annual mean air temperature is close 25 °C. The study was carried out in the experimental field of Embrapa-Acre, and authors concluded that the best yield performance was the Obatã cultivar with significant difference in relation to the other cultivars. Obatã presented a mean yield of 49 sacks per hectare of clean coffee. This is an example of genetic improvement based on selective breeding of species arabica and robusta and of how improvement can contribute to the sustainability of coffee cultivation even under marginal lands with unfavorable air temperature.

These agronomic techniques can be used, alone or in a complementary way to mitigate extreme meteorological events and to face the challenge of climatic variability or global warming on coffee crop. Fundamental scientific research using different coffee crop management, genetic breeding and new molecular tools and focusing on this subject is high recommended, and the impact of the agronomic technologies on coffee copping systems particularly in marginal lands, is a challenge to be handled within the near future.

For the next decades we can expected that meteorological adversities will continue to happen, such as high air temperatures, frosts, droughts, excessive rains, hail etc. But, the agriculture, especially the coffee crop will be more developed and protected with agronomic techniques of adaptation and mitigation that certainly will continue to be developed by the technical and scientific world coffee crop community.

REFERENCES

Assad E.D., Pinto H.S., Zullo Jr. J., Avila A.M.H. Impacto das mudanças climáticas no zoneamento agroclimático do café no Brasil. Pesquisa Agropecuária Brasileira, Brasília, v.39, n.11, p.1057-1064, 2004.

Bergo C.L., Pereira R.C.A., Sales F. Avaliação de genótipos de cafeeiros arábica e robusta no estado do Acre. Ciência Agrotec., Lavras, v.32, n.1, p.11-16, 2008.

Camargo A.P. Florescimento e frutificação de café arábica nas diferentes regiões cafeeiras do Brasil. Pesq. Agropec. Bras., Brasília, v.20, n.7, p.831-839, 1985.

Camargo A.P., Camargo M.B.P. Definição e esquematização das fases fenológicas do cafeeiro arábica nas condições tropicais do Brasil. Bragantia, Campinas, v.60, n.1, p.65-68, 2001.

Camargo M.B.P., Santos M.A., Pedro Jr. M.J., Fahl. Agrometeorological model for monitoring and predicting coffee (*Coffea arabica* L.) productivity in Sao Paulo State, Brazil. In: 21st International Conference on Coffee Science, September/2006. Montpellier, France. ASIC, Proceedings, p.1125-1131, 2006.

Camargo M.B.P., Rolim G.S., Souza P.S., Gallo P.B. Air temperature in different *Coffea arabica* microclimates arborized with dwarf coconut palm and rubber tree in Mococa, SP, Brazil. In: 22st International Conference on Coffee Science, September/2008. Campinas, Brazil. ASIC, Proceedings, p.1-4, 2008.

Caramori P.H., Grodzki L., Morais H., Juliato H. Efeito da geada em mudas de café sob diferentes métodos de proteção. In: Simpósio de Pesquisa dos Cafés do Brasil, 3., 2003. Porto Seguro, Anais. Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café, p.1-6, 2003.

Carr M.K.V. The water relations and irrigation requirements of coffee. Exp. Agric., v.37, p.1-36, 2001.

Coste R. Coffee - The plant and the product. MacMillan Press, London, 1992.

Damatta F.M., Rena A.B. Ecofisiologia de cafezais sombreados e a pleno sol. In: Zambolim L. (ed), O estado da arte de tecnologias na produção de café. Universidade Federal de Viçosa, Viçosa, p.93-135, 2002.

Damatta F.M. Ecophysiological constraints on the production of shaded and unshaded coffee: a review. Field Crops Res. v.86, n.1, p.99-114, 2004.

Damatta F.M., Ramalho J.D.C. Impacts of drought and temperature stress on cofeee physiology and production: a review. Brazilian Journal of Plant Physiology, Campinas, v.18, n.1, p.55-81, 2006.

Fazuoli L.C., Thomaziello R.A., Camargo M.B.P. Aquecimento global, mudanças climáticas e a cafeicultura paulista. O Agronômico, Campinas, v.59, n.1, p.19-20, 2007.

Halenka T. New sources of high resolution climate change scenarios for impact studies in agriculture. In: Symposium on climate change and variability – agro-meteorological monitoring and coping strategies for agriculture. Oscarsborg, Norway, Abstracts. WMO, p.49, 2008.

Maestri M., Barros R.S., Rena A.B. Coffee. In: Last, F.T. (ed), Tree crop ecosystems. Elsevier Publishers, Amsterdam, p.339-360, 2001.

Motha R.P. Developing an adaptation strategy for sustainable agriculture. In: Symposium on climate change and variability–agrometeorological monitoring and coping strategies for agriculture. Oscarsborg, Norway, Abstracts. WMO, p.46, 2008.

Pinto H.S., Assad E.D., Zullo Jr. J. et al. Aquecimento global e a nova geografia da produção agrícola no Brasil. Embrapa/Unicamp. Campinas, 81p. Agosto, 2008.

Sivakumar M.V.K., Stefanski, R. Climate change mitigation, adaptation and sustainability in agriculture. In: Symposium on climate change and variability – agrometeorological monitoring and coping strategies for agriculture. Oscarsborg, Norway, Abstracts. WMO, p.44, 2008.

Smith W., Desjardins R., Grant B. Some perspectives on agricultural GHG mitigation and adaptation strategies with respect to the impact of climate change-variability in vulnerable areas. In: Symposium on climate change and variability – agrometeorological monitoring and coping strategies for agriculture. Oscarsborg, Norway, Abstracts. WMO, p.45, 2008.

Influence of Soil Properties on Cup Quality of Wild Arabica Coffee in Coffee Forest Ecosystem of SW Ethiopia

A. YADESSA[1*], J. BURKHARDT[2], M. DENICH[1], T. WOLDEMARIAM[3], E. BEKELE[3], H. GOLDBACH[2]

[1]University of Bonn, ZEF Bonn, Walter-Flex-Str. 3, D-53113, Bonn, Germany
[*]Correspondence and permanent address: Ethiopian Institute of Agricultural Research, Forestry Research Center, P.O. Box 30708, Addis Ababa, Ethiopia.
E-mail: abebeyadessa@yahoo.com
[2]University of Bonn, INRES-PE, Karlrobert-Kreiten-Straße 13, 53115 Bonn, Germany
[3]Addis Ababa University, P.O. Box 28513, Addis Ababa, Ethiopia

SUMMARY

A study to establish a relationship between cup quality of coffee and soil properties was conducted in the coffee forest ecosystem of south western Ethiopia, the home of wild arabica coffee. Cup quality of coffee depends on different factors such as the type of coffee, soil conditions, climatic conditions, processing methods etc. The present paper assessed the influence of soil conditions of the afromontane rainforests in SW Ethiopia on cup quality of wild arabica coffee. The study was based on 74 sample plots collected from Sheko (40 samples) and Yayu (34 samples) forests. From each plot, red cherries were hand picked and dry processed, and soil samples (0-20 cm depth) were also collected. Soil texture, cation exchange capacity (CEC), pH, major-nutrients and micro-nutrients were analyzed following the standard procedures. The sensorial analysis was made in Ethiopia by 5 professional tasters (3 from Ethiopia and 2 from Germany). Results showed that the overall cup quality of wild arabica coffee was not correlated with total N and available P levels of the soil at Sheko, but significantly and inversely correlated with N: P ratio. At Yayu, however, it was neither correlated with N or P and nor with N: P ratio, but rather significantly correlated with K, Ca, CEC and pH values. The effect of micronutrients on coffee quality was more of site-specific. Soil Zn content was negatively correlated with cup quality at Sheko, but positively correlated at Yayu; that is, higher Zn concentration was associated with poor coffee quality at Sheko but with better quality at Yayu. Although the influence of soil properties varied according to the criteria and from site to site, generally coffees with better cup quality were those collected from plots with higher levels of available P, K, clay and silt, but inversely correlated with sand content. Higher levels of soil pH, Mg, Mn and Zn were also associated with improved coffee aroma. This indicates that the quality of the soil is a very important factor for the production of quality coffee, and specifically the balance between the different nutrients is of paramount importance for the cup quality of coffee.

INTRODUCTION

The composition and productivity of an ecosystem including the forest ecosystem is markedly affected by the physical and chemical properties of its soil (Raghunbanshi, 1992). The coffee forest ecosystem in Ethiopia, the home of wild arabica coffee, may have distinct ecological conditions that have favored the growth of wild Arabica coffee. Coffee is a tropical plant which grows between the latitudes of 25° N and 25° S (ICO, 2008), but requires very specific environmental conditions for production of quality coffee. The beverage quality of coffee

depends on the type of coffee, soil conditions, climatic conditions, processing methods, etc. Among these factors, the influence of soil conditions on cup quality of coffee is the focus of the present study. It is hypothesized that coffee plots differ considerably in soil characteristics and these differences would impart differences in cup quality of coffee. Soils usually vary in their nutrient concentrations based on the parent material and other soil-forming factors. Thus, the concentrations of nutrients in the soil are associated with biological and geochemical cycles (Slagle et al., 2004), and they are also influenced by anthropogenic factors such as deforestation and land management.

Soil consists of both mineral particles and organic matter, and the nature and amount of these components in the soil influences its characteristics. Soil nutrients may be inherited from the parent materials or added through the use of external inputs (organic and inorganic fertilizers, rain, etc.) (Pastor and Post, 1986; Castrignano et al., 2000). In the natural habitat of coffee, soils are acidic to slightly acidic with limited phosphorus availability (Senbeta, 2006; Kufa, 2006). Ideal soils for coffee should be deep, permeable, slightly acidic and porous (D'Souza and Jayarama, 2006). To achieve optimum yield and quality of coffee, the nature and properties of the soil are of paramount importance. Nutrients are required for both vegetative growth of coffee trees and production of high quality beans and hence nutrient imbalances can affect coffee quality (Njorge, 1998). And deficiencies in nutrients lead to lower quality coffees (Feria-Morales, 1990 cited in Feria-Morales, 2002).

Many studies have identified soil nutrient availability to be an important factor controlling net primary productivity (Pastor and Post, 1986) and biochemical contents of plants (Mazzafera, 1999), and hence quality of the product. Some works on the influence of soil properties on coffee quality has been reported, especially in coffee plantations (Njorge, 1998; Pinkert, 2004). Literature show that volcanic soils often produce a potent acidity and a good body, and such soils can lead to a more balanced cup (CRI, 2001; Harding et al., 1998 cited in Bertrand et al., 2006). But the influence of soil properties on the quality of wild Arabica coffee in general and on its cup quality in particular has not so far reported under its natural ecosystem. Therefore, the objective of the study was to assess the correlation between the soil properties and cup quality of wild arabica coffee in the Afromontane rainforests in SW Ethiopia.

MATERIALS AND METHODS

Study sites

The study was carried out in Sheko and Yayu Afromontane rainforests of SW Ethiopia, where wild populations of Arabica coffee are the common features of the forest ecosystem.

Sheko (also known as Berhane-kontir) fores

It is located in Sheko district of Bench-Maji zone, South Nations, Nationalities and Peoples Regional State. The name of Sheko forest is inherited from the Sheko ethnic group living in the area. Sheko and Mejenger are the major ethnic groups, and Menit, Bench, Amhara and Kaffa are also living in the area. The altitude in Sheko forest ranges from 950-1800 m above sea level. The total rainfall is 2200 mm per annum and the mean annual temperature 22 °C. It represents the transition between the montane moist forest and the lowland dry forest, located west of the Great Rift Valley (Senbeta et al., 2006; Taye, 2006).

Yayu forest

It is located in the Yayo district of Illu Ababor Zone, Oromia Regional State, 550 km due west from Addis Ababa, capital of Ethiopia. Yayu has got its name from the word Yayo, the name of the Oromo sub-clan living in the Yayo district of Illu Ababor Zone. The soils of the area are red or brownish Ferrisols derived from volcanic parent material. The forest area is characterized by a rolling topography, and is highly dissected by small streams and two major rivers (Geba and Dogi rivers). The altitude in Yayu forest ranges from 1200-2150 m above sea level. The area has warm and humid climate. The mean annual temperature is about 20 °C, with mean minimum and mean maximum values of about 13 °C and 27 °C, respectively. The rainfall pattern is unimodal with mean annual rainfall of 1800 mm/annum (Gole et al., 2003).

Coffee sampling and Sensory analysis

Coffee cherries were harvested at full maturity, between October and December 2006 in Ethiopia, which is usually when the coffee is at better quality. Red cherries were hand picked and dry processed. The dried cherries were depulped and the beans were made ready for cup tasting. Cup tasting was done at Coffee Quality Inspection and Auction Center, commonly known as Coffee Liquoring Unit (CLU) under the then Ministry of Coffee and Tea Development, in Addis Ababa, Ethiopia. Sensory evaluation was done using seven quality criteria: fragrance, aroma, flavour, acidity, body, aftertaste and overall quality; and scoring was based on a scale of 1-10, corresponding to the total absence (or presence) of the criterion in the coffee. The coffee samples were medium roasted and medium ground. The beverage was prepared by brewing 12 g roasted coffee in 250 millilitres of hot water. And the coffee brews were evaluated by a panel of five experienced tasters (3 from Ethiopia and 2 from Germany).

Soil sampling and analysis

Soil samples were collected from the top soil layers (0-20 cm depth). Five samples were collected per each plot and then bulked together to get the representative sample per plot. Since most of the root system of the coffee tree develops in the upper soil layer, the properties of the top soil are more crucial to the coffee plants than those of the deeper subsoil (D'Souza and Jayarama, 2006). Taye (2006) also reported that most of the root hairs concentrate on the first 20-30 cm soil layers. The soil samples were analyzed following the standard procedures as described in Yadessa et al. (2001).

Data analysis

The soil data from the soil analysis laboratory and the sensory data from the panel experienced tasters were analyzed by using SPSS computer software. The associations between cup quality traits and the soil characteristics were examined by using correlation and regression analyses.

RESULTS AND DISCUSSION

Results showed that soil organic matter was not significantly correlated with the cup quality of wild coffee at both sites and for all the quality traits except that it was positively and significantly correlated with coffee aroma ($r = 0.389$, $p = 0.034$) at Yayu. At Sheko site, the overall cup quality of wild Arabica coffee was not significantly correlated with total N and available P levels, but significantly and inversely correlated with N: P ratio (Table 1),

indicating that an increase in soil total N without commensurate increase in available P may not improve coffee quality. Thus, the balance between them is very essential to have fine quality coffee. At Yayu, overall cup quality was neither correlated with N or P and nor with N: P or P: N ratios, but rather significantly correlated with K and Ca levels of the soil. The effects of soil Ca, CEC, pH and micronutrients were more of site-specific. Higher Ca, CEC and pH values were associated with better cup quality of coffee at Yayu, but no significant correlation between these soil properties and cup quality at Sheko. This could be due to the fact that Sheko soil is relatively more weathered as compared to that of Yayu as evidenced by significantly lower silt-to-clay ratio, 0.95 and 1.06, respectively. Advanced weathering is commensurate with a low silt-to-clay ratio as compared to normal less weathered soils (FAO, 2001). Moreover, Sheko is characterized by higher mean temperature and rainfall as compared to Yayu, which are important factors of soil weathering.

Although the influence of soil properties varied from site to site, generally cup quality was positively correlated with available P, K, clay and silt, negatively correlated with sand content. This indicates that the role soil P and texture in influencing the cup quality of wild coffee, which are related to the nature of parent material of the soil and stage of weathering.

When compared across both sites, except for acidity the cup quality traits of the coffee beverage were positively correlated with soil available P and P: N ratio, but not significantly correlated with soil total N, organic matter and C:N ratio (Table 3).

Table 1. Pearson correlation coefficients between soil properties and overall cup quality of wild arabica coffee from Sheko and Yayu Afromontane rain forests in SW Ethiopia.

Soil parameter	Overall cup quality			Remarks
	Sheko (n = 40)	Yayu (n = 34)	Both (n = 74)	
OM, % DM	-0.043	0.291	-0.065	
Total N, % DM	-0.203	0.269	-0.031	
Available P, ppm	0.136	0.277	0.289*	
C: N ratio	0.25	0.021	-0.084	
P:N ratio	0.194	0.303	0.310*	
N:P ratio	-0.339*	-0.167	-0.295*	
Na, meq/100g	-0.022	0.043	0.055	
K, meq/100g	-0.036	0.412*	0.242*	
Ca, meq/100g	-0.100	0.373*	0.125	Site specific
Mg, meq/100g	-0.112	0.263	0.141	
CEC, meq/100g	-0.177	0.410*	0.149	Site specific
pH	-0.206	0.525**	0.221	Site specific
Sand, % DM	0.018	-0.297	-0.367**	
Silt, % DM	-0.059	0.385*	0.387**	
Clay, % DM	0.014	0.067	0.276*	
Fe, ppm	-0.268	-0.167	-0.157	
Mn, ppm	-0.324*	0.198	0.172	Site specific
Zn, ppm ppm	-0.321*	0.370*	0.107	Site specific

***, * = Correlation significant at 1% and 5% level of significance; n = number of observations*

Soil Zn was negatively correlated with cup quality at Sheko, but positively correlated at Yayu (Table 2 and Figure 1). This may be because micronutrients are required in small quantities, but Zn concentrations was considerably higher at Sheko as compared to that of Yayu .Soil Zn

concentration ranged from 0.66-7.22 ppm (mean of 6.56 ppm) in Sheko area, and from 0.52-3.04 ppm (mean of 1.41 ppm) in Yayu area. In most cases, the soil micronutrients studied had positive correlations with cup quality at Yayu, but an inverse relationship at Sheko, which might be related to the nature of parent material and environmental conditions. The natural content of soil microelements is mainly determined by parent materials and by the soil-forming environment (Liu et al., 1996).

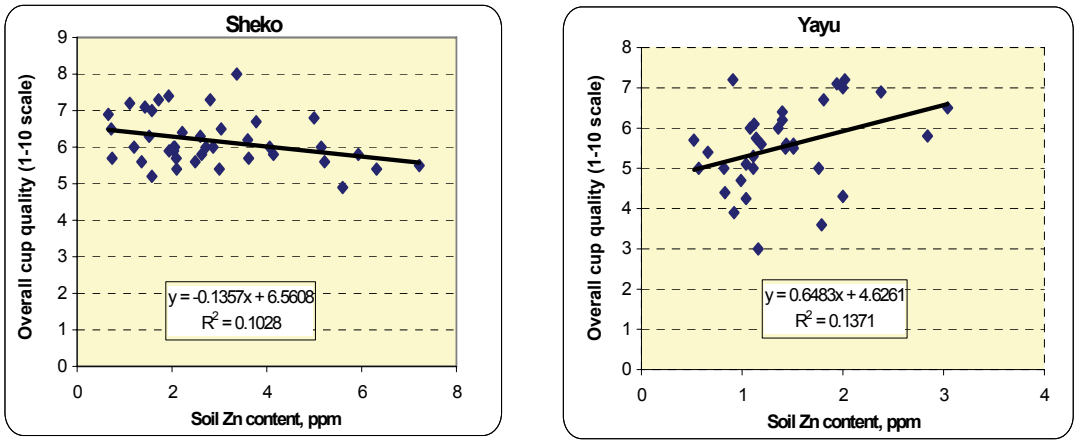


Figure 1. Overall cup quality of wild Arabica coffee as influenced by soil Zn content in Sheko and Yayu Afromontane rainforests in SW Ethiopia.

Table 2. Pearson correlation coefficients between ratios of different cations and cup quality of wild arabica coffee from Sheko and Yayu Afromontane rainforests in SW Ethiopia.

Trait	Ca:K ratio	Mg:K ratio	Mg:Ca ratio	Ca+Mg:K ratio
Fragrance	-0.101	-0.202	-0.006	-0.129
Aroma	-0.254*	-0.346**	0.054	-0.284*
Acidity	-0.036	-0.153	0.003	-0.065
Flavour	-0.202	-0.332**	0.067	-0.240*
Body	-0.159	-0.261*	-0.008	-0.188
Aftertaste	-0.214	-0.350**	0.069	-0.253*
Overall	-0.165	-0.299**	0.01	-0.202

***, * = Correlation significant at 1% and 5% level of significance, respectively.*

As clearly indicated in Table 2, the balance between the different soil nutrients, especially the balance between cations of different valency (between monovalents and bivalents) also matters for cup quality rather than differences within the same valency number. The relative proportion between Mg and K was the most important factor in this case; it was inversely related with most of the organoleptic properties of coffee assessed except for acidity. The ratio between Ca and Mg was of no or little importance for coffee cup quality. The ratio between the cations is very important for coffee because K is antagonistic to Mg and Ca (Snoeck and Lambot, 2004). K and Mg promoted the aroma of the coffee brew (Table 4), and thus Mg: K ratio is very important parameter for coffee quality. Potassium augments the body of a coffee (CRI, 2001), and the present study also confirmed this fact.

Table 3. Pearson correlation coefficients between soil OM, total N, available P, C:N and P:N rations versus cup quality of wild Arabica coffee from Sheko and Yayu Afromontane rainforests in SW Ethiopia.

	Fragrance	Aroma	Acidity	Flavour	Body	Aftertaste	Overall	OM	Total N	Avail. P	C:N ratio	P:N ratio
Fragrance	-											
Aroma	0.814**	-										
Acidity	0.633**	0.709**	-									
Flavour	0.684**	0.781**	0.891**	-								
Body	0.641**	0.699**	0.840**	0.839**	-							
Aftertaste	0.638**	0.754**	0.847**	0.947**	.853**	-						
Overall	0.702**	0.803**	0.913**	0 .946**	0.893**	0.928**	-					
OM	-0.112	-0.087	0.013	-0.130	-0.074	-0.158	-0.065	-				
Total N	-0.121	-0.074	0.067	-0.107	-0.025	-0.115	-0.031	0.774**	-			
Avail. P	0.242*	0.327**	0.188	0.343**	0.229*	0.356**	0 .289*	-0.207	-0.171	-		
C:N ratio	-0.025	-0.092	-0.089	-0.094	-0.068	-0.127	-0.084	0.510**	-0.100	-0.124	-	
P:N ratio	0.252*	0.302**	0.195	0.364**	0.256*	0.375**	0.310**	-0.341**	-0.348**	0.942**	-0.094	-
	Fragrance	Aroma	Acidity	Flavour	Body	Aftertaste	Overall	OM	Total N	Avail. P	C:N ratio	P:N ratio

***, * = Correlation significant at 1% and 5% level of significance, respectively.*

Table 4. Pearson correlation coefficients between soil cations, pH, CEC and micro-nutrients versus cup quality of wild Arabica coffee from Sheko and Yayu Afromontane rainforests in SW Ethiopia.

	Fragrance	Aroma	Acidity	Flavour	Body	Aftertaste	Overall	Na	K	Ca	Mg	CEC	pH	Fe	Mn	Zn
Fragrance	-															
Aroma	0.814**	-														
Acidity	0.633**	0.709**	-													
Flavour	0.684**	0.781**	0.891**	-												
Body	0.641**	0.699**	0.840**	0.839**	-											
Aftertaste	0.638**	0.754**	0.847**	0.947**	0.853**	-										
Overall	0.702**	0.803**	0.913**	0.946**	0.893**	0.928**	-									
Na	0.073	0.101	0.053	0.083	0.096	0.107	0.055	-								
K	0.108	0.322**	0.143	0.200	0.246*	0.225	0.242*	-0.130	-							
Ca	0.012	0.159	0.133	0.074	0.161	0.099	0.125	-0.198	0.640**	-						
Mg	0.059	0.233*	0.135	0.122	0.127	0.141	0.141	-0.179	0.643**	0.689**	-					
CEC	-0.011	0.165	0.159	0.125	0.176	0.122	0.149	-0.208	0.591**	0.829**	0.631**	-				
pH	0.092	0.269*	0.176	0.185	0.155	0.242*	0.221	-0.105	0.467**	0.460**	0.369**	0.365**	-			
Fe	0.000	-0.139	-0.119	-0.158	-0.052	-0.140	-0.157	-0.077	0.065	0.106	0.312**	0.140	-0.369**	-		
Mn	0.206	0.285*	0.105	0.239*	0.131	0.262*	0.172	0.135	0.104	-0.040	0.208	-0.057	0.324**	0.057	-	
Zn	0.141	0.247*	0.079	0.125	0.051	0.160	0.107	-0.091	0.400**	0.166	0.269*	0.172	0.494**	0.187	0.628**	-
	Fragrance	Aroma	Acidity	Flavour	Body	Aftertaste	Overall	Na	K	Ca	Mg	CEC	pH	Fe	Mn	Zn

***, * = Correlation significant at 1% and 5% level of significance, respectively.*

Table 5. Pearson correlation coefficients between soil texture and cup quality of wild arabica coffee from Sheko and Yayu Afromontane rainforests in SW Ethiopia.

	Fragrance	Aroma	Acidity	Flavour	Body	Aftertaste	Overall	Sand	Silt	Clay
Fragrance	-									
Aroma	0.814**	-								
Acidity	0.633**	0.709**	-							
Flavour	0.684**	0.781**	0.891**	-						
Body	0.641**	0.699**	0.840**	0.839**	-					
Aftertaste	0.638**	0754**	0.847**	0.947**	0.853**	-				
Overall	0.702**	0.803**	0.913**	0.946**	0.893**	0.928**	-			
Sand	-0.329**	-0.494**	-0.307**	-0.438**	-0.331**	-0.461**	-0.367**	-		
Silt	0.358**	0.521**	0.369**	0.400**	0.348**	0.419**	0.387**	-0.850**	-	
Clay	0.238*	0.371**	0.195	0.377**	0.249*	0.398**	0.276*	-0.911**	0.557**	-
	Fragrance	Aroma	Acidity	Flavour	Body	Aftertaste	Overall	Sand	Silt	Clay

***, * = Correlation significant at 1% and 5% level of significance, respectively.*

At Sheko, coffee cup quality was not significantly correlated with soil texture except that fragrance was positively correlated with sand ($r = 0.351$, $p = 0.026$) but inversely correlated with clay ($r = -0.329$, $p = -0.038$). At Yayu, however, sand content was negatively correlated with coffee aroma ($r = -0.341$, $p = 0.049$) and acidity ($r = -0.342$, $p = 0.047$), but silt content and silt-to-clay ratio were positively correlated with most cup quality traits except for fragrance. Generally across both sites, in most cases cup quality was positively correlated with silt and clay content, but negatively with sand content (Tables 2 and 5).

CONCLUDING REMARKS

The present study demonstrated that soil properties considerably influenced cup quality of wild Arabica coffee in its natural habitat. Among the organoleptic properties of the coffee assessed, aroma was the most affected attribute of coffee by soil properties. And among the major soil nutrients, available P, K and soil texture and also the proportions between Mg and K were the most important parameters influencing the cup quality of coffee. The effects of micronutrients were more of site-specific. Sodium and total N had no influence on the coffee quality at all sites and for all cup quality attributes. Generally coffees with better cup quality were those collected from plots with higher levels of available P, K, clay and silt, but inversely correlated with sand content. Higher levels of soil pH, Mg, Mn and Zn were also associated with improved coffee aroma. This indicates that the quality of the soil is a very important factor for the production of quality coffee, and specifically the balance between the different nutrients is of paramount importance for the cup quality of coffee. Above all, the balance between P and N and the balance between Mg and K are very essential to have fine quality coffee.

This finding adds evidence to the importance of soil factors for coffee quality and verifies the hypothesis that the distinct coffee varies depending on the soil characteristics of the farm where the coffee is grown. Thus, the coffee forest ecosystem in south west Ethiopia as a home of wild Arabica coffee with distinct soil conditions can be used a model for simulating suitable soil conditions for commercial production of Arabica coffee in other parts of the country or elsewhere.

REFERENCES

Abebe Yadessa, Fisseha Itanna and Mats Olsson. 2001. Contribution of indigenous trees to soil properties: the case of scattered trees of *Cordia africana* Lam. in croplands of western Oromia. Ethiopian Journal of Natural Resources, 3: 245-270.

Bertrand B., Vaast P., Alpizar E., Etienne H., Davrieux F., Charmetant P. 2006. Comparison of bean biochemical composition and beverage quality of Arabica hybrids involving Sudanese-Ethiopian origins with traditional varieties at various elevations in Central America. Tree Physiology, 26:1239-48.

Castrignano A., Giugliarini L., Risaliti R., Martinelli N. 2000. Study of spatial relationships among some soil physico-chemical properties of a field in central Italy using multivariate geostatistics. *Geoderma*, 97: 39-60.

D'Souza M.V. and Jayarama. 2006. Soil analysis to maintain the productivity in coffee plantations. March 2006 issue of Indian Coffee: the monthly Magazine of Coffee Board.

FAO (Food and Agriculture of United Nations). 2001. Lecture notes on the major soils of the world. http://www.fao.org/DOCREP/003/Y1899E/y1899e08a.htm

Feria-Morales A.M. 2002. Examining the case of green coffee to illustrate the limitations of grading systems/expert tasters in sensory evaluation for quality control. Food Quality and Preference, 13: 355–367

Liu Z., Cai Z.C., Wang J.F. 1996. Microelements of Soils in China: Nanjing, Jiangsu Science and Technology Press, 329 p.

Mazzafera P. 1999. Mineral nutrition and caffeine content in coffee leaves. Bragantia, Campinas, 58:387-391.

Njorge J.M. 1998. Agronomic and processing factors affecting coffee quality. *Outlook on Agriculture*, 27: 163-166.

Pastor J., Post W.M. 1986. Influence of climate, soil moisture and succession on forest carbon nitrogen cycles. Biogeochemistry, 2: 3-27.

Pinkert C. 2004. Nutrient and quality analysis of coffee cherries in Huong Hoa district, Vetnam. Plant Research International B.V. Wageningen, Note 280.

Raghubanshi A.S. 1992. Effect of topography on selected soil properties and nitrogen mineralization in a dry tropical forest Soil Biology and Biochemistry, 24: 145-150.

Senbeta F. 2006. Biodiversity and ecology of Afromontane rainforests with wild *Coffea arabica* L. populations in Ethiopia. Ecology and Development Series No. 38, 2006.

Slagle A., Skousen J., Bhumbla D., Sencindiver J., McDonald L. 2004. Trace element concentrations of three soils in central Appalachia. Soil Survey Horizons, 45: 73-85.

Snoeck J., Lambot C. 2004. Crop maintenance. In: J, N., Wintgens. Eds. Coffee: Growing, Processing, Sustainable Production. WileyVCH. p 246-323.

Tadesse W. 2003. Vegetation of the Yayu forest in SW Ethiopia: impacts of human use and implications for in situ conservation of wild *Coffea arabica* L. populations. Ecology and Development 10.

Taye Kufa. 2006. Ecophysiological diversity of wild Arabica coffee populations in Ethiopia: Growth, water relations and hydraulic characteristics along a climatic gradient. Ecology and Development Series No. 46.

Carbon Sequestration in an Agroforestry System of Coffee and *Mimosa scabrella* (Bracatinga) in Southern Brazil

P.H. CARAMORI, A. CARNEIRO LEAL, W. DA SILVA RICCE, H. MORAIS, F. SUANO DE SOUZA, A. ANDROCIOLI FILHO

Instituto Agronômico do Paraná (IAPAR), Rodovia Celso Garcia Cid, km 375, CEP 86001-970, Londrina, PR, Brazil

SUMMARY

Coffee shading has been a common practice used for frost protection in southern Brazil. The opportunities of the Clean Developing Mechanism established in the Kyoto Protocol opened the possibility of obtaining carbon credits for reforestation. Agroforestry Systems in tropical regions have the potential of producing significant amounts of biomass and could also become eligible in the near future. In this work the biomass production and carbon sequestration was evaluated in an agroflorestry system of coffee and *Mimosa scabrella*, compared to an open-grown coffee plantation in Londrina, Paraná state, Brazil (23^{o} 22´ S).

INTRODUCTION

Coffee shading with *Mimosa scabrella* (bracatinga) has been recommended for frost protection of coffee plantations in Paraná state (22 to 27^{o} S). In areas with frequent frost occurrence, experimental results indicated that a moderate shade can minimize frost damages and contribute to obtain stable productions (Caramori et al., 1996).

Bracatinga is a native tree from colder regions of southern Brazil that can be used for firewood, charcoal and also in the industries of construction and furniture. The species has been used for coffee shading in Guatemala (Standley and Steyermark, 1946), Costa Rica (Picado, 1985) and México (Sampieri, 1988; Angel Musálem, 1995), with very good results. The objective of this work was to compare biomass production and carbon sequestration of an open grown coffee plantation with an Agroforestry system of coffee and bracatinga.

METHODOLOGY

The experiment was carried out at the experimental farm of IAPAR in Londrina (23^{o} 22' S) from 1998 to 2007. Seedlings of the cultivar of *Coffea arabica* IAPAR 59 were planted in an area of approximately one hectare in January 1998, and were destroyed by a severe frost in July 2000. After pruning, the plants recovered and seedlings of bracatinga were intercropped at the spacing of 4.0 m x 4.5 m (555 trees/ha) in October 2001. In December 2002 half of the area had the population of bracatinga reduced to 139 trees/ha (8.0 x 9.0 m). Coffee production was assessed yearly during the experimental period. In September of 2007 the experiment was concluded and four trees of bracatinga from the density of 139 trees/ha were sampled to quantify the biomass accumulated. Four replications of 10 coffee plants from both under shade and open-grown conditions were also sampled to estimate the total contribution of each system. Biomass was separated in the components roots, litter, branches and leaves. Fresh weight was assessed in the field and samples were oven dried at 65 °C under forced circulation and used to estimate the total dry weight of the biomass. Dry samples were used to

quantify nutrients and carbon content according to the method of Walkley-Black, as described by Page et al. (1982).

RESULTS AND DISCUSSION

Table 1 presents biomass production of the coffee component in both open-grown and shaded systems. In Table 2 are presented the values of biomass production of the single coffee and of the agroforestry system of coffee and bracatinga.

Table 1. Production of above ground biomass (leaves and branches) eight years after planting (kg of dry matter/ha) of the coffee component in the open-grown and shaded systems in Londrina-PR, Brazil (Accumulated from 2000 to 2007).

Treatments	Leaves		Branches		Plagiotropic Branches		Orthotropic Branches	
	Kg dry matter/ha		Kg dry matter/ha		Kg dry matter/ha		Kg dry matter/ha	
Open-grown coffee	4488.30	a	24193.12	a	8000.59	a	16192.54	a
Shaded coffee	4737.24	a	16104.22	b	5537.94	b	10566.28	b

Means followed by the same letter in the column do not differ between each other by the Tukey test at 5% significance.

Table 2. Mean values of production of biomass (leaves, branches, liter and roots) in kg of dry matter/ha of the open-grown coffee and of the agroforestry system of coffee+bracatinga.

Treatments	Leaves		Branches		Roots		Liter	
Open-grown coffee	4488.30	b	32193,71	a	16675.94	a	9350.00	a
Coffee + bracatinga	7346.02	a	69103.44	a	22008.07	a	11210.00	a
Percent increment*	63.7		214.6		132.0		119.9	

*Means followed by the same letter in the column do not differ between each other by the Tukey test at 5% significance. * Percent of increment of biomass in the agroforestry system of coffee+bracatinga relative to the open-grown treatment.*

The average total of carbon estimated in the biomass was 65.57 ton C/ha for the shaded treatment and 32.73 ton C/ha for the open-grown. Carbon in the soil was 6.47 ton/ha in the open-grown and 6.86 ton/ha in the shaded system. Vilcahuamán et al. (2004) estimated a sequestration of 231.64 t/ha in a native field of *Mimosa scabrella* with eight years in the metropolitan region of Curitiba, Brazil.

Nutrient concentration in the leaves and plagiotropic branches was higher in the open-grown treatments and lower in the plagiotropic branches (Tables 3 and 4). Carbon content was similar in both systems.

Coffee production on the average of five harvests were 2280 and 1440 kg/ha of clean coffee for the open-grown and shaded systems, respectively. Therefore, the competition between coffee and *Mimosa scabrella* resulted in an average reduction of 37% of coffee production compared to the open-grown treatment. During the period analyzed there were no occurrences of frost in the area. The results show the potential of this agroforestry system to obtain carbon

credits, but also indicate the need of further studies to adjust plant population and system management.

Table 3. Macronutrient content (g/kg of dry matter) for the open-grown and shaded coffee in September 2007.

Treatments	N		P		K		Ca		Mg		C	
	g/kg											
	Leaves											
Open-grown	23.38	a	1.19	a	18.50	a	11.04	a	3.41	a	612.45	a
shaded	16.39	b	0.91	b	11.25	b	6.09	b	1.13	b	615.23	a
	Plagiotropic branches											
Open-grown	21.55	a	1.25	a	14.00	a	8.43	a	2.15	a	628.93	a
shaded	13.88	a	0.47	b	7.63	b	4.30	b	1.01	a	617.68	a
	Orthotropic branches											
Open-grown	17.20	a	0.58	b	7.00	b	7.31	a	1.60	b	591.28	a
shaded	21.31	a	0.92	a	13.75	a	10.43	a	3.04	a	563.21	a

Means followed by the same letter in the column do not differ between each other by the Tukey test at 5% significance.

Table 4. Micronutrient content (mg/kg of dry matter) for the open-grown and shaded coffee in September 2007.

Treatments	Cu		Zn		B		Mn	
	mg/kg							
	Leaves							
Open-grown	11.78	a	10.68	a	35.80	a	190.63	a
shaded	8.26	a	9.00	a	11.38	b	56.48	b
	Plagiotropic branches							
Open-grown	10.05	a	10.10	a	19.55	a	98.83	a
shaded	2.87	b	6.21	b	6.22	a	25.16	b
	Orthotropic branches							
Open-grown	6.75	a	11.40	a	8.10	b	188.55	a
shaded	10.71	a	16.90	a	23.65	a	295.03	a

Means followed by the same letter in the column do not differ between each other by the Tukey test at 5% significance.

CONCLUSION

Biomass production and carbon sequestration was higher in the agroforestry system of coffee+bracatinga, but the competition with shade trees caused production loss. Further research is needed to adjust plant population and management for this system in southern Brazil.

REFERENCES

Angel Musálen M. La bracatinga: introducción, crescimiento, manejo y utilización en asociación con cafetal: una combinación promisoria para los trópicos de Mexico. In: EVANS, D. O.; SZOTT, L. T. (Ed.). Nitrogen fixing trees for acid soils. Morrilton: Winrock International; Waimanolo: Nitrogem Fixing Tree Association, 1995. p. 113-129.

Page A.L., Miller R.H., Keeney D. R. Methods of soil analysis: chemical and microbiological properties. 2. ed. Madison, WI: American Society of Agronomy, 1982. pt. 2.

Picado W. *Mimosa scabrella* espécie com potencial para sombra y producción de leña em cafetales de Costa Rica. In: SALAZAR, R. (Ed.). Actas de los simpósio sobre técnicas de producción de leña em fincas pequeñas y recuperación de sitios degradados por medio de silvicultura intensiva. Turrialba: CATIE, 1985. p. 227-239.

Sampieri Z.M.R. Adaptación y crecimiento inicial de Mimosa scabrella Benth., bajo cuatro densidades de plantación en la región de Tepatlaxco, Ver. 57 f. 1988. Tesis (Profesional) - Facultad de Ciencias Agrícolas, Universidad Veracruzana, Córdoba.

Standley P.C., Steyermark J. A. Flora of Guatemala. Chicago: Chicago Natural History Museum, 1946. 502 p. (Fieldiana: Botany, v. 24, p. 5).

Vilcahuamán L.J.M., Viera A.R.R., Feistauer D., Medrado M.J.S. Sequestro de carbono de um sistema agroflorestal com bracatinga na Região Metropolitana de Curitiba. In: V Congresso Brasileiro de Sistemas Agroflorestrais, 2004, Curitiba - PR. v. 1. p. 175-177.

Relative Humidity of Coffee Canopy under Different Types of Pruning in Mococa, SP, Brazil

G.S. ROLIM[1*], P.S. SOUZA[2], M.J. PEDRO-JÚNIOR[1], P.B. GALLO[2], J.R.M. PEZZOPANE[3]

[1]Instituto Agronômico, Campinas, SP, Brazil. [*]E-mail: rolim@iac.sp.gov.br
[2]Pólo APTA Nordeste Paulista, Mococa, SP, Brazil
[3]Universidade Federal do Espírito Santo, São Mateus, ES, Brazil

SUMMARY

The pruning process changes significantly the coffee microclimate conditions. The relative humidity is directly related to water requirements and occurrence of plagues and diseases. The objective of the paper is to analyze the influence of different pruning types on the microclimate in coffee canopy. The cultivar growing in the area used for this work was Ouro Verde, with 9 years old and 3.5 m x 0.75 m of spacing. Three different types of pruning were tested: traditional (or 'decote', the most common in Brazil, consists basically of removing the apical part of the orthotropic branch at 1.6m height), skeleton cut (equal to traditional and plus the plagiotropic branches) and trunking (the most drastic, because it is removed all above-soil part of the plant). The experiment was performed in Mococa (21º 28' S, 47º 01' W, 665 m), a region of subtropical climate and a representative area of the most important coffee production zone in São Paulo State. Data of air relative humidity (RH) were collected in three heights inside coffee canopy at each 15 minutes: 50 cm above canopy (RH1), 1.5 m inside the canopy (RH2) and 50 cm inside the canopy above soil (RH3). These measurements started 3 days before pruning and ended 3 days after, during 2007/August with two repetitions in the experimental plot of about 10.5 m x 7.5 m. As a result it was observed that there were no differences in average daily RH before and after the pruning for any height, however there were differences in hourly values (Figure 1). For all measuring days it was observed the influence of the coffee canopy on RH: near the soil, from 15 h 00 on, it remained high, while at the same hour above the canopy the RH showed always the minimum values observed. After pruning there was a decrease in RH1 from 7 h 00, corresponding to the hottest period of the day for all pruning kinds. The trunking pruning decreased in up to 10% of RH1 between 11 h 00 and 14 h 30, while for the skeleton cut and tradition pruning the average decrease was of 5%. Another effect produced by all pruning systems was the reduction of RH3 inside the canopy, mainly between 8 h 00 and 15 h 00. Finally, we can verify that any pruning types performed caused a reduction in RH during the day.

INTRODUCTION

The pruning process changes significantly the coffee microclimate conditions. The relative humidity is directly related to water requirements and occurrence of plagues and diseases. These modifications are consequences of phisiology modification in the plans mainly due to the source/drain relationship (Kramer and Boyer, 1995; Alves and Livramento, 2003; Laviola et al., 2007).

There are many types of pruning in coffee, among them, the most used (traditional) in Brazil consists basically of removing the apical part of the orthotropic branch at 1.6 m height of the lateral shoots. The skeleton cut is equal to traditional pruning but the plagiotropic branches is

removed as well. Finally, the trunking is the most drastic because it is removed all above-soil part of the plant, use only in special occasions, normaly when the coffee plants are in bad conditions (Serra, 2008).

When pruning is used there are modifications in the canpoy microclimate, nevertheles few studies are found quantifying these changes. This quantification could be used for a better pruning planing in the area avoiding extremes weather.

The objective of the paper is to analyze the influence of different pruning types on hourly relative humidity in coffee canopy.

MATERIAL AND METHODS

The cultivar growing in the area used for this work was Ouro Verde, with 9 years old and 3.5 m x 0.75 m of spacing. Three different types of pruning were tested: traditional, skeleton cut and trunking. The experiment was performed in Mococa (21º 28' S, 47º 01' W, 665 m), a region of subtropical climate and a representative area of the most important coffee production zone in São Paulo State. Data of air relative humidity (RH) were collected in three heights inside coffee canopy at each 15 minutes: 50 cm above canopy (RH1), 1.5m inside the canopy (RH2) and 50 cm inside the canopy above soil (RH3). These measurements started 3 days before pruning and ended 3 days after, during 2007/August with two repetitions in the experimental plot of about 10.5 m x 7.5 m.

RESULTS AND DISCUSSION

As a result it was observed that there were no differences in average daily RH before and after the pruning for any height, however there were differences in hourly values (Figure 1). For all measuring days it was observed the influence of the coffee canopy on RH: near the soil, from 15 h 00 on, it remained high, while at the same hour above the canopy the RH showed always the minimum values observed. After pruning there was a decrease in RH1 from 7 h 00, corresponding to the hottest period of the day (Figure 1) for all pruning kinds.

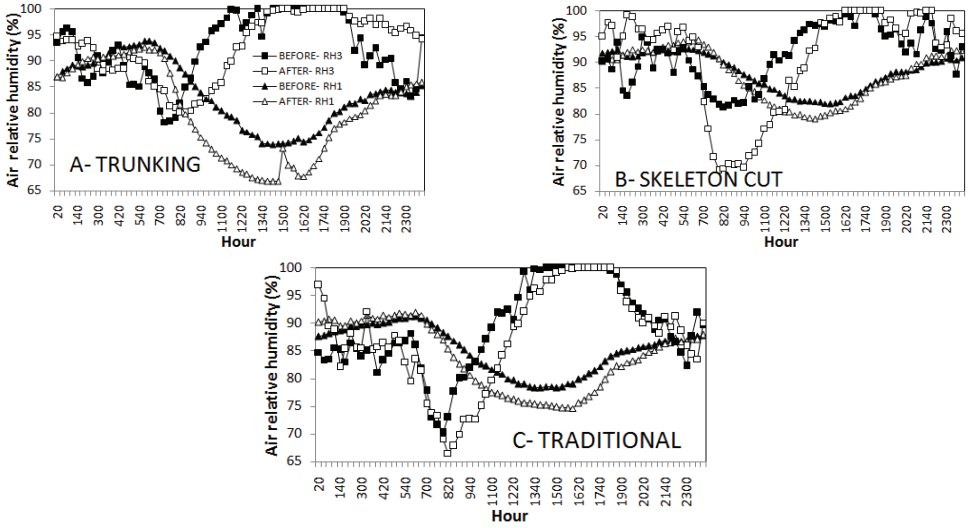


Figure 1. Hourly average relative humidity at 50 cm above the canopy (RH1) and 50 cm inside the canopy (RH3), before (black points) and after (white points) in pruning of trunking (A), skeleton cut (B) and traditional (C) in 2007/August, Mococa, SP, Brazil. Each point is an average of two repetitions inside the experimental plot.

The trunking pruning decreased in up to 10% of RH1 between 11 h 00 and 14h30, while for the skeleton cut and tradition pruning the average decrease was of 5%. Another effect produced by all pruning systems was the reduction of RH3 inside the canopy, mainly between 8 h 00 and 15 h 00. Finally, we can verify that any pruning types performed caused a reduction in RH during the day.

CONCLUSION

The pruning in coffee promoted the reduction of relative humidity in the area between 8 h 00 and 15 h 00, indicating the shelter effect of the canopy before the pruning. There were no evidence differences between pruning types.

REFERENCES

Alves J.D., Livramento D.E. Morfologia e fisiologia do cafeeiro. Lavras: UFLA/FAEPE. 46p. 2003. 46p. (Especialização em Cafeicultura Empresarial: Produtividade e Qualidade)

Laviola B.G., Martinez H.E.P., Salomao L.C.C. et al. Alocação de fotoassimilados em folhas e frutos de cafeeiro cultivado em duas altitudes. Pesq. agropec. bras., Nov. 2007, vol.42, no.11, p.1521-1530. ISSN 0100-204X.

Serra, J.R.M. Poda de café: decote, recepa e esqueletamento, o que fazer.Informativo Garcafé, dezembro de 1998. http://www.coffeebreak.com.br/ocafezal.asp?SE=8&ID=60. Acessed in August,25, 2008.

Water Relations of Ethiopian Wild Coffee Populations: Genetic Fixation and Phenotypic Plasticity

A. BEINING[1,2], J. BURKHARDT[1], M. FETENE[3]

[1]University of Bonn, University of Bonn, INRES-PE, Karlrobert-Kreiten-Str. 13, D-53115 Bonn, Germany
[2]Present address: Institute of Sugar Beet Research, University of Göttingen, Holtenser Landstr. 77, 37079 Göttingen, Germany
[3]Addis Ababa University, P.O. Box 28513, Addis Ababa, Ethiopia

SUMMARY

Drought is a wide-spread limiting factor in coffee (*Coffea arabica* L.) production, but the development of adapted cultivars is hampered both by a limited understanding of the physiological mechanisms of drought tolerance, and by the extremely narrow genetic database of material used in plant breeding. The study reported here evaluates the ecophysiological diversity of wild coffee populations in Ethiopia, which is the center of genetic diversity and the primary gene pool. *Coffea arabica* populations within four fragments of the afromontane rainforests of Ethiopia under contrasting climatic conditions were chosen for the experiments. Plant water relations under drought conditions were studied in naturally generated stands of wild coffee (*in-situ*), and in an *ex-situ* experiment where seedlings from the original populations were raised under controlled conditions and subjected to different watering and light regimes. Plant ecophysiological behavior was assessed by water potential, gas exchange, and carbon isotope discrimination measurements. When responding to soil moisture, vapor pressure deficit, temperature and light, drought-exposed *Coffea arabica* generally avoided desiccation and maintained leaf turgor by fine adjustment of stomata resulting in reduced water loss and improved water use efficiency. No active osmotic adjustment was observed. Populations from different sites showed different water use strategies *in-situ*. This origin-specific variability was confirmed by the seedling experiment under common-garden conditions, underlining the genetic basis of the observed differences and indicating the different selection pressure under which the populations have developed. The coffee plants exhibited substantial phenotypic plasticity for all ecophysiological traits in response to varying soil moisture conditions, with differing magnitude for the different sites. Plants from drier, variable climates had the highest plasticity suggesting fast adaptation to rapidly changing environmental conditions. The ecophysiological diversity and the specific adaptations to drought stress suggest a high potential for breeding of improved cultivars and underlines the need for *in-situ* conservation of *Coffea arabica* within the evolutionary dynamic ecosystems of the natural habitats.

INTRODUCTION

Due to the low level of genetic diversity of modern cultivars of *C. arabica*, interest is growing in the wild relatives of this species, which can be found in Ethiopia, its primary center of origin and center of genetic diversity (FAO, 1968). The wild populations occurring there are believed to show high diversity that can be used as a valuable raw material from which breeders can craft more productive varieties to meet the needs of world-wide efforts to improve the productivity, resilience and quality in cultivated Arabica coffee (Hein and Gatzweiler, 2006). Especially with regard to enhancement of drought adaptability, a high

potential can be expected, since wild *C. arabica* populations occupy a broad habitat range, above all characterized by the spatio-temporal variation in water availability. Due to this predominant feature and the tight interrelation with plant ecophysiological behavior (Blum, 1988), the contrasting environmental conditions of the distant habitats are likely to create distinct selection pressures related to water-use, which promotes the diversification of ecophysiological traits among the wild coffee populations (Parsons, 1988; Sandquist and Ehleringer, 1998). However, it is unclear, if the mechanisms, which allow wild *C. arabica* to successfully occupy this broad habitat range with contrasting environmental conditions, results from patterns of phenotypic plasticity of genetically similar populations or from the subdivision of genetically distinct ecotypes for each habitat type, which both represent alternative means of adaptation (Bradshaw and Hardwick 1989; Schlichting and Smith 2002). Though these benefits of biodiversity are obvious it still remains largely unknown how much useful ecophysiological diversity exists in wild coffee progenitors and to date there has been no sufficient evaluation of their potential value. In fact, management of the last remaining wild progenitors of *C. arabica* is rather poor, and anthropogenic activities such as deforestation and land-use changes (Gole, 2002) are among the main reasons why the Afromontane rainforests, which provide these highly valuable natural resources, are disappearing at an alarming rate. In addition, Ethiopian coffee farmers tend to replace wild coffee plants by improved and uniform varieties. As a consequence, there is a permanent loss of diversity of Arabica coffee (Solbrig, 1992), and though some efforts to preserve the wild relatives of this species have been made, the destruction of their natural habitats is still ongoing. Consequently, urgent action has is necessary to manage the finite genetic resources in a sustainable way. With growing awareness of the irreversible loss of plant genetic resources, conservation strategies are obligatory if the remaining wild populations of Arabica coffee are to continue to serve as the principal source of sustenance to meet current and future unforeseen needs.

MATERIALS AND METHODS

The study was conducted in natural stands of *C. arabica* in patches of the Afromontane rainforest on the western and eastern highland plateaus of Ethiopia, which represent major residual areas of wild arabica coffee. Specifically, four distant habitats spanning the species natural range were chosen, namely Berhane-Kontir, Bonga and Yayu and Harenna Forest. While the latter is located on the eastern plateau of the highlands, all other sites can be found in the south-western part of the country, separated from Harenna by the Great Rift Valley.

All study sites are classified as Afromontane rainforests (Friis, 1992). Dystric Nitisols are the dominant soil association in this Afromontane rainforest region (FAO, Iscric et al., 1998). The natural stands were selected over a broad gradient of environmental conditions and located between altitudinal ranges of 1000 to 1800 m a.s.l.

The maximum air temperatures fluctuate between 26 and 34 °C, while the minimum temperatures lie between 10 and 15 °C. The distant habitats exhibit a high variability with respect to water availability (Table 1). A broad precipitation gradient extends from the relatively dry southern part, which receives 950 mm annual precipitation (Harenna) to the south-western regions with intermediate amounts (Bonga and Yayu), and annual precipitation as high as 2,100 mm in Berhane-Kontir.

In all habitats, more than 85 % of the annual rainfall falls within the wet season; nevertheless, the geographic variability is accompanied by a high temporal variability with respect to precipitation and drought. Whereas the south-western habitats experience conditions of a mono-modal rainfall type, with a single dry and wet season per year, the Harenna Forest lies

in an area with a bi-modal rainfall pattern showing a long rainy season in the beginning of the year and a prolonged phase of reduced water availability followed by a second wet period later in the growing season.

Table 1. Precipitation gradient spanning the habitats of wild *C. arabica* chosen for the study.

	annual precipitation		mean monthly precipitation		maximum drought length	
	mean	CV	dry season	wet season	mean	CV
habitat	*(mm)*	*(%)*	*(mm)*	*(mm)*	*(days)*	*(%)*
Harenna	950 (20)	26	22	130	43	33
Bonga	1700 (44)	16	62	184	22	61
Yayu	1800 (32)	11	40	227	30	41
Berhane-Kontir	2100 (25)	13	72	222	18	41

**CV = (standard deviation x 100) mean (thus expressed as percentage).*
Source: (NMSA 2004).

In order to evaluate the response of wild *C. arabica* to drought stress, two experiments were carried out by combining *in-situ* field measurements with an experimental approach under common-garden conditions *ex-situ*. The first experiment was designed to determine seasonal differences in the ecophysiological behavior of populations during a single dry and wet season in their natural habitat under varying weather conditions. In a second experiment, the plants examined under field conditions were used as seed collection sites for a drought-stress experiment where seedlings were grown under identical environmental conditions.

The *ex-situ* experiment was established at the experimental nursery site at the Ethiopian Agricultural Research Subcenter in Jimma (JARC), Ethiopia (7°36'N, 36°48'E at 1,750 m a.s.l.) in spring 2005. The site is located on former pasture land with a slope to the east with less than 4°. In March, mean daily minimum and maximum temperatures are 12 °C and 28 °C, respectively, whereas long-term annual precipitation is about 1600 mm (years recorded: 1986-2003).

Four different treatments were applied in the experiment. Two light intensity regimes (shade and open sun) as well as two soil moisture treatments (irrigated and non-irrigated) were imposed. For the shade treatment, shade nets were erected horizontally above the seedlings while unshaded plants were grown under natural open sun conditions. Water was withheld from half of the pots in order to simulate a fast soil drying period, whereas the well watered plants were irrigated regularly in 4-day-intervals to ensure moisture conditions corresponding to soil water availability in the field during wet periods.

Gas exchange measurements were obtained using a porometer (LCpro, ADC Bioscientific Ltd., Hoddesdon, UK). Net photosynthetic rate (A_{net}, µmol CO_2 m^{-2} s^{-1}), stomatal conductance to water vapor (g_s, mol H_2O m^{-2} s^{-1}) and transpiration (E, mmol m^{-2} s^{-1}) were measured. Instantaneous water-use efficiency (WUE_i) was calculated as the ratio of net assimilation (A_{net}) to transpiration (E). For both experiments, two different types of leaf gas exchange measurements were carried out, i.e., diurnal and seasonal variation of gas exchange activity. During the wet season in the *in-situ* study, frequent heavy rainfall over the day made measurements of diurnal change in gas exchange parameters impossible. Therefore, the

comparison of dry and wet season values is based only on midday measurements of porometry. Diurnal variation of gas exchange parameters were conducted in the *ex-situ* study as well as on field-grown plants during the dry season at 2-h intervals from 8:00 to 18:00 h over 2 consecutive days per population. All measurements were made on 5 individuals per population during the dry and the wet season (*in-situ*) and on 2 plants per population per treatment (*ex-situ*), respectively. Two new fully expanded leaves were excised from each plant and measured by placing the mid-portion of the fascicles in the cuvette, while values were allowed to stabilize before measurements were taken.

Leaf water potential (ψ) was estimated using a pressure chamber (SoilMoisture Equipment Corp., Santa Barbara, CA, USA) as described by Scholander (1965). On each sampling date, ψ measurements were taken from the terminal twigs of selected plants and monitored at midday (ψ_{md}; 12:00 to 13:00 h, local time) and the following predawn (ψ_{pd}; 05:00 h) on the uppermost fully expanded leaves. Measurements were made on 8 trees per population with 3 leaves per tree (*in-situ* study) and on 2 seedlings per population (*ex-situ* study), respectively.

The samples for measurements of osmotic potential were taken from the same branches as the samples for the measuring water potential. The leaves were killed in order to avoid enzymatic changes in the cells (Mitlöhner, 1998). Sample leaves were then immediately sealed in plastic bags, placed in an insulated container and transported to the laboratory. Dried leaf samples were ground to a fine powder in a ball mill and extracted with hot water (55 °C for 12 h) and processed following the method of (Mitlöhner, 1998). The sap was removed by centrifugation (40,000 g for 15 min) and collected in tubes that were immediately sealed and stored on ice. Leaf osmotic potential of the extracted sap was determined by freezing-point depression with a freezing-point osmometer according to Kreeb et al. (1989) with a measuring error of ± 0.2 MPa.

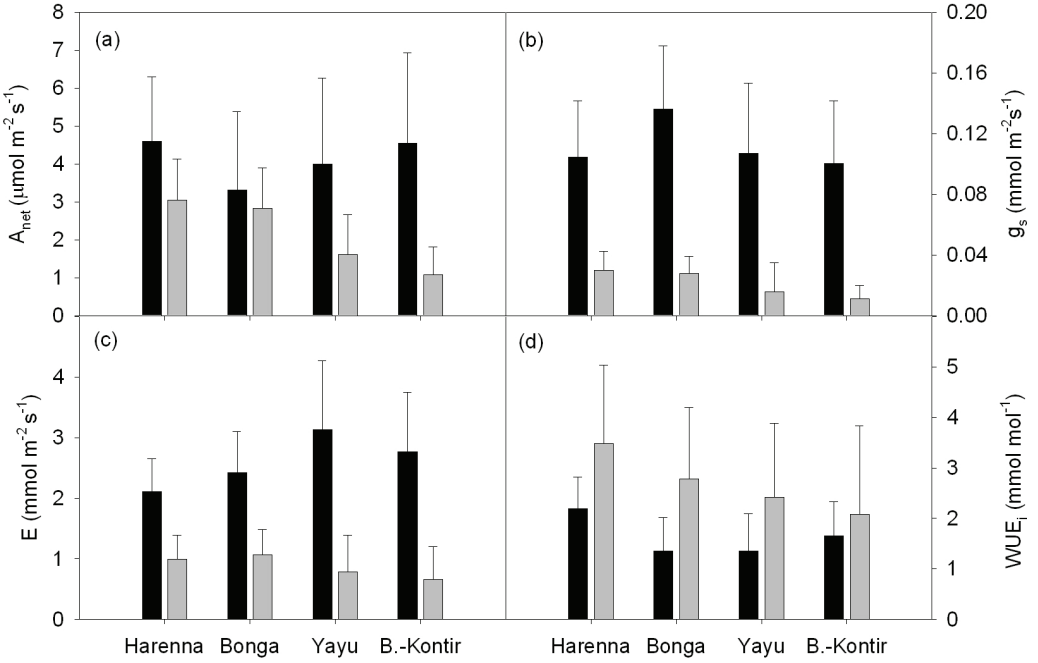
Foliar carbon isotope discrimination ($\delta^{13}C$) was determined on leaves collected at the end of each field campaign in the *in-situ* study, thus comprising dry and wet season estimates of variation in water-use efficiency of the coffee plants, as well as at the end of the drought-stress experiment in the *ex-situ* study. Specifically, 20 plants were selected at each population, and 4 leaves per tree collected from all cardinal directions were bulked into a single sample per plant. Samples were oven dried at 70 °C for 72 h and the dried pooled samples were ground into a fine powder in a matrix mill. Analyses of $^{13}C/^{12}C$ were carried out with a mass spectrometer (Finnigan Delta-S). The carbon isotope ratios were expressed as $\delta^{13}C$ (‰) against the Chicago Pee Dee Belemnite (PDB) standard (Farquhar, Ehleringer et al. 1989) computed as $\delta^{13}C = [(R_{sample}/R_{standard}) - 1]\ 1000)$, and $R = {}^{13}C/{}^{12}C$ (Craig, 1957). Reproducibility was 0.1 with well-homogenized cellulose standards.

RESULTS AND DISCUSSION

Generally, net photosynthetic rate (A_{net}), transpiration (E) and stomatal conductance (g_s) measured during period of sufficient soil moisture supply were higher at all study sites compared to the dry season (Figure 1). Average wet season A_{net} at midday ranged from 3.3 to 4.6 µmol m^{-2} s^{-1} while there was a reduction to 1.07 to 3.05 µmol m^{-2} s^{-1} during the dry period. Transpiration rates also decreased in dry season, however reduction due to reduced soil moisture availability was more pronounced than in A_{net}, resulting in greater instantaneous water-use efficiency (WUE_i) during dry season.

Carbon stable isotope signatures of plant foliage as an indicator of long-term WUE ($\delta^{13}C$) of distant *C. arabica* were significantly affected by seasonal variation in moisture availability (Figure 2). During wet season plants displayed low $\delta^{13}C$ values, while having high $\delta^{13}C$ in

poor rainfall seasons. Furthermore, results of $\delta^{13}C$ showed that intrapopulation variance of carbon isotope discrimination correspond to the degree of spatio-temporal heterogeneity of water availability of the distant habitats. Specifically, a negative correlation existed between total annual precipitation and foliar $\delta^{13}C$ among distant habitats. However, population



variation was less profound in wet season than under drought conditions.

Figure 1. Seasonal changes in midday gas exchange parameters in wild *C. arabica* populations from different habitats measured *in-situ*; (a) net photosynthetic rate (A_{net}, µmol m^{-2} s^{-1}), (b) stomatal conductance (g_s, mol m^{-2} s^{-1}), (c) transpiration rate (E, mmol m^{-2} s^{-1}), and (d) instantaneous water-use efficiency (WUE_i, µmol mmol^{-1}).

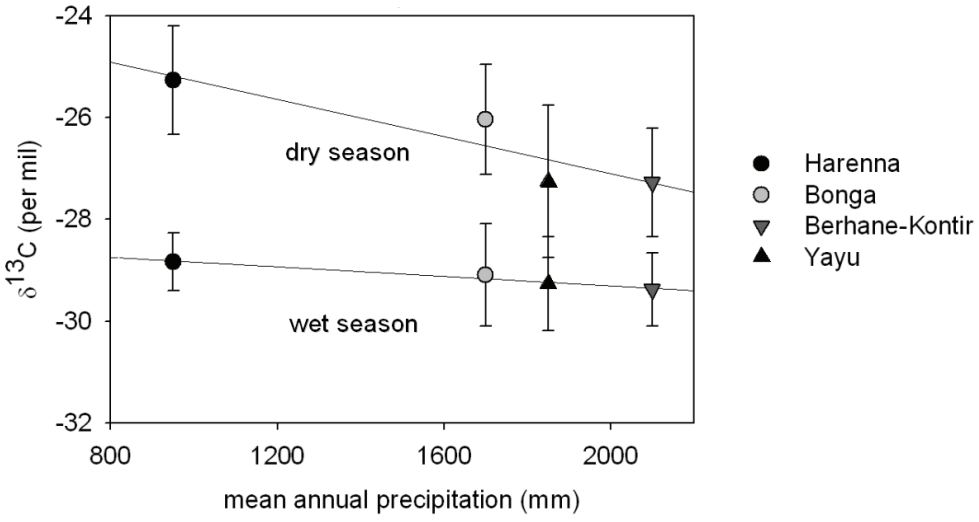


Figure 2. Relationship between foliar carbon isotope discrimination ($\delta^{13}C$) and total annual precipitation among wild C. arabica populations from different habitats.

The results further support that integrated WUE as measured by $\delta^{13}C$ signatures reflects population differences similar to instantaneous water-use efficiency (A_{net}/E, WUE_i) as shown in Figure 3. Both the gas exchange and carbon isotope data indicated that the trees responded to declining moisture availability by increasing WUE.

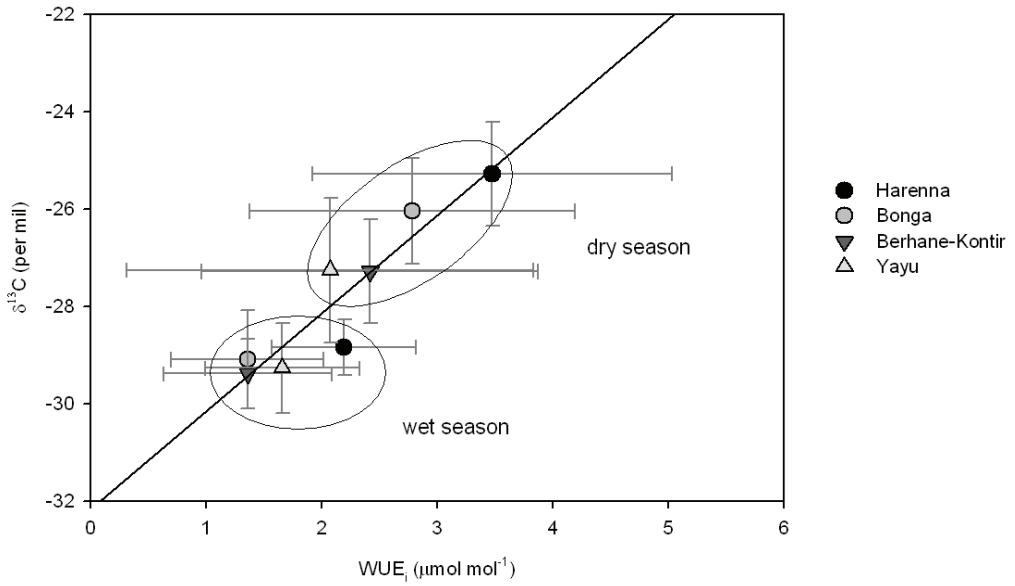


Figure 3. Relationship between instantaneous water-use efficiency (WUE_i) obtained from gas exchange measurements and long-term water-use efficiency ($\delta^{13}C$) taken from carbon isotope discrimination measures of wild *C. arabica* populations from different habitats.

The measurements of the osmotic potential (Π) of the coffee seedlings under common-environmental conditions show drought-induced solute changes (Figure 4); however, this was mainly passive due to dehydration of the leaves rather than an active solute accumulation mechanism because neither turgor maintenance nor a pattern of diurnal changes, both indications of active osmoregulation, became evident.

Hence, the coffee plants did not show osmotic adjustment as a possible strategy in drought conditions, which would allow turgor maintenance in dehydrating tissues (Ludlow, 1989). However, no detectable mechanism with respect to tolerance to dehydration under extreme water deficit conditions was in agreement with the findings of low cell-wall elasticity and no pattern of osmotic adjustment in other experiments (Meinzer, Goldstein et al. 1990; DaMatta, Chaves et al., 2003). The rapid soil drying in this study may have hindered the synthesis and/or translocation of osmotic solutes, and the rate of increase in osmotic adjustment was therefore unable to match the rate of decline in leaf water potential. Consequently, the plants expressed less osmotic adjustment than field-grown plants as presented in many other common-garden studies where an active osmotic adjustment only developed in gradually developing drought stress (Turner and Jones, 1980; Abrams, 1988; Basnayake et al., 1996). Therefore, a reduced rate of development of water deficit would provide a greater opportunity for osmotic adjustment and thus minimize the decrease in relative water content (RWC) per unit fall in Ψ.

Summarizing, the drought resistance strategy of wild *C. arabica* could thus be clearly classified following the known categories of tolerance to and avoidance of tissue dehydration (Ludlow, 1989). The coffee plants exhibited a strong desiccation avoidance strategy by means of efficient water-use and transport, which has been documented in many other studies on drought adaptation in modern coffee cultivars (Meinzer et al., 1990; DaMatta et al., 1993). Hence, Arabica coffee should be considered a dehydration-avoiding rather than a dehydration-tolerant species as reported elsewhere (Nunes, 1976; DaMatta et al., 1993).

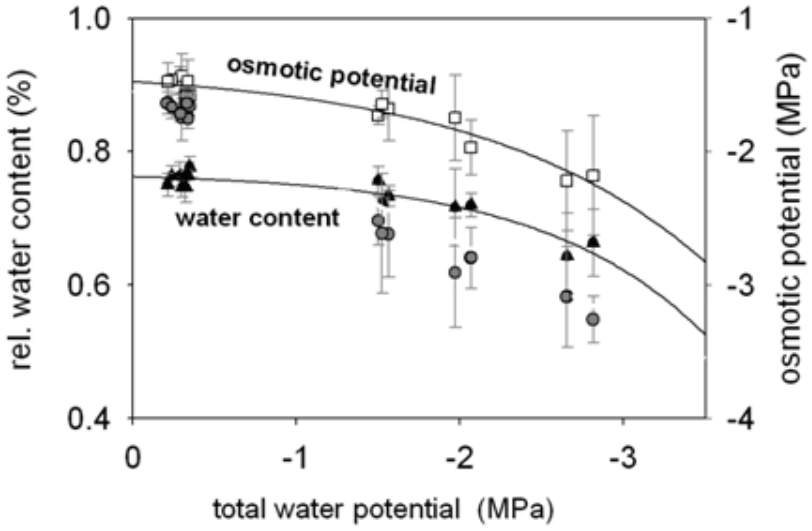


Figure 4. Relative leaf water content and osmotic potential measured at predawn as a function of predawn leaf water potential in wild *C. arabica* populations grown under open sun over a drought stress period in the *ex-situ* study. Signature as in Figures 2 and 3.

In order to estimate the degree of phenotypic plasticity, the norms of reactions diagrams for each physiological parameter were plotted along the water availability treatments for the two light regimes (shade and open sun), separately for populations from contrasting habitats at each measuring date over the whole drought stress period (Figure). While a slope of zero indicates that the environmental variable had no effect on the phenotypic expression of the trait, a non-zero reaction norm revealed phenotypic plasticity. Crossing or non-parallel reaction norms, however, are a sign of interaction.

The reaction norms plotted for ecophysiological parameters of shade grown plants measured 13 days after treatment start revealed a high degree of plasticity in plants behavior due to progressed soil drying as it can be seen by a significant effect of the watering level for all traits considered (Figure 5). Additionally, populations differed in their magnitude in plasticity and the most profound differences were found for plants originating from Harenna habitats in contrast to the south-western ones. While exchange activity and leaf water status of Harenna plants showed to be highly plastic and therefore most variable under changing environment conditions, the other populations responded rather minimally to a change in environment. Whereas all populations showed to respond to severe water restrictions by similar patterns of plasticity in gas exchange parameters revealed by parallel reaction norms, tissue water relations (Ψ_{pd}, Π_{pd}) depicted differences in magnitude of response among plants from distant habitats. Plants from Harenna habitats revealed strongest decrease in their leaf osmotic as well as water potentials.

As sessile organisms, plants experience spatially and temporally varying environmental conditions, and the capacity for plastic responses to the environment is consequently regarded as an important determinant in plant's adaptation to spatial heterogeneity and temporal environmental change (Bradshaw, 1965; Schlichting, 1986; Sultan, 1987). Moreover, high phenotypic plasticity allows efficient capture of available natural resources, therefore it is not only important for individual plant fitness, but also for the persistence of plant populations in response to climate changes and their ability to evolve in response to novel selection pressures. Consequently, expression of phenotypic plasticity has major implications for the

stability and diversity of populations as well as for community and ecosystem functioning in changing environments (Schlichting and Pigliucci, 1998; Sultan, 2001).

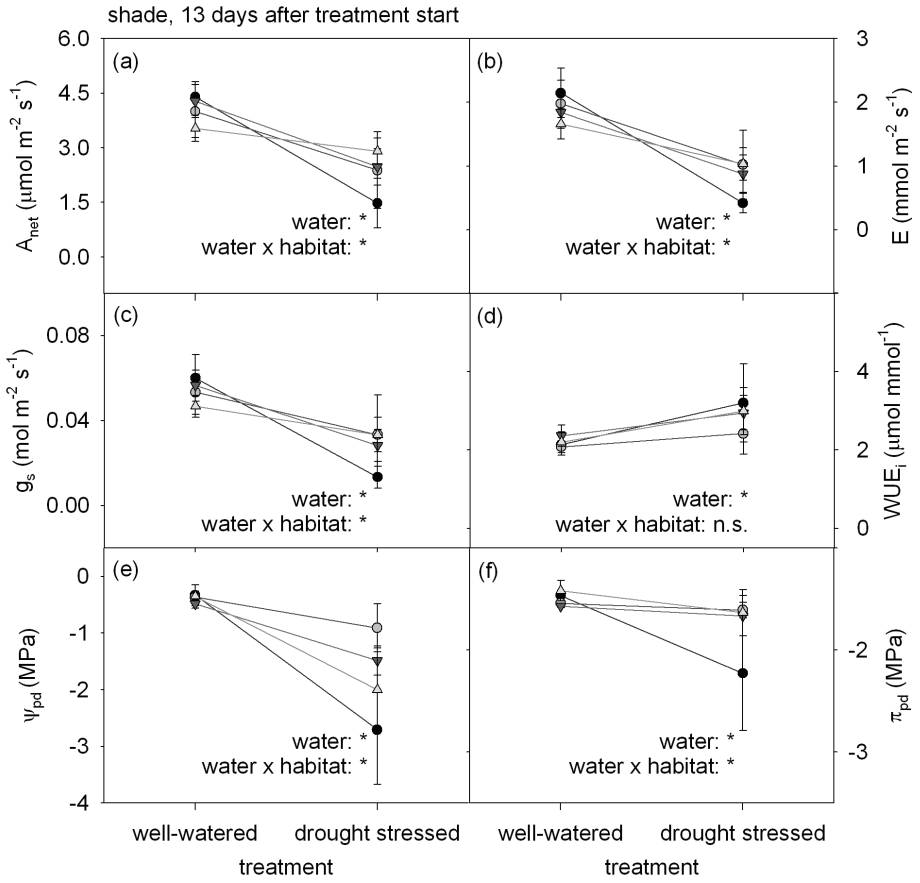


Figure 5. Reaction norms of net photosynthetic rate (A_{net}), transpiration rate (E), stomatal conductance (g_s), instantaneous water-use efficiency (WUE_i), predawn leaf water potential (Ψ_{pd}) and predawn leaf osmotic potential (π_{pd}) of wild *C. arabica* populations from different habitats in response to soil moisture conditions (well watered and drought-stressed); under shade (left panel) and open sun (right panel), 13 days after treatment start. Signature as ion Figures 2 and 3.

In addition, the degree of phenotypic plasticity in response to soil drought was different among populations revealed by a significant habitat-by-trait interaction for nearly all ecophysiological characters as reported for other species characterized by high ecological breadth (Schlichting, 1986; Linhart and Grant, 1996). Hence, the observed pattern of contrasting phenotypic plasticity provides evidence of differentiation between populations of *C. arabica* growing in different habitats, which evidently select on these traits differently. Generally, in more variable environments such as the Harenna habitat with higher level of uncertainty of precipitation, natural selection should favor high phenotypic plasticity for characters that are adaptations to these heterogeneous soil moisture conditions, thereby buffering the plant against variable growing conditions (Bradshaw, 1965; Sultan, 1987). In contrast, low level of phenotypic plasticity is predicted for characters that are adaptation to environmental conditions that are likely to remain fairly constant during the lifespan of the coffee plants, as can be found in Berhane-Kontir. Accordingly, differences in degree of plasticity ranged from very low plasticity for all ecophysiological parameters in the seedlings from Berhane-Kontir to a very high level of plasticity in the Harenna seedlings, and intermediate values for the Bonga and Yayu populations. Plants from Harenna consistently exhibited a flexible water-use pattern in response to soil drought and a good control in gas

exchange, which indicates increased investment in water saving characteristics. However, the higher of phenotypic plasticity in gas exchange in the Harenna plants was not followed by a fitness advantage under drought stress as revealed by the reaction norms of leaf water status.

In contrast, Berhane-Kontir seedlings exhibited a flat reaction norm of leaf water status and thus less dehydration stress; thereby this fitness homeostasis was maintained although no visual adjustment through rapid adjustments in gas exchange to soil moisture variation was observed. However, a flexible gas exchange activity was advantageous for Harenna under sufficient moisture supply but non in-stressful environments, whereas the consistently low level of gas exchange in Berhane-Kontir suggests that the mechanisms that improved plant performance under resource limitation may incur costs that reduce productivity in unstressful conditions and as a result adaptation to low-resource environments may preclude successful occupation of high-resource environments and vice versa (Chapin, 1980). Consequently, there was no superiority or inferiority of the different populations along the water availability gradient, which highlights the need to evaluate the plants drought resistance mechanisms under the specific environmental conditions and to clearly define the target environment, to which the coffee plant should be adapted to. Generally, low plasticity in leaf water relations rather than high plasticity will be an advantage in plant fitness for desiccation avoiders like *C. arabica*.

CONCLUSION

The study reveals high ecophysiological diversity in the primary gene pool of *C. arabica*, with plants' ability to avoid rather than tolerate tissue dehydration seeming to the most important drought resistance mechanism. Incorporating genetic material from wild populations in breeding programs provides scope for improving drought survival by selecting for more efficient mechanisms of dehydration postponement. The existing evidence of the high value of the wild coffee populations with regard to their ecophysiological diversity emphasizes the need to protect them from threats through habitat degradation in order to assure that genetic resources are available for present and future needs.

REFERENCES

Abrams M.D. (1988). Sources of variation in osmotic potentials with special reference to North American tree species. Forest Science 34: 1030-1046.

Basnayake J., Cooper M., Henzell R.G. and Ludlow M.M. (1996). Influence of rate of development of water deficit on the expression of maximum osmotic adjustment and desiccation tolerance in three grain sorghum lines. Field Crops Research 49: 65-76.

Blum A. (1988). Plant breeding for stress environments. Boca Raton, Florida, CRC Press.

Bradshaw A.D. (1965). Evolutionary significance of phenotypic plasticity in plants. Advances in Genetics 13: 115-155.

Bradshaw A.D. and Hardwick K. (1989). Evolution and stress - genotypic and phenotypic components. Biological Journal of the Linnean Society 37: 137-155.

Chapin F.S. (1980). The mineral nutrition of wild plants. Annual reviews of Ecology and Systematics 11: 233-260.

DaMatta F.M., Chaves A.R.M., Pinheiro H.A., Ducatti C. and Loureiro M.E. (2003). Drought tolerance of two field-grown clones of *Coffea canephora*. Plant Science 164: 111-117.

DaMatta F.M., Maestri M., Barros R.S. and Regazz A.J. (1993). Water relations of coffee leaves (*Coffea arabica* and *C. canephora*) in response to drought. Journal of Horticultural Science 68: 741-746.

FAO, ISCRIC and I. (eds) (1998). World reference base for soil resources. World Soil Sources Reports 84.

Farquhar G.D., Ehleringer J.R. and Hubick K.T. (1989). Carbon isotope discrimination and photosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 40: 503-537.

Friis I. (1992). Forests and forest trees of Northeast tropical Africa. London, HMSO.

Gole T.W. (2002). Human impacts on Coffea arabica genetic pool in Ethiopia and the need for its in situ conservation. Managing plant genetic diversity. M. Jackson, CAB International/IPGRI: 237-247.

Hein L. and Gatzweiler F. (2006). The economic value of coffee (*Coffea arabica*) genetic resources. Ecological Economics 60: 176-185.

Kreeb K.H., Richter H. and Hinckley T.M. (1989). Structural and functional responses to environmental stresses: Water shortage. The Hague, SPB Academic Publishing.

Linhart Y. and Grant M.C. (1996). Evolutionary significance of local genetic differentiation in plants. Annual Review of Ecology and Systematics 27: 237-277.

Ludlow M.M. (1989). Strategies in response to water stress. Structural and functional responses to environmental stresses: water shortage. T. M. Hinckley. The Hague, SPB Academic Press, The Netherlands: 269-281.

Meinzer F.C., Goldstein G. and Grantz D.A. (1990). Carbon isotope discrimination in coffee genotypes grown under limited water supply. Plant Physiology 92: 130-135.

Meinzer F.C., Grantz D.A., Goldstein G. and Saliendra N.Z. (1990). Leaf water relations and maintenance of gas exchange in coffee cultivars grown in drying soil. Plant Physiology 94: 1781-1787.

Mitlöhner R. (1998). Pflanzeninterne Potenziale als Indikatoren für den tropischen Standort. Aachen, Shaker Verlag.

Nunes M.A. (1976). Water relations in coffee: significance of plant water deficits to growth and yield: a review. Journal of Coffee Research 6: 4-21.

Parsons P.A. (1988). Adaptation. Analytical biogeography. P. S. Giller. London, Chapman & Hall.

Sandquist D.R. and Ehleringer J.R. (1998). Intraspecific variation of drought adaptation in brittlebush: leaf pubescence and timing of leaf loss vary with rainfall. Oecologia 113: 162-169.

Schlichting C.D. (1986). The evolution of phenotypic plasticity in plants. Annual Review of Ecology and Systematics 17: 667-693.

Schlichting C.D. and Pigliucci M. (1998). Phenotypic Evolution: A reaction norm perspective. Sunderland, M A.

Schlichting C.D. and Smith H. (2002). Phenotypic plasticity: linking molecular mechanisms with evolutionary outcomes. Evolutionary Ecology 16: 189-211.

Senbeta F. 2006. Biodiversity and ecology of Afromontane rainforests with wild *Coffea arabica* L. populations in Ethiopia. Ecology and Development Series No. 38, 2006.

Solbrig O.T. (1992). The IUBS-SCOPE-UNESCO program of research on biodiversity. Ecological Applications 2: 131-138.

Soto-Pinto L., Perfecto I., Castillo-Hernandez J. and Caballero-Nieto J., 2006. Shade effect on coffee production at the northern Tzeltal zone of the state of Chiapas, Mexico. Agriculture, Ecosystems and Environment, 80: 61-69

Sultan S.E. (1987). Evolutionary implications of phenotypic plasticity in plants. Evolutionary Biology 21: 127-178.

Sultan S.E. (2001). Phenotypic plasticity for fitness components in Polygonum species of contrasting ecological breadth. Ecology 82: 328-343.

Life-Cycle Based Sustainability Indicators of the Brazilian Coffee

L. COLTRO, A.L. MOURAD

Institute of Food Technology – ITAL / CETEA, Av. Brasil, 2880,
13070-178 Campinas, SP, Brazil

SUMMARY

Coffee is one of the largest consumed internationally traded commodities. Approximately sixty countries produce coffee in the world. Brazil and Colombia together command approximately half of the world market, while the remaining countries have small market shares. In 2006, Brazil produced 2.57 million tons of coffee in a total cultivated area of 2.33 million hectares, obtaining an average yield of 1,112 kg/hectare. So, the goal of this study was to assess the regional differences of coffee cultivation for the reference crops 2001/2002 and 2002/03 by means of Life Cycle Assessment − LCA in order to generate the environmental indicators of this tillage that are fundamental for evaluating the sustainability of this product.

INTRODUCTION

Brazil and Colombia together command approximately half of the world market, while the remaining countries have small market shares. In 2006, Brazil produced 2.57 million tons of coffee in a total cultivated area of 2.33 million hectares, obtaining an average yield of 1,112 kg/hectare. Brazil is also a great consumer of coffee, having consumed approx. 40% of its production in this same year.

In 2005, three states produced about 92% of green coffee exported by Brazil: Minas Gerais (71%), São Paulo (11%) and Espírito Santo (10%), being also produced in smaller amount in the states of Paraná and Bahia (IBGE-Brazilian, 2004).

So, four Brazilian coffee producer regions located at Minas Gerais and São Paulo States were evaluated in this study: Cerrado Mineiro and South of Minas Gerais regions in Minas Gerais State, Alta Paulista and Mogiana regions in São Paulo State. There are several differences in all the production aspects among them as size of the coffee production areas, edafoclimatic differences, cultivated varieties, adopted spacer, crop management, local topography and processing technology conditions (Aguiar et al., 2001; Igreja and Bliska, 2002; Fazuoli et al., 1999; Kashima, 1990; Verdade et al., 1996).

Several factors like climate change, biodiversity, land use, economic growth, labor conditions, etc. are considered important regarding the sustainable development of our society. The sustainable development means fulfilling the needs of the present generation without endangering the fulfillment of the needs of future generations. Companies integrated responsibilities for mankind, the economy and the environment are becoming a prerequisite for good enterprise. The sustainability issue is increasing at the chain level. Several countries, e.g. the Netherlands, USA, Sweden, Spain among others, are encouraging the agro food business sector to take innovative steps towards sustainable development from the chain point of view (Kramer and Meeusen, 2004; Keoleian and Heller, 2004; Sanjuán et al., 2005).

According to the standard model interpretation of sustainability, the "three pillars", for achieving sustainability, the environmental, economic and social aspects have to be tuned and checked against each other. One proposed method for assessing the life cycle sustainability of a product or a process is based on the following equation:

$$LCSA = LCA + LCC + SLCA$$

where LCA is the environmental Life Cycle Assessment; LCC is an LCA-type ("environmental") Life Cycle Costing assessment and SLCA stands for societal or social Life Cycle Assessment.

The most important requirement for using this scheme is that the system boundaries of the three assessments are consistent (ideally identical). LCA is the only internationally standardized environmental assessment method. Despite being older than LCA, LCC is not yet standardized except for very special purposes. On the other hand, SLCA is generally considered to be still in its infancy, although the idea is not new (Kloepffer, 2008).

So, indicators are useful instruments to determine the level of a product´s or a company´s sustainability. Table 1 presents a matrix of sustainability indicators developed for the agro food chains concerning the agricultural growing and production life cycle stage (Kramer and Meeusen, 2004; Keoleian and Heller, 2004).

OBJECTIVE

The goal of this study was to assess the regional differences of coffee cultivation for the reference crops 2001/2002 and 2002/03 by means of Life Cycle Assessment − LCA in order to generate detailed production inventory data as well as environmental indicators of this tillage that are fundamental for evaluating the sustainability of this product.

METHODS

General Considerations

This study has been conducted in accordance with the recommendations of the International Standard ISO 14040 (2006) – Environmental Management – Life Cycle Assessment – Principles and Framework (ISO, 2006).

Data storage and modeling were performed by means of the PIRA Environmental Management System – PEMS4 software purchased from Pira International.

System Evaluated and the Functional Unit

The system evaluated includes crop cultivation at commercial farms, harvesting, storage and transport by trucks until the exportation harbors. The adopted functional unit was the production of 1,000 kg of green coffee destined for exportation. This unit is not related to the function of the green coffee, since the use stage was not included in the system. Thus, the cradle to gate LCI basis was adopted. The varieties of coffee beans considered in this study were Mundo Novo, Catuaí (yellow and red), Icatu (yellow and red), Catucaí (yellow and red) and Obatã.

Table 1. Sustainability indicators for the agro food chain at the agricultural growing and production life cycle stage (Kramer and Meeusen, 2004; Keoleian and Heller, 2004).

Indicators	Category	Aspect	Task
Environmental	Transportation	Reducing freight transport	
	Energy	Reducing energy use (energy input / unit of production) Renewable energy (ratio of renewable to non-renewable energy)	Promoting its use
	Materials	Reuse of materials % waste utilized as a resource	
	Water	Water quality Water withdrawal vs recharge rates Number of contaminated or eutrophic bodies of surface water or groundwater	Reducing emissions
	Air	Air quality (air pollutants / unit of production)	Reducing emissions
	Soil	Rate of soil loss vs regeneration Quantity of chemical inputs / unit of production	
	Fauna	Biodiversity (number of species / ha)	Preventing the reduction in diversity of sorts and types of animals
Economic	Costs and efficiency	Price / quality ratio (output / input productivity) % return on investment	Increasing the price / quality ratio of products and services
	Ethics in B2B context	Control and certification	Checking whether demands have been met
	Employment	Quantity of employment	Increasing the number of jobs
Social	Working conditions	Workplace	Improving the location, interior (ergonomic) and safety
	Food safety		Reducing food-pollution components
	Norm and values	Emancipation	Stimulating integration of the elderly, handicapped, immigrants, women, etc.
	Social responsibility	Welfare	Contributing to the health, housing, safety, education, etc. of the community

Geographical Extension and Temporality

The selection of the studied regions was done taking into account the geographical boundaries. The four main Brazilian coffee producer regions included were: Cerrado Mineiro and South of Minas Gerais regions in Minas Gerais State, Mogiana and Alta Paulista regions in São Paulo State.

The time-related coverage of this study was 2 complete crops (2001/02 and 2002/03), including the higher and subsequent lower productive periods.

Data Collection

All information considered in this study was taken up in-depth data collection by means of specific questionnaires applied on farm level and/or sent by mail considering the inputs of water, fossil based energy, fertilizers, chemicals and correctives, water usage, energy consumption and residue disposal. These data reflect the cultivation profile of 56 coffee farms grouped in 28 questionnaires.

Boundaries of the Study

Only the inputs and outputs relative to the coffee tillage were considered in this study as a cradle-to-gate system. The production of fertilizers, correctives and pesticides were not included in the boundary, but only their amount and the transportation of them up to the farms.

RESULTS AND CONCLUSION

The data refer to a production of approx. 25,200 tons of green coffee and a productive area of approx. 14,300 ha, supplied by 56 coffee growers. The final LCI was previously published by the group authors in the paper *Environmental Profile of Brazilian Green Coffee* (Coltro et al., 2006). The present work shows some environmental indicators for each Brazilian coffee region evaluated in this study.

Important environmental indicators related to energy use through the life cycle of the coffee growing in Brazil for the functional unit of 1,000 kg of green coffee is showed in Figures 1 to 4. The results showed a great variation among the regions.

The total energy (Figure 1) accounts the extraction of the oil and the production of the specific fuels considering the upper calorific value of them. Energy is required both in the growing and in the processing stages.

Wood and electric energy (Figures 2 and 3) are used mainly at the coffee processing and the coffee beans drying in order to reduce the moisture content of the product up to 11%. The environmental performance of the green coffee is improved due to the use of this type of energy resources since these inputs are from renewable resources (electricity in Brazil are mainly generated by hydropower stations) and then do not contribute to global warming.

Diesel consumption (Figure 4) is due to the fuel use by the agricultural machinery and the considered transport stages.

The sun drying in yards is favorable for Cerrado Mineiro region, resulting in good indicators for energy consumption in this region. Besides, this drying method allows the migration of the sugars from the pulp to the grain, providing a more sweetened drink.

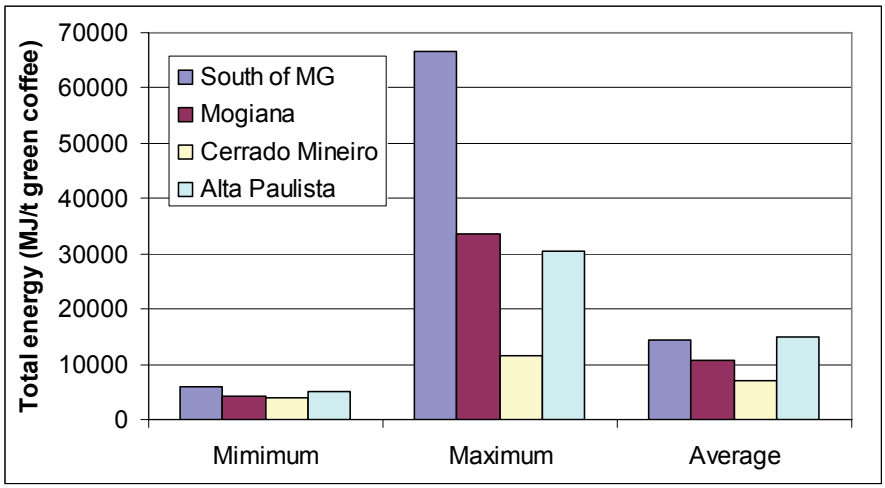


Figure 1. Total energy input by the four Brazilian coffee producer regions (MJ/t green coffee).

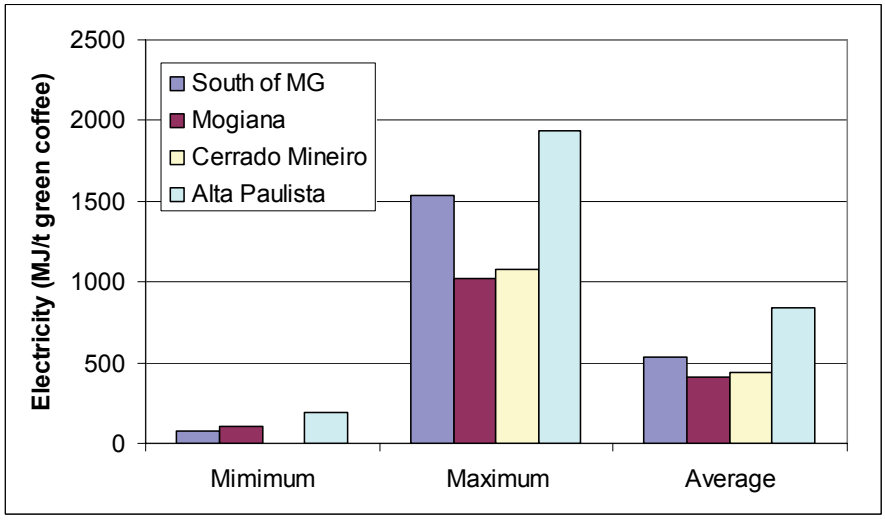


Figure 2. Electricity input by the four Brazilian coffee producer regions (MJ/t green coffee).

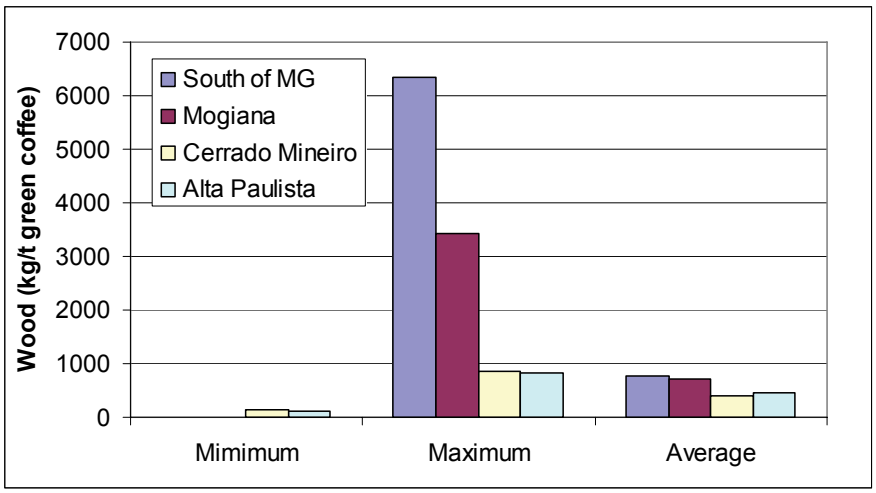


Figure 3. Wood input by the four Brazilian coffee producer regions (kg/t green coffee).

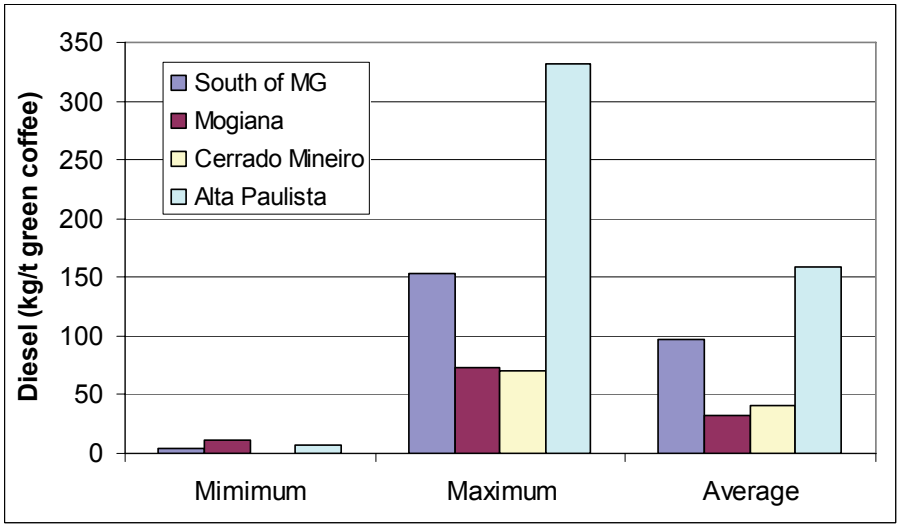


Figure 4. Diesel input by the four Brazilian coffee producer regions (kg/t green coffee).

The use of agricultural equipment for harvesting and some tillage treatments is usual for Alta Paulista region, mainly due to the favorable topography of the soil resulting in high diesel consumption in this region.

Other environmental indicators were published elsewhere (Coltro et al., 2006; Coltro et al., 2007). Combining all of them to social and economic indicators can help coffee growers to evaluate their sustainable performance.

The inputs are mainly related to impact categories as resource depletion, eutrophication, human toxicity, ecotoxicity, land use and can be used as sustainability indicators of the Brazilian coffee.

Although the inputs are directly related to the specific characteristics of each farm and the climatic and topographic conditions of its location, this study has identified some farms that can probably reduce the amount of some inputs and enhance their environmental performance.

As the farmers realize that there are farms showing lower consumption of some inputs in the same region they are located, a driving force for trying to reduce the consumption of these

inputs is established, both for environmental and economical reasons, towards the sustainability.

Besides the total amount of these inputs, good agricultural practices should also be considered for having a good yield and improving the sustainability of the coffee production.

ACKNOWLEDGMENTS

The authors are grateful to FINEP (Research and Projects Financing), CNPq (National Board of Technologic and Scientific Development) and MCT (Brazilian Science and Technology Ministry) for the financial support and the fellowships.

REFERENCES

Aguiar A., Maluf M., Gallo P., Mori E., Fazuoli L. C. in proceedings of International Conference on Coffee Science, Trieste, 2001, Vol. 19, 5 pp. CD-ROM.

Coltro L., Mourad A.L., Oliveira P.A.P.L.V., Andrade J.P.B.O., Kletecke R.M. Environmental Profile of Brazilian Green Coffee. International Journal of Life Cycle Assessment, Heidelberg - Alemanha, v. 11, n. 1, p. 16-21, 2006.

Coltro L., Mourad A.L., Oliveira P.A.P.L.V., Andrade J.P.B.O., Vilela G. B. Environmental profile of several green coffee producers assessed by LCA. In: IV Global Conference on Sustainable Product Development and Life Cycle Engineering − Sustainaible Manufacturing, 2006, São Carlos. Annals of IV Global Conference on Sustainable Product Development and Life Cycle Engineering - Sustainaible Manufacturing, 2006. v. CD-Rom. p. 1-7.

Coltro L., Mourad A.L., Oliveira P.A.P.L.V., Andrade J.P.B.O., Vilela G. B. Regional differences of coffee cultivation assessed by LCA. In: CILCA2007 International Conference of Life Cicle Assessment, 2007, São Paulo. Proceedings of the CILCA2007 International Conference of Life Cicle Assessment. São Paulo : ABCV − Associação Nacional de Ciclo de Vida, 2007. p. 1-4.

Fazuoli L.C., Medina Filho H.P., Guerreiro Filho O., Gonçalves W., Silvarolla M.B., Lima M.M.A. in proceedings of Association Scientifique International du Café – In: 18 éme Colloque, Helsinki, Finlândia, 1999, 396.

IBGE-Brazilian Institute of Statistics and Geography, 2004, Produção agrícola municipal. Electronic address: <http://www.ibge.gov.br/>. Access in Jul 2006.

Igreja A., Bliska F. in proceedings of 2º Encontro de Estudos Regionais e Urbanos, São Paulo, 2002, CD_ROM.

International Organization for Standardization – ISO (2006): Environmental management – Life cycle assessment – Principles and framework – ISO 14040. Genève: ISO, 20p.

Kashima T. in proceedings of 4º Ciclo de Estudos sobre Mecanização Agrícola, Campinas, 1990

Keoleian G.A., Heller M.C. Life cycle-based sustainability indicators for assessment of the U.S. food system. In: 4th International Conference on Life Cycle Assessment in the Agri-food Sector, 2003, Bygholm, Denmark. Proceedings from the 4th International Conference on Life Cycle Assessment in the Agri-food Sector. DIAS Report, Animal Husbandry, n. 61, 2004. p. 190-196.

Kloepffer W. Life cycle sustainability assessment of products. International Journal of Life Cycle Assessment, Heidelberg - Alemanha, v. 13, n. 2, p. 89-95, 2008.

Kramer K.J., Meeusen M. Sustainability in the Agrofood Sector. In: 4th International Conference on Life Cycle Assessment in the Agri-food Sector, 2003, Bygholm, Denmark. Proceedings from the 4th International Conference on Life Cycle Assessment in the Agri-food Sector. DIAS Report, Animal Husbandry, n. 61, 2004. p. 182-189

Sanjuán N., Úbeda L., Clemente G., Mulet A., Girona F. (2005): LCA of integrated orange production in the Comunidad Valenciana (Spain). Int. J. Agricultural Resources, Governance and Ecology, 4 (2), 15 p.

Verdade F., Dias C., Silva G., Mello M., Victor M. in , Levantamento Censitário de Unidades de Produção Agrícola do Estado de São Paulo, F.A. PINO, Ed.; IEA/CATI/SAA, São Paulo, 1996, 4v.

Nutrient Utilizations of *Coffea arabica* Seedlings Under Integrated Use of Organic and Mineral Fertilizers

T. KUFA

Ethiopian Institute of Agricultural Research, Jimma Research Center,
P. O. Box 192, Jimma, Ethiopia. E-mail: kufataye@yahoo.com

SUMMARY

The study was conducted in south-western Ethiopia at Jimma Agricultural Research Centre (JARC) with the objective to optimise nitrogen (N) and phosphorus (P) utilizations of Arabica coffee seedlings under an integrated use of organic and mineral fertilizers. A factorial experiment in a split–split-plot design with three replicates was used. To this end, DAP at three rates (0, 2 and 4 g pot^{-1}), undecomposed coffee husk (UCH) and decomposed coffee husk (DCH), farmyard manure (FYM), UCH+FYM and DCH+FYM and the ratios of these organic manure (M) with topsoil (TS), 0M: 4TS, 1M: 4TS, 2M: 4TS, 3M: 4TS and 4M: 4TS, were assigned as main, sub-and sub-plot treatments, respectively. Soil and plant tissue sampled from the same central pots and analysed for total nitrogen and available phosphorus. The results depicted that nitrogen and phosphorus uptake and use-efficiency of coffee seedling were differently influenced due to the main and interaction effects. Consequently, DAP at 2 g pot^{-1} had the highest total nitrogen applied; maximum nitrogen use-efficiency and high total dry matter yield of coffee seedlings. Among the organic sources, the highest nitrogen recoveries were observed from seedlings on DCH and UCH+FYM. Whereas, the least and highest nitrogen use-efficiency were determined for seedlings grown on potting blended with UCH and FYM alone, respectively. In contrast, the highest phosphorus uptake and use-efficiency were obtained from seedlings grown in media without DAP and UCH alone. FYM, on the other hand, resulted in the increased apparent phosphorus recovery, the highest phosphorus amounts applied and the greatest total dry matter yield. Unlike available phosphorus, the total leaf nitrogen content was significantly increased with increased ratios of organic fertilizers. But, maximum nitrogen and phosphorus use-efficiencies were obtained at 1M: 4TS and 2M: 4TS and the effectiveness were reduced thereafter. The interaction of organic resources by their mix ratios as well as the combination of DAP and organic ratio significantly affected the concentration of nitrogen and phosphorus in coffee leaves. As a whole, the use of decomposed organic manure and inorganic fertilizers at modest rates had significantly improved N and P uptake of coffee seedlings, though the relationship was noted to be antagonistic. It can be therefore concluded that N and P uptake and utilization patterns differed with potting media types (source and decomposition stages) and hence can be used as selection criteria to evaluate the performances and resource use-efficiencies of Arabica coffee populations under specific agro-ecological conditions.

INTRODUCTION

The nature and properties of soils are of vital concern in the production of coffee since it ranks among those tropical crops with high nutrient demand. Even within the genus, *Coffea arabica* L. has greater nutrient requirement than the other species (Michori, 1981). Yet, the crop is grown on highly weathered soil types (Alfisols, Oxisols and Ultisols), which require intense management (Mesfin, 1998). These “typical” red and brown soils with a depth of 1.5

to 2.0 meters often have pHs of 5.3 to 6.6 and are low in cation exchange capacity with prospects of phosphate fixation (Tekalign and Haque, 1991; Mesfin, 1998).

As a corollary to this, mineral stress phenomena of coffee soils are related to a low nutrient retention capacity and a strong fixation of phosphates by free sesquioxide and clay components. This is in contrast to the higher nutrient requirement of Arabica coffee than that of other coffee species (Michori, 1981). Notwithstanding, the specific mineral stress phenomena include deficiency in bases (Ca, Mg, K), inadequate capability to retain bases that are applied as fertilizers or amendments and presence of free manganese, iron and exchangeable aluminum, elements which when in high concentrations could be toxic to plants and highly active in the fixation of phosphorus and deficiency of molybdenum, especially for the growth of legumes and fixation of P on sesquioxide (Mesfin, 1998).

The use of organic fertilizers, singly or in combination, with mineral fertilizers has been well elaborated elsewhere. As a case in point, there are ample direct and indirect evidences on the positive effects of well-decomposed manure in complementary with various regimes of mineral N-P fertilizer on coffee under Kenya conditions (Jones, 1971). In addition, he also elaborated on the merits of manure application on such soils to slightly decrease acidity along with the mobility of aluminum, iron, and manganese and the buffering capacity of the soil becomes more pronounced. Muller and Kotschi (1994) emphasized on the use of coffee compost as a potential renewable source of organic fertilizer since it offers a means of ensuring long-term soil fertility without the need for external inputs. In Ethiopia, there is an immense potential of the by-products of coffee processing, which can contribute to environmental pollution unless properly disposed of or recycled in the form of compost. According to Janssen (1993), integrated plant nutrient management was the best approach to mitigate the inherent soil fertility problems for sustainable crop production.

The productivity of coffee cultivation also depends on the production of high quality coffee seedlings. This in turn relies on the production of not doubtful coffee seedlings produced under optimum nursery practices, which primarily include media amendment, moderate shade and moisture and pest control (IAR, 1996). Such a focus is sought because, the early growth potential of coffee seedlings puts the most imprints and increase the chance of survival of young coffee plants. The need therefore to know how coffee seedlings utilize the nutrients under integrated soil management cannot be over emphasized. The study has the purpose to minimize the ever-increasing dependency on inorganic fertilizers and provide insights on the agronomic effectiveness of coffee plant under specific locality. It was hypothesized that the combined use of soil applied mineral and organic fertilizers would complement and improve N and P use-efficiency and performance of *Coffea arabica* cultivars grown on acidic soils. The specific objective of the study was, therefore, to investigate the effects of different nitrogen and phosphorus levels, coffee husk composts and farmyard manure, singly or in combinations, on N and P uptake and utilization of Arabica coffee seedlings.

MATERIALS AND METHODS

Site description

The study was conducted in southwestern Ethiopia, at the Jimma Agricultural Research Centre (JARC) (7° 46’ N and 36° E). It is situated within the Tepid to cool humid highlands agro-ecological zone of the country at an altitude of 1750 meters above sea level. The site receives high amount of rainfall with a long-term mean total of 1573.6 mm per annum, which is distributed into 166 days. The driest months usually last between November and February. The mean maximum and minimum air temperatures are 26.3 and 11.6 ^{o}C, respectively

December being the coldest month. The predominant coffee soil is *Nitosols* (Mesfin, 1998) and the experimental soil was determined to be acidic (Taye et al., 2003) and thus needs amendment.

Treatment and design

A one-year undecomposed coffee husk (UCH) and two-years decomposed coffee husks (DCH) were collected from the dry coffee processing heaps. The well-decomposed farmyard manure (FYM) was collected from the dairy development enterprise in Jimma. The topsoil was collected from an open field free of any vegetation cover. These materials were separately dried, manually crushed and passed through a 2 mm sized sieve to prepare five organic fertilizer treatments (UCH, DCH, FYM) and their combinations (UCH+FYM, DCH+FYM). Then, these organic manure sources (M) were thoroughly mixed on the same proportion of topsoil (TS) at the ratios of 1M: 4TS, 2M: 4TS, 3M: 4TS and 4M: 4TS, mimicking soil incorporation at the ground surface. A check plots without organic manure (0M: 4TS) was also included.

A factorial experiment arranged in a split–split- plot design with three replicates was used to lay out the study. In this case, the rates of DAP (0, 2 and 4 g pot^{-1}); organic sources and their rations were assigned as main, sub- and sub-sub-plot treatments, respectively. The so prepared media were properly filled in black diothene 200 gauge polythene bags of 12 cm x 24 cm size. These were arranged on nursery beds at 50 cm spacing and each plot consisted of sixteen pots. Then, coffee seeds from the released coffee berry disease resistant Arabica coffee cultivar (7440) with intermediate growth habit were prepared and sown. All routine nursery operations were properly applied as per the recommendation of the research center (IAR, 1996).

Data collection and laboratory analyses

When the seedlings reach normal transplanting stage, soil and leaf samples from the central four seedlings were for the laboratory determinations on major plant nutrients (N and P). The different parts of the seedlings were oven-dried at $70^{o}C$ for 24 hours and weight measurements were taken using a digital sensitive balance. The total biomass yield (g) was used to estimate nutrient utilization of coffee seedlings with special attention to nitrogen (N) and phosphorus (P).

Soil analysis

Soil samples were taken from the four central pots of each plot and bulked. About 500 g soil samples were air-dried and ground to pass through a 2 mm sieve size. These were used for the determinations of nitrogen and phosphorus both at the beginning and end of the experiment. Total nitrogen (TN) was determined by the modified Kjeldahl method as described by Jackson (1958). Six ml of concentrated H_2SO_4 was added and mixed carefully to one gram of sample passing 0.5 mm sieve plus sulphuric salicylate and potassium salt extractant mixture to which is added two g of catalyst mixture. This was digested and distilled with 45% NaOH. The NH_3 was allowed to react with a 0.20N H_2SO_4 using methyl red as an indicator. This was further titrated with 0.10N NaOH. A blank was run in exactly the same manner. The total nitrogen was then calculated as: TN (%) = (t-b) 1.4N/w. Where, t - volume of H_2SO_4 sample, b - volume of H_2SO_4 blank, N - normality of H_2SO_4, and w – weight of sample.

Available phosphorous was determined on a five-gram sample following the procedure of Olsen et al. (1954) using the $NaHCO_3$ extracting solution. Ammonium molybdate and stannous chloride solution to complete the reaction and enhance colour development,

respectively. A standard curve was prepared from an aliquot of dilute P solution containing 2 to 20 ppm of P. The transmittance of the solution was measured at 660 nm with a spectrophotometer using red filter per cent transmittance was plotted against P concentration and the results expressed as P (ppm) = (standard curve*ES*f)/weight of sample. Where, ES = extracting solution and f = dilution factor.

Plant tissue analysis

Leaf samples were taken from third to fourth pair of the four central pots. The leaves were oven-dried at 70 °C for 24 hours and ground with a stainless steel Wiley mill to pass through a 1.5 mm sieve. A dilute sulfuric-salicylic acid and 30% hydrogen peroxide digested these. Subsequently, total nitrogen was determined by the modified-Kjeldahl method (Black, 1965), phosphorus spectrophotometrically by the methods of Olsen et al. (1954).

Nutrient use efficiency

From the foregoing, nutrient use-efficiency (N and P uptake and utilization) of *Coffea arabica* seedlings grown on the various potting media blends was calculated using such components as apparent nutrient recovery (ANR), nutrient use efficiency (NUE) and total nutrient applied (TNA). The Apparent Nutrient Recovery (ANR) was estimated in accordance with the methodology described by Guillard *et al.* (1995) where the difference between the nutrient uptake by the nutrient fertilized plot and the nutrient uptake by the control plots divided by their respective rate of nutrients applied was employed. That is, [(N at N_x-N at N_0)/ (Applied N at N_x)*100]. Total Nutrient Applied (TNA) was calculated as dry matter times total nutrient concentrations. Again, Nutrient Use Efficiency (NUE): gram dry matter (DM) produced per gram nutrient applied was calculated as: (DM at N_x-DM at N_0)/ Applied N at N_x (Guillard *et al.,* 1995). However, the nutrients that can be lost due to other factors such as by leaching, microbial immobilization and others were not considered to make the analysis more precise. Finally, analysis of variance and comparison of treatment means were made according to Fisher's LSD at 5% probability level.

RESULTS AND DISCUSSION

Nitrogen and phosphorus uptakes

Nitrogen

Nitrogen uptake of coffee seedlings was significantly influenced by inorganic resource rates and highly significantly by the various organic ratios, but no by organic resource sources (Table 1). The status of nitrogen in the leaf of coffee seedlings, therefore, increased with increasing the rates of DAP and thus, the highest (2.65%) and the lowest (2.50%) nitrogen status were found at the highest and the lowest rates of DAP with increments of leaf total nitrogen by 12.43% and 19.46%, respectively. This is quite similar to the close relationships between leaf nitrogen and inorganic nitrogen from DAP (Table 1). Though not significant, the greatest (2.62%) and the lowest (2.43%) nitrogen contents of coffee leaf were from DCH plus FYM and UCH, respectively (Table 1) where the highest (18.11%) and the lowest (9.46%) increments were also recorded. This could be due to the variations in the decomposition stage and thus, the low organic matter tied up nitrogen of the undecomposed organic sources. There was also a highly significant responses in the nitrogen status of coffee seedlings to the various organic proportions where 3M: 4TS, 2M: 4TS and 4M:4TS treatments revealed the respective total nitrogen increments of 21.98 and 20.63% over the

control that received neither DAP nor organic resource. However, the lowest nitrogen uptake was observed from those seedlings with low organic manure and topsoil (Table 1).

The interaction effect of different organic resources and ratios with various regimes of DAP (Figure 1) was significant ($P < 0.05$) on the status of leaf total nitrogen. Consequently, high values recorded due to the use of DAP on decomposed materials (DCH and DCH plus FYM) and moderate organic ratio (2M: 4TS to 3M: 4TS). This is in contrast to the low values noticed under UCH and without or low rate of DAP, which largely suggests the immobilization of nitrogen. This variation in the nitrogen status of coffee leaves could in part reflect the differences in the nitrogen and organic matter concentrations of the potting media compositions. This may be justified from the positive and significant correlations between leaf nitrogen and soil media nitrogen and organic carbon status (Taye et al., 2003).

Phosphorus

Phosphorus in coffee seedlings revealed significant responses due to the main effects of DAP. Consequently, the highest (0.38%) and the lowest (0.29%) values were obtained from the lowest and the highest DAP treatments (Table 1) where the respective increase (13.34%) and decrease (11.97%) were determined. This is in line with our previous report (Taye et al., 2003) in that phosphorus has been inversely and significantly associated with fertilizer rates and with the soil media phosphorus content.

Table 1. Concentration of N and P in potting media and coffee leaves due to use of fertilizer treatments.

Treatment	Total Nitrogen (%)		Available Phosphorus	
	Soil	Leaf	Soil (ppm)	Leaf (%)
Rate of DAP (g pot^{-1})				
0	0.310	2.50b	80.83b	0.38a
2	0.304	2.51b	253.05a	0.32b
4	0.303	2.65a	270.90a	0.29c
Organic resource				
UCH	0.24d	2.43	178.14c	0.37a
DCH	0.37a	2.59	173.67c	0.33bc
FYM	0.31bc	2.53	240.09a	0.34ab
UCH+FYM	0.26cd	2.59	213.26b	0.32bc
DCH+FYM	0.35ab	2.62	202.81b	0.30c
Organic Manure (M) to Topsoil (TS) ratio				
0M: 4TS	0.13d	2.22b	181.97b	0.33
1M: 4TS	0.23c	2.43ab	196.99ab	0.34
2M: 4TS	0.32b	2.71a	202.95ab	0.33
3M: 4TS	0.40a	2.73a	213.21a	0.31
4M: 4TS	0.44a	2.68a	212.86a	0.32

Means followed by the same letter(s) with in a column are not significantly different from each other at $P < 0.05$ probability level.

The contributions of the different organic sources to coffee leaf P were also significant. As a result, those seedlings on FYM gave the highest results (0.34%) and the lowest (0.30%) in leaves from DCH plus FYM where phosphorus showed an increase of 0.87% and decrease of 3.98%, respectively (Table 1). This could be attributed to the high available phosphorus contents of FYM (Taye et al., 2003). Thus, the application of UCH was noticed to enhance

the status of leaf phosphorus by 10.52% which is much higher than that for other organic treatments, possibly due to minimized risk of P fixation.

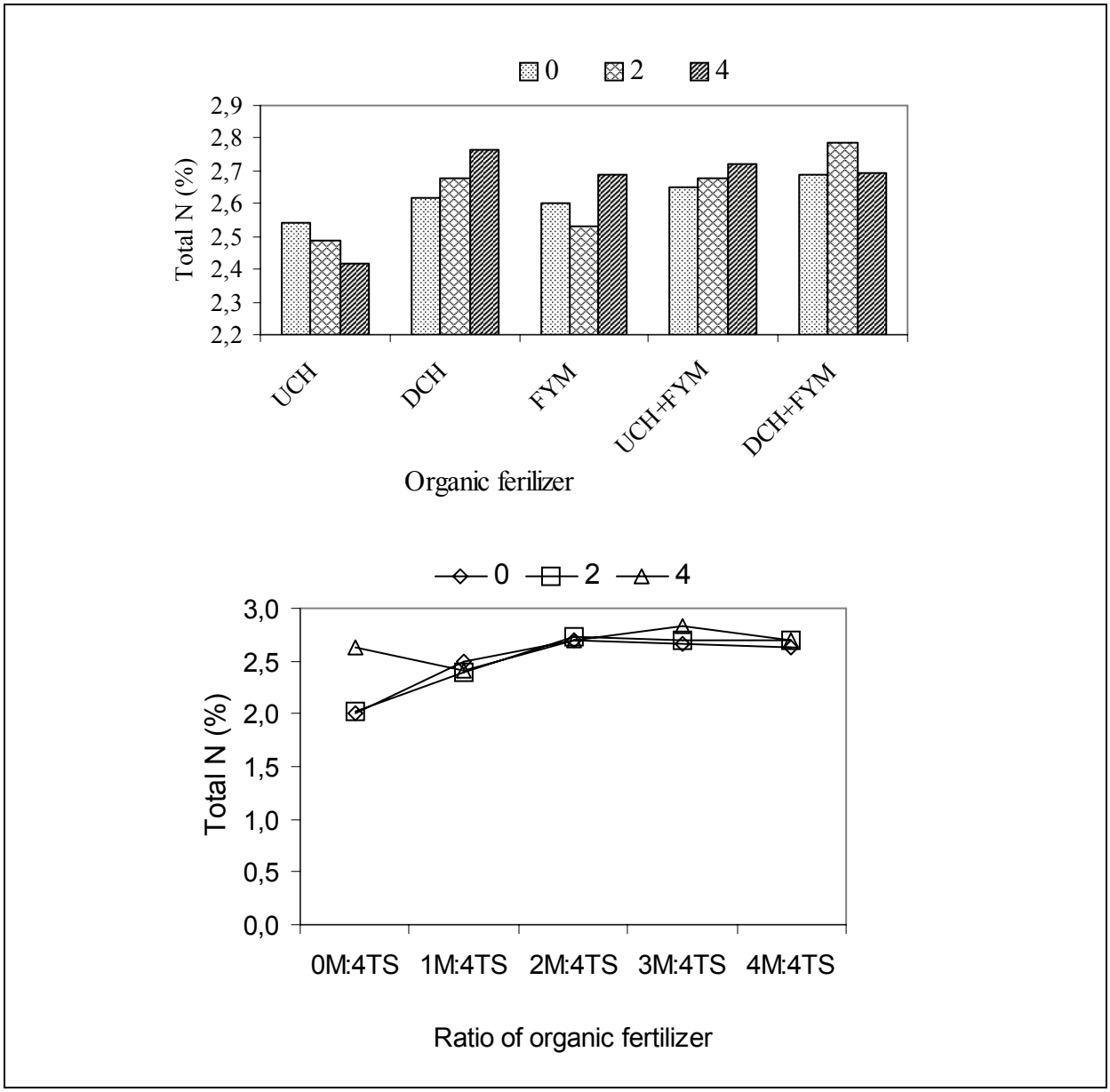


Figure 1. Nitrogen uptake by coffee seedlings as influenced by integrated fertilizer regimes.

Phosphorus removal from the various proportions of organic resources was not significant. However, phosphorus tends to decrease with the increased organic ratio where the highest (0.34%) and the lowest (0.31%) values were determined for 1M: 4TS and 3M: 4TS treatments; with the respective P increment of 0.16% and reduction of 6.16%, respectively (Table 1). The integrated use organic fertilises and DAP had significant ($P < 0.05$) effects on the contents of available phosphorus in coffee leaves. In contrast to nitrogen uptake, the available P status in coffee leaves due to the interaction effect of organic and mineral fertilizer was significantly maximum for coffee seedlings grown on pots filled with undecompsed materials and did not receive DAP (Figure 2). This could largely indicate variations in the P affinity of the media compositions and thus, its uptake by coffee seedlings. In other words, the association between P in potting media and in coffee leaf was negative. This is in agreement with the observation of Ofori (1980) who suggested that organic colloids prevent dissolved

phosphate coming into contact with aluminium and iron; organic phosphorus is less strongly fixed by the soil and the micro-organisms can mineralise the organic phosphate compounds and the decay of organic matter releases carbonic acids that forms dissolved phosphate. The finding corroborates with the reports of Paulos and Moorby (1995) and Asefa (1996), who respectively found low phosphorus concentrations in coffee leaves and husks. This could justify either the relatively low phosphorus requirement of coffee plant or P fixation problem, particularly on the more decomposed organic materials. According to Taye et al. (2003), leaf phosphorus revealed negative relationships with most of the soil chemical parameters and with other nutrient status in the leaves, say nitrogen. Singh and Bhanadari (1992) also reported similar findings.

Nitrogen and phosphorus utilizations

Nitrogen

Apparent nitrogen recovery was appreciably increased by the applications of DAP where the highest result (132.24%) was from coffee seedlings that received the highest rate of DAP (4 g/pot) as compared to the other DAP levels. However, the addition of DAP at the rate of 2 g/pot had high total dry matter yield (6.08 g/pot), highest nitrogen use efficiency (10.91 g/g), and subsequently total nitrogen applied (1.99 g/pot) (Table 2). This could possibly be attributed to the improved physical and chemical conditions of the media (Taye *et al.,* 2003) which favoured the production of vigorous leaves that would enhance photosynthesis, and lateral roots with much more feeders and thus, increased the effectiveness of the seedlings to obtain nutrients from the soil and assimilates more carbohydrates.

Among the different organic resources, the highest nitrogen recoveries were observed from seedlings on DCH and UCH plus FYM with the respective results of 110.32 and 112.42%. In contrast, the least and highest nitrogen use-efficiency was noted for those seedlings grown on soil media composed of the UCH and FYM alone, respectively (Table 2). This resulted in low total dry matter yields of coffee seedlings, which may be attributed to the inadequate physical and chemical status of the potting media (Taye et al., 1999, 2003).

This suggests that the use of UCH, as a major component of soil media for nursery potting is not advisable, unless and otherwise it is blended with some other decomposed organic materials, particularly with that of animal manure. In this regard, Howard (1943) was quoted by Muller and Kotschi (1994) and recommended a mixture of plant and animal residues to produce a better compost and high plant response.

On the other hand, the increased proportions of organic manures in the media did not show better responses and ratio greater than 2M: 4TS reduced nitrogen utilizations (Table 2) and hence, a curvilinear growth was observed. This could be because of the increased carbon to nitrogen ratio, which may cause nitrogen immobilization and ultimately reduced coffee leaf productions. This supports the work of Murwira (1994) and Mburu and Mwaura (1996), who elaborated that the availability of nitrogen depends on the amount and type of organic matter present, and on the presence of microbial populations and conditions favouring their activity. Myers et al. (1994) also reported that a C: N ratio greater than 25 can lead to temporary immobilization of mineral nitrogen in the soil and adversely affect plant growth. In addition, Guillard et al. (1995) found such a declined apparent nitrogen recovery with the increasing rates of fertilizers.

Phosphorus

In contrast to the nitrogen utilizations, the highest recovery (+698.74 %) from the control treatment and was reduced with increasing rates of DAP (Table 2). This could largely be attributed to the high phosphorus fixation capacity of coffee soils (Mesfin, 1998; Tekalign and Haque, 1991). However, root growth of coffee seedlings was enhanced with increased rates of P (Taye et al., 1999), indicating the promotion of coffee root growth activity by P as it has been documented by authors (Wrigley, 1988).

The effect of organic resources on the P use-efficiency of coffee seedlings was also found to be different. Accordingly, the highest (+4986.81%) and the lowest (-396.18%) apparent phosphorus recoveries were from seedlings on soil media containing UCH and DCH+FYM, respectively (Table 2). The highest P-use efficiency (0.19 g/ppm) was recorded under poor media blended with UCH where the least total applied phosphorus and subsequently, reduced seedling growth responses were also noticed. This might probably be explained in terms of reduced soil contact area for P fixation, plant immobilization of phosphorus, low P and poor seedling growth.

FYM, on the other hand, resulted in increased apparent phosphorus recovery by 27.73% and showed the highest amounts applied (621.86 ppm/pot) with the greatest total dry matter yield of 5.88 g/pot (Table 2). Such variations could largely be related to the exceptionally high phosphorus content of FYM, high base saturation and divalent cations as shown in our previous report (Taye et al., 2003). In contrast to UCH, the use of FYM either singly or in combination with coffee compost had reduced P use-efficiency of coffee seedlings (Table 2), largely indicating the increased surface area to fix P. Against this, the adequate physical status of the media have been found to favour both shoot and root systems of the coffee seedlings (Taye et al., 1999) may help as strategy to optimise the exploitations of the available immobile soil phosphorus. Similar findings have been reported by Njoro (1988) and Jama et al. (1997), who described increased crop yields on phosphorus deficient acidic soils with the application of cattle manures.

Like nitrogen, phosphorus utilization by the coffee seedlings was enhanced with the increased proportions of organic manure in the potting media mix. Hence, the greatest use-efficiency to the maximum phosphorus application was obtained at the ratio of 2M: 4TS and the effectiveness were reduced thereafter (Table 2). This is despite the increased available phosphorus and improved chemical conditions of the potting media due to the same treatments (Taye et al., 1999, 2003). This may in part reflect the increased P contact area of the media and thus, its maximum fixation with increased decomposed organic resources. This is in agreement with several authors (Ofori, 1980; Hsieh and Hsieh, 1990; Singh and Bhandari, 1992; Mesfin, 1998) who reported such reduced plant phosphorus uptakes due to the accumulation of other nutrients (for instance high nitrogen status) that showed an antagonistic relationship with phosphorus, unlike that of potassium.

Table 2. Nitrogen and phosphorus utilizations of Arabica coffee seedlings due to fertilizer treatments.

Treatment	ANR (%)		NUE		TNA		TDM (g pot^{-1})
	N	P	N (g g^{-1})	P (g ppm^{-1})	N (g pot^{-1})	P (ppm pot^{-1})	
Rate of DAP (g pot^{-1})							
0	84.51	+698.74	4.73	0.02	1.327	260.19	4.06
2	89.39	-218.07	10.91	0.06	1.987	391.95	6.08
4	132.24	-618.87	9.32	0.05	1.817	360.69	5.56
Organic source							
UCH	77.20	+4986.81	4.90	0.19	1.05	27.24	3.85
DCH	110.32	-153.95	8.64	0.12	1.85	136.83	5.45
FYM	102.32	+27.73	11.13	0.03	1.78	621.86	5.88
UCH+FYM	112.42	-149.21	8.84	0.03	1.79	485.56	5.43
DCH+FYM	103.34	-396.18	7.83	0.03	2.17	517.02	5.57
Organic Manure (M) to topsoil (TS) ratio							
0M: 4TS	0.00	0.00	0.00	0.00	0.28	3.37	2.52
1M: 4TS	84.74	+46.86	12.98	0.06	1.43	294.47	5.75
2M: 4TS	132.97	+6.84	11.71	0.06	2.50	531.67	6.82
3M: 4TS	115.91	-221.52	8.98	0.05	2.85	568.65	6.47
4M: 4TS	98.49	+7.86	8.05	0.04	2.91	637.30	6.26

Abbreviations: ANR = Apparent Nutrient Recovery, NUE = Nutrient Use Efficiency, TNA = Total Nutrient Applied, TDM = Total dry matter, UCH = Undecomposed Coffee Husk, DCH = Decomposed Coffee Husk, FYM = Farmyard Manure.

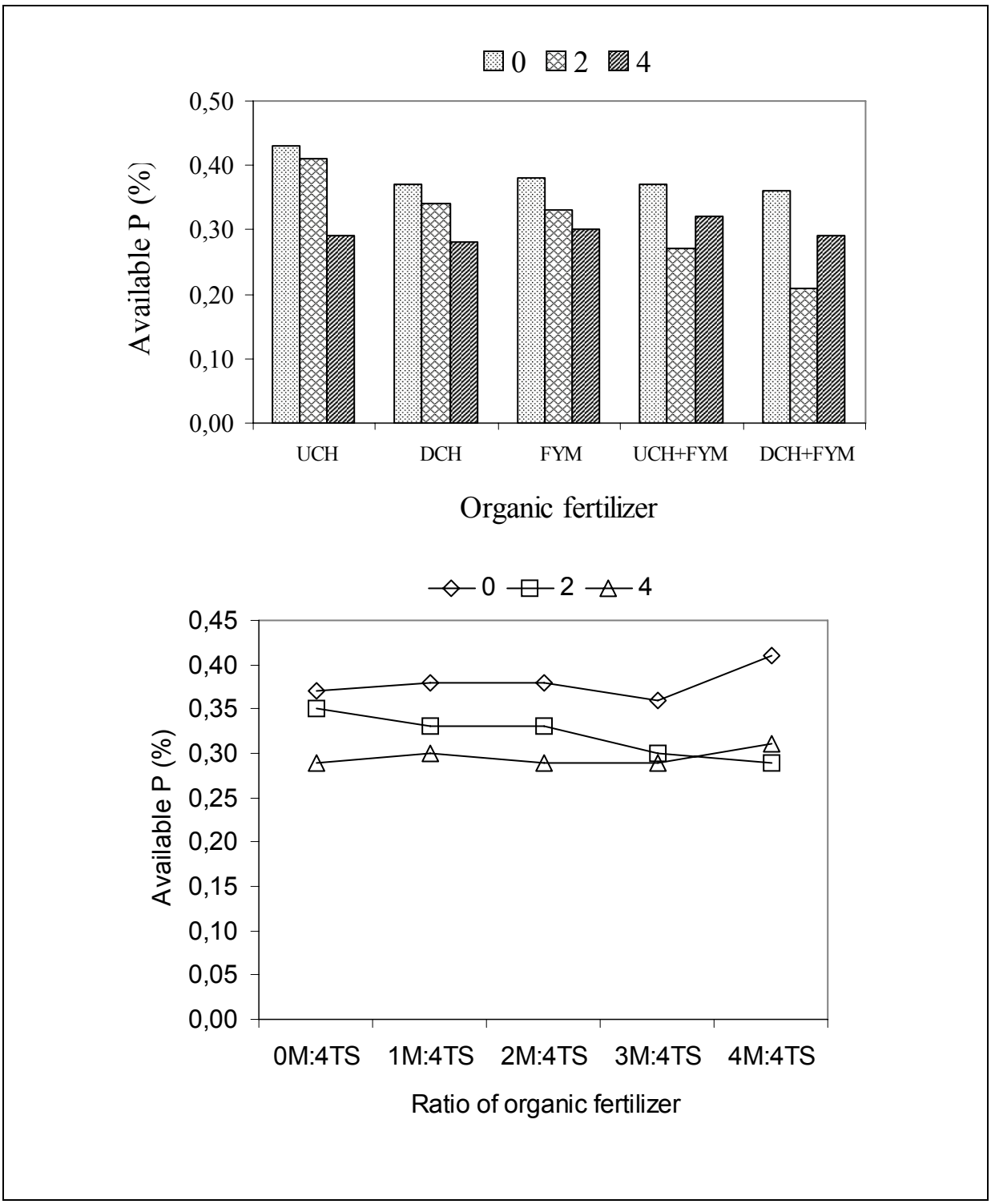


Figure 2. The status of available P in coffee leaves due to an integrated use of fertilizers (above) and application regimes (below).

In general, the present results on nutrient uptake and utilizations had a close relationship with the soil physical and chemical conditions as well as the shoot and root growth performances of coffee seedlings (Taye et al., 1999, 2003). Elsewhere, the use of inorganic resource alone has also shown to cause a damaging effect on soil nutrient balances and thus reduced crop yields (Schwab et al., 1990; Zake et al., 1996). Therefore, there is a need to focus on integrated plant nutrient management with the recognition that organic matter plays a role in the supply of major and minor plant nutrients, improvement of physical and chemical

constraints, and the enhanced efficiency of inorganic resources through reduced leaching and fixation loses, as a store-house for plant nutrients and a buffering against adverse conditions (Mesfin, 1998; Palm, 1995).

CONCLUSION

The nitrogen uptake and utilization of *Coffea arabica* seedling differ due to the integrated use of organic and inorganic fertilizers under controlled nursery conditions. The lowest total biomass yield was obtained from the control plot without use of any fertilizer sources. This suggests that the soil is acidic and poor in terms of the inherent N and P contents, requiring ameliorations. The results revealed marked variations among the organic resources in their nutrient contents where high total nitrogen and available phosphorus were obtained from coffee composts and farmyard manure, respectively, though the values varied with decomposition stages.

In contrast, available P was more fixed due to the increased ratio of decomposed organic sources. The mixed use of coffee composts and farmyard manure as potting media ingredients helped to maintain proportional plant nutrients, which reflects the possible roles of bulk farm residues to amend soil constraints for coffee production. The incorporation of DAP also significantly enhanced N and P utilizations of the seedlings. As a whole, the finding would shade a light on the integrated use of mineral and organic fertilizers at the modest rates to improve N and P use-efficiency by coffee seedlings on the problematic acid soils. Hence, the findings suggest the need to consider both plant nutrient uptake and its use-efficiency as selection criteria to evaluate the diverse Arabica coffee gene pools under different climate and soil conditions. This calls for further studies under nursery and field environments with a view to optimizing use of fertilizer resources and promote sustainable production of organic coffees.

ACKNOWLEDGEMENTS

The author would like to acknowledge the financial support from the European Union- Coffee Improvement Project (CIP-IV) to cover all expenses to participate and present this paper in the International Scientific Conference (ASIC 2008).

REFERENCES

Asefa Z. 1996. Nutritional status and optimum NP rate studies for different organic source. Pp 47-152. In: Proceedings of the 3rd conference of Ethiopian society of soil science, February 28-29, 1996, Addis Ababa, Ethiopia.

Black C.A. 1965. Methods of Soil Analysis. Part 2. Am.Soc Agron.Madison, Wisconsin, U.S.A.

Guillard K., Griffin G.F., Allinson D.W., Rafey M.R., Yamartino W.R., Pietrzyk S.W. 1995. Nitrogen utilization of selected cropping systems in the U.S. Northeast I. Dry matter yield, N uptake, apparent N recovery and use efficiency. Agron. J. (87): 193-199.

Hsieh S.C., Hsieh C.F. 1990. The use of organic matter in crop production food and fertilizer technology Centre, Extension Bulletin No. 315 November 1990, Republic of China of Taiwan. Jackson, M.L. 1958. Soil Chemical Analysis. Englewood clifs, N.J., Prentice Hall.

IAR (Institute of Agricultural Research). 1996. Recommended production technologies for coffee and associated crops. Jimma Research Center Task Force, Addis Ababa, Ethiopia.

Jama B., Swinkels R.A., Buresh R.J. 1997. Agronomic and economic evaluation of organic and inorganic source of Phosphorus in Western Kenya. Agron. J. (89): 597-604.

Janssen B.H. 1993. Integrated nutrient management: the use of organic and mineral fertilizers. Pp 89-105. In: Realer and Prins (ed.). The Role of Plant Nutrients for Sustainable Food Production in Sub-Saharan Africa, The Dutch Association of Fertilizer Producers (VKP), The Netherlands.

Jones M.J. 1971. The maintenance of soil organic matter under continuous cultivation at Samaru, Nigeria. J.of Agric. Sci.(77): 473-482.

Mburu J.K., P.K.Mwaura. 1996. Environmentally sound management of coffee processing by-products: A Review. Kenya Coffee, 61(716): 2237-2244.

Mesfin Abebe. 1998. Nature and Management of Ethiopian Soils. Alemaya University of Agriculture, ILRI, Addis Ababa, Ethiopia.

Michori P.K. 1981. Trend of coffee nutrition Research in Kenya. Kenya Coffee, 46(545):247-260.

Muller-Smann K.M., Kotschi J. 1994. Sustaining Growth: Soil Fertility Management in Tropical Smallhodings. [CTA; GTZ. Transl.:Christine Ernsting and Simon chater].

Murwira H.R. 1994. Synchrony relationships of nitrogen release and plant uptake in a Zimbabwean sandy soil amended with manure and fertilizer nitrogen. Africa Crop Science Journal, 2(1): 69-77.

Myers R.J.K., Palm C.A, Ceuvas E., Gunatilleka J.U.N., Brossand M. 1994. The synchronization of nutrient mineralization and plant nutrient. Pp 81-116. In: P.L. Woomer and P.L.Swift (Eds.). The biological management of tropical soil fertility, Nairobi, Kenya.

Njoro J.M. 1988. Effects of methane gas plant residues, cattle manure and artificial fertilizers on coffee yield and quality in Kenya. Kenya Coffee, 53(615): 255.

Ofori C.S. 1980. The use of organic materials in increasing crop production in Africa. FAO soils bulletin No 43: 121-128.

Olsen S.R., Cole C.V., Watanab F.S., Dean L.A. 1954. Estimation of available phosphorus in soils by extraction with Sodium Bicarbonate. U.S.D.A.Circ.939, Washington D.C.

Palm C.A. 1995. Characterisation of organic inputs. Pp 46-54. In: Mwenja Gichuru and Paul Woomer (Eds.). TSBF programme report No. 9, 1997, Nairobi, Kenya.

Paulos D., Moorby J. 1995. Growth response of coffee (*Coffea arabica* L.) seedlings to phosphorus nutrition in nutrient solution. PP 755-764. In: Proceedings of the 16th ASIC Colloquium, Kyoto, Japan.

Schwab A.P., Owensby C.E., Kulyingyong S. 1990. Changes in soil chemical properties due to 40 years of fertilization. Soil Science, 149(1): 35.

Sharma, R.C., Sharma, H.C. 1988. Usefulness of organic manures and their nitrogen fertilizer equivalent. J. Agri. Sci. Cambridge, (111): 193-195.

Singh N., Bhandari A.R. 1992. Relation between nutrient contents of apple leaves and nutrient status of soils. J. Indian Soc. Soil Sci. (40): 483-487.

Taye Kufa, Mesfin Abebe, Paulos Dubale. 1999. Effect of nitrogen, phosphorus and organic fertilisers on growth and developments of coffee seedlings. African Crop Science Conference Proceedings, (4): 213-223.

Taye Kufa, Mesfin Abebe, Paulos Dubale. 2003. Contribution of organic amendment to physico-chemical conditions of coffee nursery media. Pp 25-40. *In*: Tilahun Amede and Eylachew Zewdie (Eds.). Proceedings of the Sixth Conference of the Ethiopian Society of Soil Science (ESSS) on Challenges of Land Degradation to Agriculture in Ethiopia, Feb 28-March 1, 2002, Addis Ababa, Ethiopia.

Tekalign M., Haque I. 1991. Phosphorus status of some Ethiopian soils. III. Evaluation of some soil test methods for available phosphorus. Tropical Agriculture, 68(1): 51-56.

Wrigley G. 1988. Coffee. Tropical Agriculture Series, London, John Wiley and Sons, Inc., N.Y.

Zake J.Y.K., Bwamiki D.P., Nkwiine C. 1996. Potential for organic and inorganic fertilization for sustainable coffee production in Uganda. Pp. 69-74. *In:* Proceedings of IACO workshop, 4-6, September 1995, Kampala, Uganda.

Development and Evaluation of a Container with Perforated Wall in the Quality of the Coffee Seedling

V.A. SILVA*, A.D. DURANTE, L. POLI, J.M. MONTEIRO, E.C. FERREIRA

Escola Agrotécnica Federal de Muzambinho, Minas Gerais, Brazil.
*E- mail:virgilio@eafmuz.gov.br

SUMMARY

The first container used in the production of coffee seedlings had sleek walls, which promoted a winding of the roots, which did not unwind after the installation of the crop, allowing a significant fall in the productivity. In order to solve this problem, the grooved wall container was created, which makes the root grow vertically in compact blocks. In spite of the non-winding, this compact and vertical form of the roots tends to remain this way after the definite planting and also with possible damages to the productivity. The present work, accomplished at the Campus of the EAFMuz (Escola Agrotécnica Federal de Muzambinho) has as objective, the development and evaluation of the effects of a container with wall in mesh form in the architecture of the coffee seedling root system. The results of the statistical analysis revealed that the dry matter of the root system of the seedlings produced in the grooved container was significantly greater. However, the same did not happen with the analysis of the shoot, without a significant difference. Visually comparing the root systems, it can be concluded that the one originated from the mesh container seedling presented a closer architecture to that considered ideal.

INTRODUCTION

Because the coffee plant is a perennial plant, it has in the seedling formation one of the most important stages in the determination of the productive and enduring success of the culture. The first container used as a recipient in the production of coffee plant seedlings had sleek walls (Figure 1), which promoted a winding of the roots (Figure 2. Parviainen, 1981), being that the spiral growth of the roots continues after planting allowing the promotion of a low stability of the future plants (Schimidt-Vogt, 1984). To solve this problem, a grooved wall container was created (Figure 3), whose format makes the root system grow in vertical compact blocks (Figure 4), preventing the winding or the spiral form growth inside the container (Guimarães et al., 1998).

Figure 1.

Figure 2.

Figure 3.

Figure 4.

OBJECTIVE

The present work, accomplished at the Campus of the EAFMuz (Escola Agrotécnica Federal de Muzambinho) has as objective, the development and evaluation of the effects of a container with wall in mesh form (Figure 5), in the architecture of the coffee seedling root system.

Figure 5.

MATERIALS AND METHODS

Due to the unavailability of container of similar kinds to the idealized in the trade, a handicraft model was used with a 1 mm nylon mesh, adapted to the extremities of a PVC container (Figure 6). Fifty-four perforated wall containers with 120 ml in volume were manufactured to be compared with other fifty-four grooved wall containers, which were distributed in four appropriate trays (Figure 7), to support the referred containers. It was considered that, as soon as the roots reached the wall of the container, they would neither suffer winding such as the one promoted by the container of sleek wall nor deformities caused by the container of grooved wall. Therefore, they would tend to escape through the holes (Figure 8) and they would suffer a natural pruning by the oxygen (Marchi, 2002).

Figure 6.

Figure 7.

Figure 8.

EVALUATION

When the seedlings reached 4 pairs of definite leaves, 24 plants of each treatment were evaluated, being considered the following features: a) root system dry matter; b) shoot dry matter; c) root system architecture.

RESULTS AND DISCUSSION

The results of the statistical analysis revealed that the dry matter of the root system of the seedlings produced in the grooved container was significantly greater. However, the same did not happen with the analysis of the shoot, without a significant difference. Visually comparing the root systems, it can be concluded that the one originated from the mesh container seedling (Fig.9) presented a closer architecture to that considered ideal. The verified means of dry matter of the root system and the shoot are presented in Table 1.

Table 1. Mean values of the features of the coffee plant seedlings in regards to two types of containers.

Container	MAPA	MSSR
Grooved	20,29 a	17,23 a
Mesh	19,71 a	12,05 b

Means followed by the same letter do not differ statistically between each other, according to the SAS test at 5% level of significance.

It can be verified in Table 1, that the MSSR of the seedling produced in the mesh container was significantly lower, which agrees with Marchi (2002), who reports the effect of the natural pruning of the roots when they go out of the holes in the container.

Mesh form - Grooved wall - Sleek wall

Figure 9.

CONCLUSION

The results show the viability of the perforated wall container in mesh form in the production of coffee seedlings, as well as the adequation of the root system. However, new experiments should be performed, including the evaluation of the seedlings in the field.

REFERENCES

Guimarães, P.T.; Andrade Neto, A. De; Bellini Junior, O.; Adão, W. A.; Silva, E.M. Produção de mudas de cafeeiro em tubetes. Informe Agropecuário, Belo Horizonte, n.193, p. 98-108, 1998.

Marchi, E.C.S. Sobrevivência de mudas de cafeeiro (*Coffea arabica L.*) no pós-plantio. Em função do recipiente, época e classe de solo no sistema convencional e plantio direto. 2002, 80 p. Dissertação (Mestrado)-Universidade Federal de Lavras, Lavras, MG.

Parviainem, J.O. desenvolvimento radicular das mudas florestais no viveiro e no local de plantio. In: Seminário de sementes e viveiros florestais, 1, 1981, Curitiba1981. Anais... Curitiba: FUPEF, 1981, v.2, p.111-130.

Shimidt-Vogt, H. Morpho-physiological quality of forest tree seedlings: the present international status of research. In: Simpósio internacional: métodos de produção e controle de qualidade de sementes e mudas florestais, 1984, Curitiba. Anais... Curitiba: FUPEF, 1984, p.366-378.

Effect of Plant Density and Pruning on Yield of Compact Disease Resistant Coffee Variety

D.A. ODENY AND J.K. KIMEMIA

Coffee Research Foundation, P.O. Box 4-00232, Ruiru, Kenya
E-mail: director@crf.co.ke, odeny.dan@crf.co.ke

SUMMARY

A study was undertaken to determine the effect of plant density and pruning management on yields of hybrid, compact and disease resistant variety of Arabica coffee cv Ruiru 11. The study was carried out from 1988 to 1998. The treatments consisted of five planting densities; 1600, 2400, 3200, 4000 and 4800 trees/ha and four pruning regimes. Results showed that the clean coffee yields increased with increase in tree density and number of stems per stump. It was concluded that it was possible to grow Ruiru 11 at densities beyond 5000 trees/ha.

INTRODUCTION

Spacing and pruning are two important aspects of coffee management. Coffee trees are often prone to biennial bearing pattern where a high production year often alternates with a year of low production. In order to ensure consistent and sustainable production, proper pruning management of coffee is necessary. Coffee may be trained to leave either a single stem or multiple (three to four stems). The precise pruning system depends on the cultivar and the growing conditions. In Kenya, the single stem was preferred but most growers now use multiple stem system in which the stems are replaced at intervals of five to seven years (Griffiths and Waller, 1971). Available knowledge is based on the taller traditional varieties which are also highly susceptible to Coffee Berry Disease (CBD) and Coffee Leaf Rust (CLR). There was need, therefore, to understand the planting density and pruning management of the hybrid variety since it is compact and resistant to CBD and CLR indicating that it can be planted at higher populations. The optimum tree density gives individual coffee trees opportunity to utilize resources and least competition with adjacent trees for growth and yield (Talopa and Kiara, 1999).

MATERIALS AND METHODS

The studies were conducted at 4 sites the Coffee Research Station (CRS) located in Ruiru (1^o 06'S, 36^o 45'E, 1620 m) and the sub-stations in Kisii (0^o 41'S, 34^o 47'E, 1700 m), Koru (0^o 07'S, 35^o 16'E, 1554 m) and Mariene (0^o 00, 37^o 39E, 1631 m). The four sites are representative of the areas where coffee is grown in Kenya. The coffee was established and managed as outlined by Mwangi (1983), except for the plant spacing and pruning.

The treatments consisted of five planting densities; 1600, 2400, 3200, 4000 and 4800 trees/ha and four pruning regimes (1 - One stem throughout, 2 - One stem in first cycle followed by two stems in second cycle, 3 - Two stems throughout, 4 - Two stems in the first cycle followed by three stems in the second cycle).

The treatments were laid out in factorial design and replicated three times. The experimental plots consisted of 30 trees with the 12 middle trees as the effective trees for data collection.

Six months after planting, the seedlings were capped to raise the required stems for the treatments that had more than one stem.

RESULTS

The results showed that coffee yield increased significantly with increase in tree density in all sites. The yield also increased with increase in number of stems per stump in all the sites, except CRS, Ruiru (Tables 1a-d). The results indicate possibility for growing coffee beyond 4800 trees/ha (Figure 1). On average, Kisii had the highest clean coffee yield, 3004 kg/ha, followed by Koru 2788 kg/ha, Ruiru 1319 kg/ha and Mariene 993 kg/ha. The effect of pruning was more evident in Kisii and Koru, but less so in Mariene, in Ruiru however, the effect was not significant (Figure 2).

Table 1. Effect of plant density and pruning system on clean coffee yield (Kg/ha).

a) Kisii

Pruning system	**Plant density trees/ha**					
	1600	**2400**	**3200**	**4000**	**4800**	**Mean**
1	1345	1941	2823	3295	3695	**2620 b**
2	1700	2499	2778	3564	3943	**2897 ab**
3	1933	2780	2908	3558	3904	**3017 ab**
4	1959	3730	3716	4100	3908	**3482 a**
Mean	**1734 d**	**2737 c**	**3056 bc**	**3629 ab**	**3862 a**	**3004**
CV %	23.4					

b) Koru

Pruning system	**Plant density trees/ha**					
	1600	**2400**	**3200**	**4000**	**4800**	**Mean**
1	1292	1732	2220	3131	3134	**2302 b**
2	1319	2236	2435	3220	3487	**2539 b**
3	1515	2379	2846	3044	3826	**2722 b**
4	2298	2530	3671	5276	4176	**3590a**
Mean	**1606 c**	**2220 bc**	**2793 b**	**3668 a**	**3656 a**	**2788**
CV %	24.2					

c) Mariene

Pruning system	**Plant density trees/ha**					
	1600	**2400**	**3200**	**4000**	**4800**	**Mean**
1	472	781	839	1052	1268	**882 b**
2	581	690	926	1026	1466	**938 ab**
3	627	825	1281	1152	1101	**997 ab**
4	741	1119	924	1212	1783	**1156 a**
Mean	**605 d**	**854 c**	**993 bc**	**1110 b**	**1405 a**	**993**
CV %	23.4					

d) CRS, Ruiru

Pruning system	**Plant density trees/ha**					
	1600	**2400**	**3200**	**4000**	**4800**	**Mean**
1	767	1069	1541	1323	1600	1260 a
2	922	1252	1612	1563	1587	1387 a
3	989	1044	1280	1516	1638	1293 a
4	894	1147	1610	1427	1592	1334 a
Mean	**893 b**	**1128 b**	**1511 a**	**1457 a**	**1604 a**	**1319**
CV %	14.9					

Means followed by similar letter (s) are not significantly different according to Duncan's Multiple Range Test (p = 0.05).

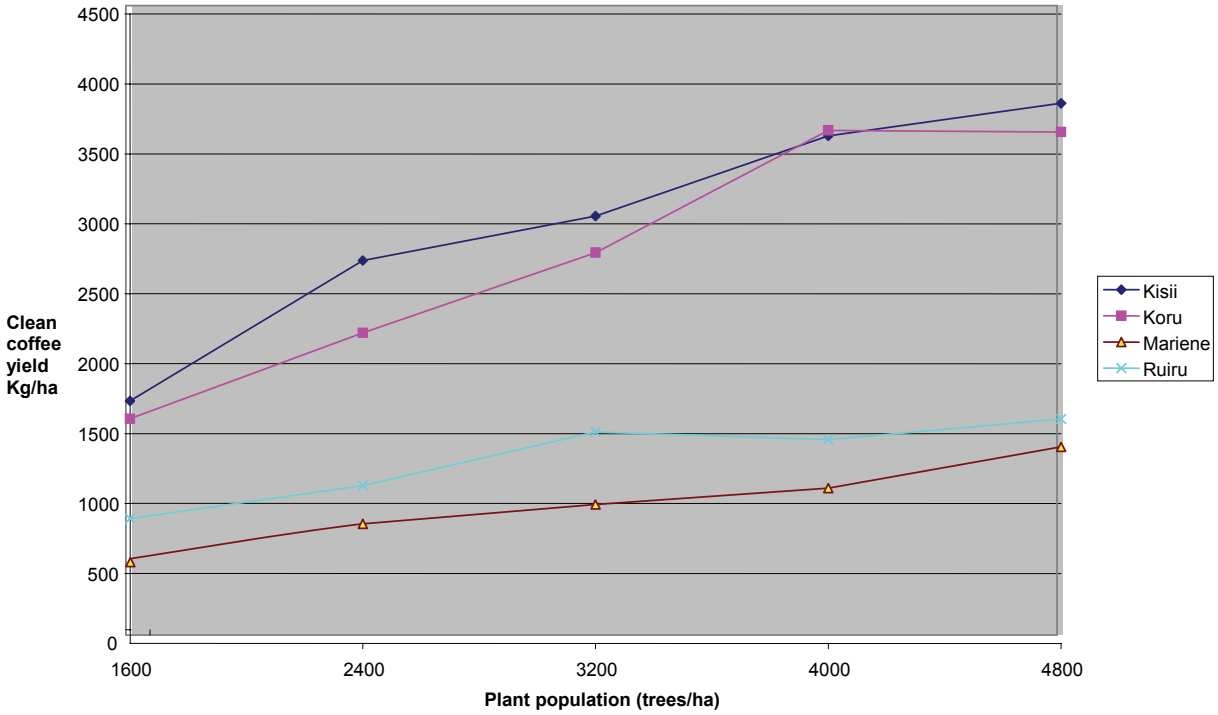


Figure 1. Effect of plant density on clean coffee yield (Kg/ha).

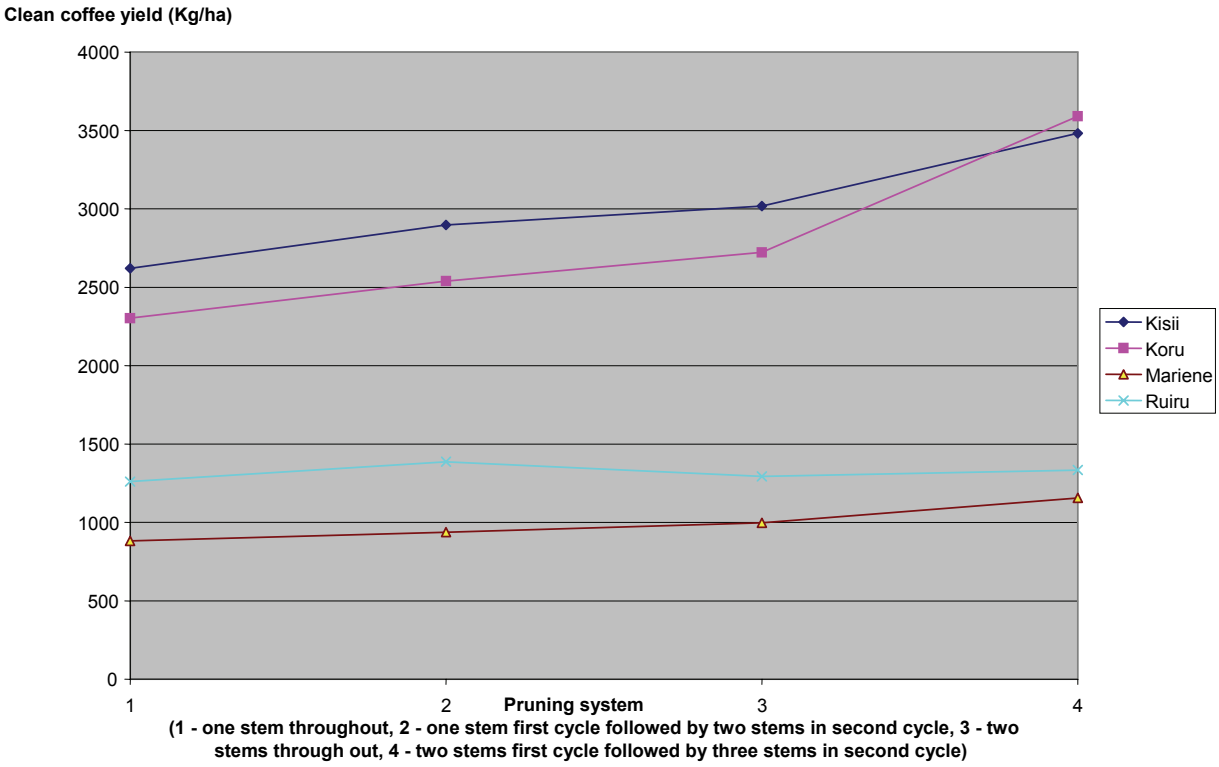


Figure 2. Effect of pruning system on clean coffee yield (Kg/ha).

DISCUSSION

A wide variety of conventional spacing, for traditional coffee varieties, exists in Kenya. The most widely adopted is the 2.74 x 2.74 m and 2.74 x 1.37 m giving densities of 1329 to 2660 trees per ha respectively (Mitchell, 1976; Wilson, 1985). The conventional spacing has, however, been considered below optimum. The limitation to higher density planting with regard to traditional varieties has been their susceptibility to disease such as Coffee berry disease (CBD) and coffee leaf rust (CLR).

With the higher plant densities comes the challenge of developing an appropriate pruning system. The results showed that in Kisii and Koru the highest yields were recorded at 4000 trees/ha where two stems in the first cycle were followed by three in the second cycle. Kiara (1981), however, recommended that high-density coffee should be trained on one or two stems per stump and stumped after three years.

The study showed that yields increased with increase in tree density at all the sites. Similar results have been observed for other arabica coffee varieties (Kiara and Stolzy, 1986) and Catimor (Gathaara and Kiara, 1990). The development of hybrid, compact and disease resistant variety of Arabica coffee cv Ruiru 11 presents the possibility of growing coffee at higher densities.

Kisii and Koru which have a unimodal rainfall pattern received 2070 mm and 1730 mm of rainfall respectively. Mariene and CRS, Ruiru on the other hand which have a bimodal pattern (two distinct rainfall seasons), received 1438mm and 1063mm of rain respectively. Further study would require to be carried out beyond 4800 trees/ha especially in high rainfall areas such as Koru and Kisii where the yields appeared on upward trend.

ACKNOWLEDGEMENT

The assistance of the staff of Agronomy department is hereby acknowledged. This paper is published with the permission of the Director of Research, Coffee Research Foundation.

REFERENCES

Gathaara M.P.H. and Kiara J.M. (1990). Density and fertilizer requirements of the compact and disease resistant Arabica coffee. Kenya Coffee 55: 907-910.

Griffiths E. and Waller J.M.1971. Rainfall and cropping patterns in relation to coffee berry disease. Annals of Applied Biology 67: 75-91.

Kiara J.M. 1981. Yield response to various pruning methods in hedgerow spaced coffee in Kenya. *Kenya Coffee* 47, 559: 247-257.

Kiara, J.M. and Stolzy, L.H. 1986. The effects of tree densities and irrigation on coffee growth and production in Kenya. Kenya Coffee 51: 259-263.

Mitchell H.W. 1976. Research on close-spacing systems for Intensive Coffee Production in Kenya. Annual Report 1974/75. Coffee Research Foundation. Pp 13-58.

Mwangi C.N. 1983. Coffee Growers Handbook. Coffee Research Foundation.

Talopa P. and Kiara J.M. 1999. Coffee Pruning and spacing – Management of tall Arabica Coffee (*Coffea arabica* L.) in Papua New Guinea. ASIC, 18e colloque, Helsinki, 1999.

Wilson K.C. 1985. Cultural methods. In: Coffee: Botany, biochemistry and production of beans and beverage, 7-207 (Ed. Clifford, M.M. and Wilson, K.C.) Avi Publishing Company INC. Westport, Connecticut.

Advances in Coffee Nursery Management in Ethiopia

A. NETSERE*, E. TAYE, T. KUFA, T. SHIMBER

Jimma Agricultural Research Center, P. 0. Box 192, Jimma, Ethiopia
*E-mail: anetsere@yahoo.com

SUMMARY

An investigations were carried out at Jima Agricultural Research Center, Ethiopia, to develop improved nursery management practices that promote and sustain the production of healthy and vigorous Arabica coffee seedlings. Research results depicted that forest soil or a mixture of topsoil (TS), compost and sand (S) in 3 : 1: 0 and 2 : 1 : 1 ratios or blends of manure (M) and TS in 1 : 4, 2 : 4 and 3 : 4 ratios resulted in vigorous growth of coffee seedlings. Applying 750 mg P or a combination of 2.31 g lime and 250 mg P pot^{-1} (each pot filled with 2.5 kg sieved TS) ensured the production of quality seedlings. Sowing coffee seeds at a depth of 1 cm with the grooved side placed down and embryo tip up had improved germination. Seedbeds covered with 3 to 5 cm thick mulch after seed sowing and watered at 2 days interval until hypocotyl emergence had higher germination percentage. After emergence, with the removal of mulch, nursery beds provided with 50% over head shade and irrigated twice and once at a week interval until seedlings attained 2 to 4 pairs of leaves and there after, respectively, produced vigorous seedlings. Soft wood single node cuttings with a pair of leaf taken from orthotropic shoots and blends of TS, S and M in 2 : 2 : 1 ratio were recommended for vegetative propagation of hybrid coffee.

INTRODUCTION

Despite the existence of enormous genetic diversity of Arabica coffee in Ethiopia and its importance in the country's economy, the productivity of the crop is very low (0.66 ton ha^{-1} green coffee) (Central Statistical Agency, CSA, 2006). Such a low level of productivity of the crop stems from, *inter alias*, the uses of weak and whippy seedlings with undesirable shoot and root growth for field planting. This emanates mainly from use of growing media not suitable for germination and seedling growth, and inadequate or excessive shading and watering during the nursery period (Yacob et al., 1996; Tesfaye et al., 2006).

In view of this, several nursery management practices. Have been tested aiming at production of healthy and vigorous coffee seedlings in the country. Thus, available research findings and techniques generated hitherto in the aforementioned areas within the country are reviewed and discussed in this paper.

RESEARCH FINDINGS

Nursery Media

Coffee seedlings can be grown on raised conventional beds (15 cm height) or in polythene pots (10 to 12 cm in diameter and 22 to 25 cm height) filled with forest soil (FS) collected from the top 5 to10 cm depth. However, in the absence of forest soil it was recommended to use blends of topsoil (TS), and compost (C) only or TS, C and sand (S) following the order 3TS : 1C : 0S > 2TS :1C 1S > 2TS : 1C : 0S > 6TS : 3C : 2S (Fig. 1). Likewise, results

showed that a mixture of locally available manure and TS in 1 : 4, 2 : 4 and 3 : 4 ratios had promoted both shoot and root growth of coffee seedlings (Table 1). However, if these media blends are suspected to be low in plant nutrients, addition of 2 g of DAP per seedling or per pot after the stage of two pairs of true leaves would help improve growth of the seedlings (Table 2).

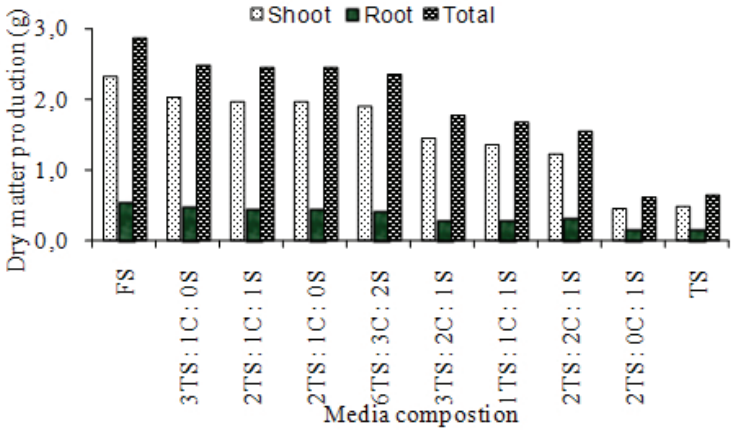


Figure 1. Effect of different media composition on dry matter yield of coffee seedlings. (Source: Taye et al., 2002)

Table 1. Effect of mixture of manure and topsoil in different ratio on growth of coffee seedlings.

Ratio of manure and topsoil	Plant height (cm)	Girth (cm)	Leaf			Shoot* dry matter (g)	Root dry matter (g)
			Number	Area (cm^{-2})	Dry matter (g)		
0 : 4	16.348^{a}	0.337^{c}	10.698^{b}	9.833^{b}	1.161^{c}	0.661^{b}	0.692^{b}
1 : 4	28.571^{b}	0.443^{a}	21.435^{a}	12.770^{a}	2.744^{b}	1.804^{a}	1.219^{a}
2 : 4	28.401^{ab}	0.424^{ab}	21.622^{a}	12.839^{a}	2.899^{ab}	1.774^{a}	1.186^{a}
3 : 4	29.049^{a}	0.442^{a}	22.834^{a}	13.022^{a}	3.096^{a}	1.998^{a}	1.161^{a}
4 : 4	26.947^{b}	0.413^{b}	21.651^{a}	12.628^{a}	2.824^{ab}	1.697^{a}	1.099^{a}

**Shoot = Stem + Branch. Figures followed by same superscript letters within a column are not significantly different at 0.01 probability level. (Source: Taye et al., 1999).*

Table 2. Effect of DAP on growth of coffee seedlings.

Rate of DAP (g/pot)	Leaf			Shoot dry matter (g)	Root dry matter (g)
	Number	Area (cm^{-2})	Dry matter (g)		
0	11.363^{b}	11.363^{b}	2.029^{b}	1.189^{b}	0.802^{b}
2	12.785^{a}	12.785^{a}	2.867^{a}	6.854^{a}	1.229^{a}
4	$12.^{517a}$	12.517^{a}	2.783^{a}	5.961^{a}	1.183^{a}

Figures followed by same superscript letters within a column are not significantly different at 0.05 probability level. (Source: Taye et al., 1999).

Media Amendment

It has been reported that the application 750 mg of P followed by a combination of 2.31 g lime and 250 mg P pot^{-1} (each pot filled with 2.5 kg sieved topsoil) produced seedlings with

higher dry matter yield (Figure 2). This was primarily attributed to the rise in soil pH and precipitation of the exchangeable Al that fixes P and increase in solubility and availability of soil P to the seedlings.

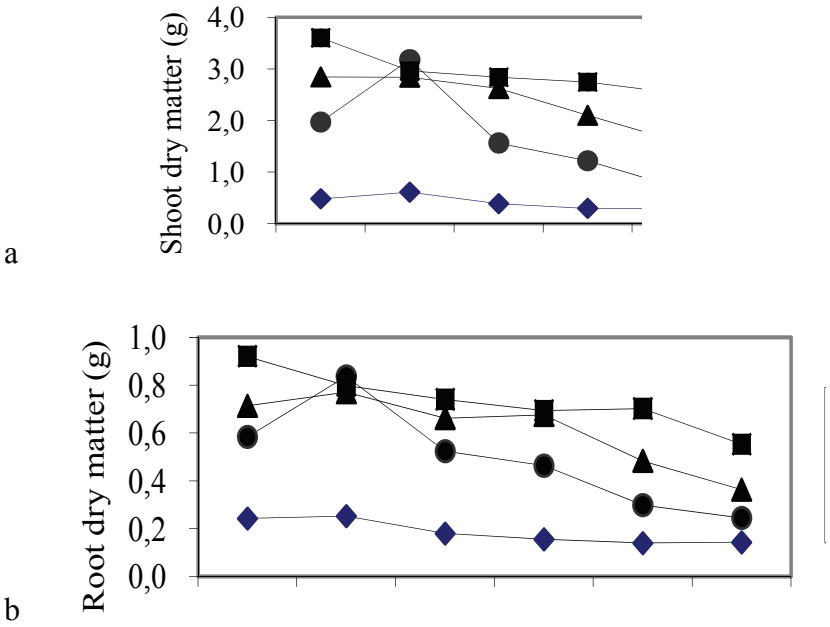


Figure 2. Effects of lime and P rates on shoot (a) and root (b) dry matter of coffee seedlings. (Source: Anteneh and Heluf, 2007).

Seed Sowing

For maximum germination, coffee seeds should be sown at a depth of 1 cm with grooved side of the seed down and the embryo tip up. However, seed germination rapidly declines with sowing depth > 1 cm (Table 3).

Table 3. Effect of sowing depth and position of seeds on percent germination of coffee seeds.

Sowing depth (cm)	Seed position				**Mean**
	Grooved side		Embryo tip		
	up	down	up	down	
0	80.1	80.9	76.2	85.7	**80.7**
1	80.9	85.7	90.7	69.0	**81.6**
2	64.3	69.1	69.0	69.0	**67.9**
5	23.8	23.8	28.6	26.2	**25.7**
Mean	**62.3**	**64.9**	**66.1**	**62.5**	

Source: Yacob (1986).

Mulching Seedbeds

Seedbeds covered with 3 to 5 cm thick mulches of straw or other dried plant materials, such as grass, banana or enset leaves, immediately after sowing have resulted in significantly higher germination percentage than did mulch plus shade and shade alone (Fig. 3). Relatively higher germination response to mulch alone could be ascribed to regulation of diurnal temperature in the nursery bed which ensued from its insulating nature against fluctuations of

soil temperature. However, the mulch should be removed and replaced by moderate (50%) overhead shade when the seedlings start to emerge (Tesfaye et al., 2006).

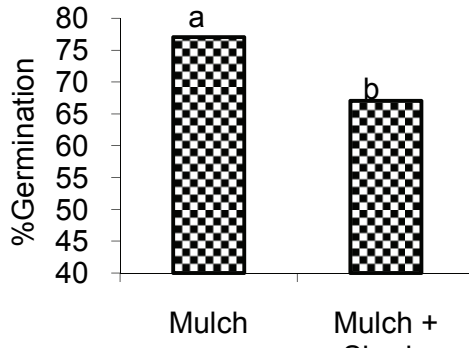


Figure 3. Germination percentage of coffee seeds as affected by mulch, shade and their combination. Bars capped with same letter are not significantly different at 0.05 probability level. (Source: Yacob, 1986).

Watering Seedbeds

It was observed that coffee seed beds covered with 3 to 5 cm thick mulch need to be watered at 2 days interval until seedling emergence during the dry season. After emergence, with the removal of mulch and provision of moderate overhead shade, watering seedbeds twice a week until seedlings produce 2 to 4 pairs of true leaves and there after at a week interval resulted in vigorous seedlings (Table Tesfaye et al., 2006).

Overhead Shade

Provision of moderate level of over head shade (25 to 75%, but 50% is the best) to coffee seedlings up on emergence and removal of mulch resulted in vigorous seedling growth with the highest total dry matter (TDM) (shoot + root) yield and relative water content (RWC) and improved moisture content of the rooting medium (MCM) (Table 4). To harden the seedlings, it is recommended that the watering frequency and the shade level should gradually be reduced one month a head of transplanting to the field at a stage of six to eight pairs of true leaves (Tesfaye et al., 2006).

.

Table 4. Effect of shade level on growth and RWC of seedlings and moisture content of the MMC.

Shade level (%)	Height (cm)	Leaf number	TDM (g)	RWC (%)	MCM (% by volume)
0	24.7^{c}	14.4^{c}	5.3^{c}	64.9^{b}	14.6^{b}
25	28.5^{b}	15.3^{b}	6.1^{b}	68.0^{a}	21.7^{a}
50	30.0^{a}	17.0^{a}	6.6^{a}	68.5^{a}	22.8^{a}
75	30.2^{a}	15.1^{b}	6.1^{b}	68.9^{a}	23.5^{a}

*Figures followed by same superscript letters within a column are not significantly different at 0.01 probability level. (Source: Yacob et al., 1996)***.**

Vegetative Propagation

Research results in mist propagation at Jimma showed that a combination of single node soft wood cuttings with one pair of leaves taken from orthotropic shoot and rooting media composed of top soil, sand and manure in 2 : 2 : 1 ratio was recommended for vegetative propagation of hybrid coffee varieties. It was observed that this practice resulted in the highest rooting (89.2%) and survival rate (63.3%) of cuttings at hardening off stage (Behailu *et al*., 2006). Van der Vossen and Op de lack (1976) also reported similar results on rooting cuttings of Riru coffee in Kenya.

CONCLUSION AND RECOMMENDATIONS

For maximum germination and seedling growth, coffee seeds should be sown in forest soil to a depth of 1 cm with the grooved side of the seed placed down. However, in the absence of forest soil divers type of alternative potting media with ideal physical and chemical properties like, forest soil, can be prepared by blending locally available manure (M) and topsoil (TS) or compost (C), TS and sand (S) in various proportions. Phosphorus at a rate of 750 mg pot^{-1} or a combination of 2.31 g lime and 250 mg P pot^{-1} is also recommended for growing Arabica coffee seedlings at Jimma. In the absence of facilities for micro-propagation using tissue culture, the practice of planting soft wood single node cuttings with a pair of leaf taken from orthotropic shoots in pots filled with a mixture of TS, S and M in 2 : 2 : 1 ratio should be exploited for multiplication of hybrid coffee varieties using mist-propagator.

After sowing, seedbeds should be covered with 3 to 5 cm thick mulch (straw or other dried plant materials) and watered at two days interval until seedling emergence. The mulch should be removed when the seedlings start to emerge. After emergence, the nursery beds should be provided with moderate (50%) shade and the watering frequency also are reduced to twice and once a week until the seedlings attain 2 to 4 pairs of true leaves and thereafter, respectively. However, both watering frequency and shade level should gradually be reduced a month before transplanting the seedlings to the field at the stage of six to eight pairs of true leaves.

REFERENCES

Anteneh Netsere and Heluf Geberekidane, 2007. Response of Arabica coffee seedling to lime and phosphorus: II. Dry matter production and distribution. p. 1095-1100. In: Int. Conf. Coffee Science, 21st , Montpellier, 11th-15th September 2007. ASIC, France.

Behailu Atero, Gibramu Temesegen and Bayetta Belachew, 2004. Effect of type of cutting and media mixture on rooting ability of Arabica coffee hybrid. p. 135-138. In: Proceedings of the Eleventh Conference of the Crop Science Society of Ethiopia, 26-28 April 2004, Addis Ababa, Ethiopia. Sebil Vol. 11.

Taye Kufa, Mesfin Abebe and Paulos Dubale, 1999. Effect of nitrogen, phosphorus and organic fertilizer on growth and development of coffee seedlings. p. 213-223. In: Proceedings of African Crop Science Conference. African Crop Science Society, Vol. 4, Kampala, Uganda.

Taye Kufa, Tesfaye Shimber and Alemseged Yilma, 2002. Influence of media mixture and watering frequency on seed germination and seedling growth of Arabica coffee in Ethiopia. *In* Int. Conf. Coffee Science, 19th, Trieste, 14-18 May 2001. ASIC, Italy.

Tesfaye Shimber, Alemseged Yilma, Taye Kufa, Endale Taye and Anteneh Netsere, 2006. "Coffee seedlings management and production." Hand Book Amharic version, Ethiopian Agricultural Research Organization, Addis Ababa, Ethiopia. 17pp.

Yacob Edjamo, 1986. Review of coffee nursery trial at Melko. Paper presented on the First National Coffee Symposium in Ethiopia, 21-26 August 1986, Addis Ababa, Ethiopia.

Yacob Edjamo, Tesfaye Shimber, Alemseged Yilma, Anteneh Netsere, Takele Negewo, Mohammednur Abachebsa and Bekele Bogale, 1996. Advances in coffee agronomy research in Ethiopia. p. 40-45. In: Proceedings of Inter Africa Coffee organization (IACO) Workshop, 4-6 September 1995, Kampala, Uganda.

Regulating Quality of Shade Tree Mulch Into Soil to Control Nitrification in a Coffee Agroforestry

JOHN BAKO BAON[1] AND PURWANTO[2]

[1]Indonesian Coffee and Cocoa Research Institute, Jl. P.B. Sudirman 90, Jember, Indonesia
[2]Agriculture Faculty, Sebelas Maret University, Surakarta, Indonesia

SUMMARY

The objective of this study was to investigate the strategy in controlling nitrification biologically by manipulating quantity and quality of shade trees mulch input into the soil. First experiment used mulch of avocado, coffee and gliricidia, represented, respectively, low moderate and high quality mulch. Quantity factor tested consisted of three levels (0, 10 and 30 Mg ha^{-1}). In second experiment, combination of those mulches was used with composition of 25%, 50% and 75% in the same quantity of 30 Mg ha^{-1}. Results of this study showed that application of low quality of mulch resulted in low NH_4^+ and NO_3^- release and low soil potential nitrification. Higher the ratio of L+P)/N content in mulch lower the nitrification activity in soil, which suggest that application of mulch with high ratio of (L+P)/N may inhibit activity of nitrification in soil. Application of single kind mulch tended to mobilize N soil, whereas mixed mulches resulted in mineralization of N. There was significant difference between combination treatments of mulch on concentration of soil NH_4^+ and NO_3^- produced. There was very close positive relationship between soil NH_4^+ concentration and potential nitrification, NH_4^+ oxidizing bacteria population and NO_2^- oxidizing bacteria population. Those results indicate that by regulating the quality of organic matter input to soil, rate of nitrification can be controlled.

INTRODUCTION

Recently, as world coffee price was not too promising; farmers try to increase their income by planting more economic value trees as shade trees in their coffee farms, such as: avocado (*Persea americana)*, banana (*Musa paradisiaca),* or *Carica papaya* (Nur et al., 2003). Variety of shade tree species would produce different kind, quantity and quality of standing litters. High quality litter has ratio of C/N < 20 (Handayanto, 1994), or ratio of (lignin+polifenol)/N < 10 (Hairiah, 2005). More diversified tree species in mixed coffee agro-forestry was predicted to reduce soil potential nitrification, thus nitrification rate could be reduced by decreasing the quality of litter input. Therefore, researches on potential nitrification in coffee agro-forestry systems using various shade trees are still needed to investigate the strategy to increase use efficiency of N. Objective of this study is to investigate the ability of mulch of various shade trees of coffee to control nitrification by combining quality and dosage of mulches.

MATERIALS AND METHODS

A glass house experiment was carried out in an incubation house size of 2.8 m X 4.7 m where humidity and temperature were relatively constant. Soil material for this experiment was an Inceptisol. Mulch used were prunned materials of gliricidia (*Gliricidia sepium)* represent high quality, avocado represent low quality mulch, and coffee represent moderate quality (Table 1).

Table 1. Chemical characteristics of several mulches used in this study.

Mulch kind	Mulch quality				
	Lignin (%)	Poliphenol (%)	C/N	(L+P)/N	Quality level
Avocado (A)	14.7	34,7	25	31.3	Low
Gliricidia (G)	32	1.12	16.53	10,35	High
Coffee (K)	13.5	6.18	11	7.46	Moderate

Notes: C = carbon, N = nitrogen, L = lignin, P = polifenol. High quality litter when C/N < 25, lignin content <15% and poliphenol < 3% (Palm and Sanchez, 1991).

Treatments were laid in completely randomized design replicated three times. First factor was mulch kind, viz. avocado, gliricidia and coffee litters. Second factor was mulch dosage, vis. No, 10 and 30 Mg ha^{-1}. In the second experiment, the first factor was similar to the First Experiment with dosage of mulch 30 Mg ha^{-1} consist of mixture composition of mulch with ratio of 25:75; 50:50 and 75:25. Soil was taken from the depth of 0-20 cm in coffee garden planted 17 years ago. Dry mulch was mixed with 300 air dried soil before putting into pots. Urea as nitrification substrate was added at the first day together with mulch. Moisture content was maintained at field capacity by adding sterile free mineral water daily, whereas the leachate was collected and put back into its pot. Parameters observed were concentrations of NH_4^+, NO_3^-, potential nitrification, population of nitrifiers and heterotroph microbes.

RESULTS AND DISCUSSION

Addition of mulch resulted in immobilization of NH_4^+. Mulch of gliricidia decomposed more quickly and produced more NH_4^+ than other mulches (Figure 1). Based on result pattern of net-ammonification, shows that varying composition of mulch coffee with avocado gliricidia mulches may slow down ammonification process. Addition of single mulch type caused immobilization of N mineral ($NH_4^+ + NO_3^+$) until sixth week. At tenth week, high dosage of gliricidia and coffee increased mineral-N, whereas at avocado immobilization of N still occured. Mixture of mulches influence the pattern of net N-mineral released. Addition of single mulch type tended resulted in immobilization, while for mixture tended to balance immobilization, ammonification and nitrification. Sixth week was the shifting time between mineralization and immobilization. There is a close relationship between mulch quality {(L+P)/N and polyphenol content} and adanya potential nitrification. High value of (L+P)/N and polyphenol content of mulch tended to decrease N-mineral content and potential nitrification (Table 2). Result of diagram analysis showed that avocado mulch inhibit more than gliricidia or coffee mulch. There was a very close positive correlation between concentration of soil NH_4^+ with concentration soil NO_3^- and potential nitrification. Avocado mulch had direct negative effect on NH_4^+ oxidiser population and indirect negative effect on potential nitrification. Heterotroph bacteria population had negative indirect effect on potential nitrification through competition on the use of NH_4^+ with NH_4^+ oxiding bacteria (Table 3). Compared with control, application of avocado mulch resulted in depression of NO_3^- release, while gliricidia mulch formed high amount of NO_3^- to soil. This indicates that quality of mulch may potentially control nitrification process.

Table 2. Matrix of correlation between soil characteristics and content of NH_4^+, NO_3^- and potential nitrification.

	Soil NH_4^+ concentration		Soil NO_3^- concentration		Potential Nitrification	
	r	P	R	P	r	P
Soil NH_4^+ concentration			0.509**	0.000	0.468**	0.000
Soil NO_3^- concentration	0.509**	0.000			0.238*	0.014
Potential nitrification	0.468**	0.000	0.238**	0.014		
NH_4^+ oxidizing bacteria population	0.482**	0.000	0.376**	0.000	0.507*	0.000
NO_2^- oxidizing bacteria population	0.387**	0.000	0.474**	0.000	0.274**	.005
Bacteria population	-0.194*	0.048	-0.498**	0.000	-0.134	0.172
Fungi population	-0.202*	0.039	-0.431**	0.000	-0.167	0.089
Actinomycetes population	-0.122	0.213	-0.256**	0.008	-0.199*	0.042

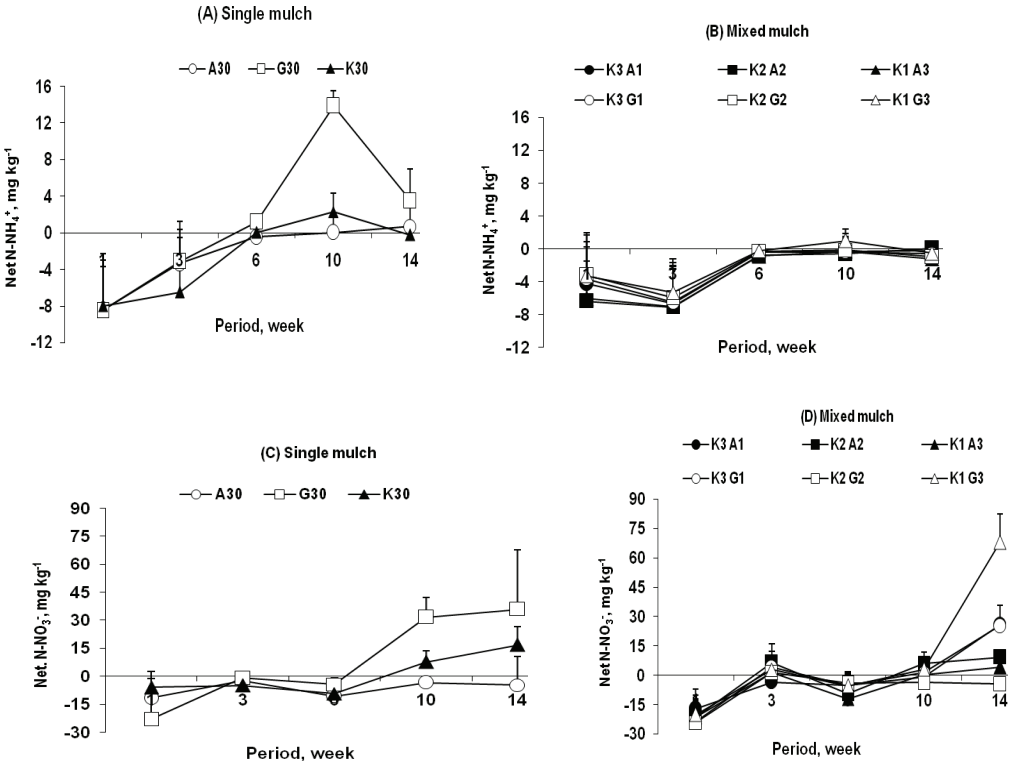


Figure 1. Concentration of net N- NH_4^+ and NO_3^- as affected by application of single (10 and 30 Mg ha-1) or mixture (1 = 25%; 2 = 50%; 3 = 75%) of three types of mulches (A = Avocado; K = coffee; G = gliricidia). Above zero line, ammonification and nitrification happened, while lower than that immobilization and depression of nitrate occured.

Table 3. Matrix of correlation between soil characteristics and mulch quality.

	Mulch lignin content (L)		Mulch polyphenol content (P)		Mulch C/N ratio		Mulch (L+P)/N ratio	
	r	**P**	**r**	**P**	**r**	**P**	**r**	**P**
Soil NH_4^+ concentration	0.118	0.269	**-0.479****	0.000	-0.198	0.061	-0.029	0.789
Soil NO_3^- concentration	-0.245*	0.020	0.168	0.114	-0.099	0.352	-0.189	0.074
Potential nitrification	-0.235**	0.000	0.130	0.223	-0.132	0.289	**-0.534****	0.000
NH_4^+ oxidizing bacteria population	-0.260*	0.13	0.110	0.302	-0.150	0.159	-0.219*	0.038
NO_2^- oxidizing bacteria population	-0.321**	0.02	0.216*	0.041	-0.132	0.215	-0.248*	0.018
Bacteria population	0.142	0.183	0.298**	0.004	0.309**	0.03	0.221*	0.036
Fungi population	-0.159	0.134	0.318**	0.02	-0.337**	0.01	-0.243*	0.021
Actinomycetes population	-0.198	0.061	0.091	0.392	-0.233*	0.027	-0.209*	0.048

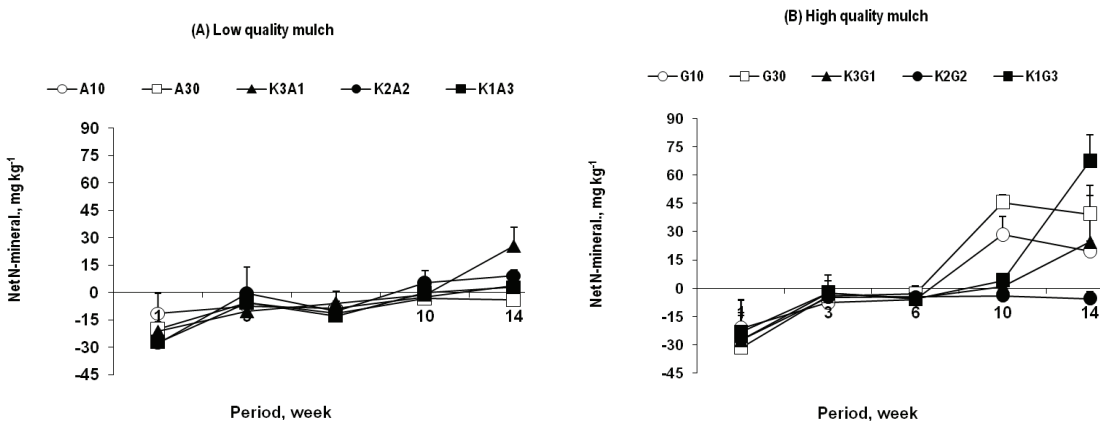


Figure 2. Concentration of net N-mineral as affected by application low quality mulch (A) and high quality mulch (B) of single (10 and 30 Mg ha-1) or mixture (1 = 25%; 2 = 50%; 3 = 75%) of three types of mulches (A = Avocado; K = coffee; G = gliricidia). Ammonification and nitrification happened above concentration of zero, while lower than that immobilization and depression of nitrate occured.

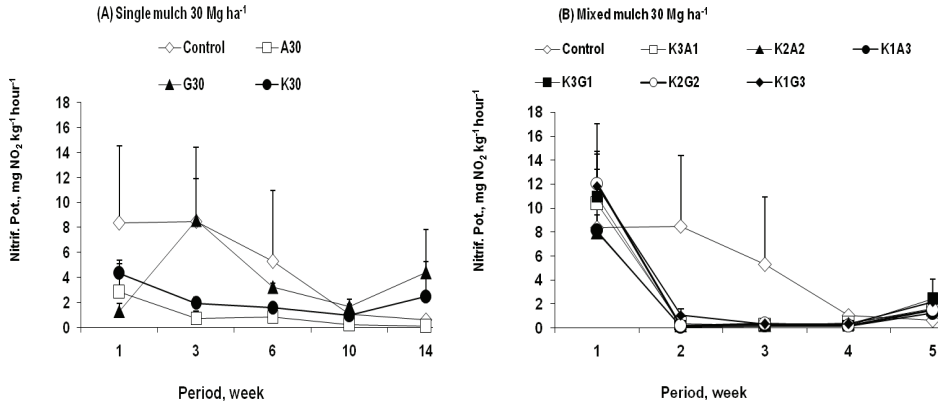


Figure 3. Soil potential nitrification as affected by application of single (30 Mg ha-1) (A) or mixture (1 = 25%; 2 = 50%; 3 = 75%) (B) of three types of mulches (A = Avocado; K = coffee; G = gliricidia).

CONCLUSION

a. There was positive correlation between quality mulch application and rate of mineralization and release of NO_3^-.
b. Mulch (L+P)/N content ratio was stronger as nitrification inhibitor than lignin content, whereas mulch polyphenol content was stronger as ammonification innhibitor, and lignin as inhibitor of NO_3^- formation compared to mulch (L+P)/N content ratio or C/N ratio.
c. Organic matter input quality control can be used to inhibit rate of nitrification and to increase N use efficiency.

REFERENCES

Handayanto E., 1994. Nitrogen Mineralization from legume tree prunings of different quality. Thesis for Doctor of Phylosophy. Department of Biological Sciences, Wye College, University of London. 176 p.

Hairiah K., 2005. Biodiversitas Tanaman Menuju Pertanian Sehat. Makalah Seminar Nasional 'Sistem Pertanian Berkelanjutan untuk Menunjang Pembangunan Nasional'. Dies Natalis Univ. Sebelas Maret ke 29. Surakarta, 15 Feb 2005. 18 p.

Nur A.M., Rahardjo P., Priyono, Wardani S., Subekti S., Sulistyono B., 2003. Diversifikasi di kebun kopi dengan tanaman industri dalam rangka menunjang keberlanjutan usaha tani kopi. Warta Puslitkoka, 19, 91-106.

Palm C.A., Sanchez P.A., 1991. Nitrogen release from some tropical legumes as affected by lignin and polyphenol contents. Soil Biol.Biochem. 23. 83-88.

Wagner G.H.,Wolf D.C., 1999. Carbon Transformations and Soil Organic Matter Formation. In Principles and Application of Soil Microbiology (Sylvia D.M., Jeffry J.F., Peter G.H., David A.Z., Eds.). Prentice Hall, New Jersey. 218-258.

Arbuscular Mycorrrhiza and Copper Uptake and Toxicity in Coffee Plants

S.A.L. ANDRADE[1], P. MAZZAFERA[1], M.A. SCHIAVINATO[1], A.P.D. SILVEIRA[2]

[1]Instituto de Biologia, Dpto. Fisiologia Vegetal, Universidade Estadual de Campinas, Brasil
[2]Centro de Solos e Recursos Ambientais, Instituto Agronômico, Campinas, Brasil

SUMMARY

Although an essential nutrient for plants, copper may be toxic at high concentrations. Excess Cu in soils is usually originated from mining and agricultural use of Cu-based fungicides and sewage sludge. On the other hand, coffee benefits from arbuscular mycorrhizal fungi (AMFs) particularly in soils with low nutritional status. Those fungi have been shown to alleviate heavy metal toxicity in plants, improving mineral nutrition or by diminishing metal translocation to the shoots. In the present study the effects of Cu addition to soil on *Coffea arabica* growth and Cu uptake under the influence of AMF inoculation was investigated. A pot culture experiment was carried out under greenhouse conditions, in a 2 × 4 factorial design. The treatments were either inoculation or non-inoculation with AMFs (*Glomus clarum*, *Gigaspora margarita* and *Acaulospora* sp.) and the addition of four Cu levels to the soil (0, 50, 150, 450 $mg.dm^{-3}$). Mycorrhizal (M) coffee showed better growth than non-mycorrhizal (NM) coffee plants in all Cu levels in soil, mainly due to their better nutritional status. In addition, Cu toxicity symptoms were more pronounced in NM plants which showed leaf chlorosis and necrosis in the highest dose of Cu. Roots were highly colonized by AMFs, suggesting a certain tolerance of the fungal species to high soil Cu concentrations. Cu was mainly accumulated in roots increasing with increased levels in the soil. However, in NM roots the increase was higher than in M roots, with almost 150 $mg.kg^{-1}$ more Cu than in M roots in the highest dose applied. Cu concentration in M shoots was not influenced by increasing concentrations of Cu in the soil, whereas NM plants had a linear response, and at the highest Cu level in soil NM plants had 50% more Cu in shoots than M ones. These results indicate that mycorrhiza benefits coffee plants under conditions of excess Cu in soil, by possible retention in extra-radical mycelium, limiting its translocation to the shoots. The capacity of M plants in maintaining good P nutrition caused a higher growth and higher P:Cu ratios and S concentrations, improving coffee tolerance to metal toxicity.

INTRODUCTION

The concentrations of heavy metals and other trace elements are increasing in soils due to human activities. In the specific case of coffee soils, pesticides, fertilizers or organic amendments are the most frequent exogenous sources of metals. Agricultural soils from many coffee-producing countries have been successively amended with high applications of Cu-containing fungicides, extensively used in orchards for disease control (Lepp et al., 1984), resulting in Cu accumulation in different soil horizons. Additionally, sewage sludge application in coffee orchards as a plant fertilizer and soil conditioner is becoming a possible practice in coffee plantations. Unfortunately, depending on the sludge origin it may be a source of metal accumulation in soils. The transfer of heavy metals to coffee plants may cause physiological disorders or excessive metal accumulation which could enter the food chain via agricultural products. In natural conditions coffee plants are usually associated with AMF fungi and it is considered to be highly mycorrhizal dependent, especially during the seedling

stage. The main AM benefit for plants is an increase in nutrient uptake, particularly P, as a consequence of the higher soil volume explored by mycorrhizal roots via fungal extra-radical hyphae. AMF may alter the capacity of plants to accumulate heavy metals, as well as the functioning of plants under contaminated soil conditions, normally beneficing plant development under metal stress conditions. The aim of this study was to evaluate the effects of mycorrhization on coffee plants development in response to Cu addition to soil

MATERIAL AND METHODS

A pot culture experiment was installed under greenhouse conditions. The experiment consisted in a 2 × 4 factorial completely randomized design, with seven replicates. The treatments were either inoculation or non-inoculation of AMFs and the addition of four Cu rates to soil (0, 50, 150 and 450 $mg.dm^{-3}$). A sample of a "loamy Typic Hapludox" soil from the surface horizon was collected and a soil sample was chemically characterized with a pH ($CaCl_2$) of 5.5; 28 $mg.dm^{-3}$ of organic matter and 6, 2, 90 and 40 $mg.dm^{-3}$ of P, K, Mg and Ca, respectively. The soil sample was autoclave-sterilized to eliminate native AMF propagules. Soil samples were placed in 2 L pots to which 40 $mg.dm^{-3}$ of P as single superphosphate and an aqueous solution of $Cu(SO_4)_2$ at a previously determined range of Cu concentrations was added. After thoroughly mixing, the soil was allowed to stabilize for 15 days and then subsamples were taken for soil chemical analyses (Table 1).

Table 1. Soil chemical characteristics after copper application. (BS: basic saturation).

Added Cu ($mg.dm^{-3}$)	BS (%)	pH $CaCl_2$	Resin ($mmol_c.dm^{-3}$)				DTPA ($mg.dm^{-3}$)				
			Ca	Mg	P		B	Cu	Fe	Mn	Zn
0	62	5.5	31	9	18		0.33	5.4	10	33.8	2.3
50	66	5.5	36	10	22		0.22	22.8	9	37.5	5.4
150	65	5.5	39	10	16		0.37	48.6	11	35.9	2.7
450	59	5.3	34	9	20		0.28	162	10	48.0	2.8

One seedling of the *Coffea Arabica* var. Obatã (IAC-1699-20) was transplanted to each 2 L pot and then allowed to grow for 30 weeks. The AMFs used was a mixture of *G. clarum*, *G. margarita* and *Acaulospora* sp. Colonized root fragments, mycelium and a sand-soil mixture containing spores were used as inoculum. At harvest, leaves, stems and roots were separated, washed, dried, weighed and ground. Root subsamples were stored in 50% ethanol for mycorrhizal colonization determination. Plant P and Cu concentrations were determined by inductively coupled plasma-optical emission spectroscopy after HNO_3-$HClO_4$ digestion. Thirty root segments were mounted on slides and observed under a compound microscope to estimate the percentage root length infected by AMF by first clearing the roots with KOH and staining with trypan blue. Data were submitted to analysis of variance, Tukey test (5%) and regression analyses.

RESULTS AND DISCUSSION

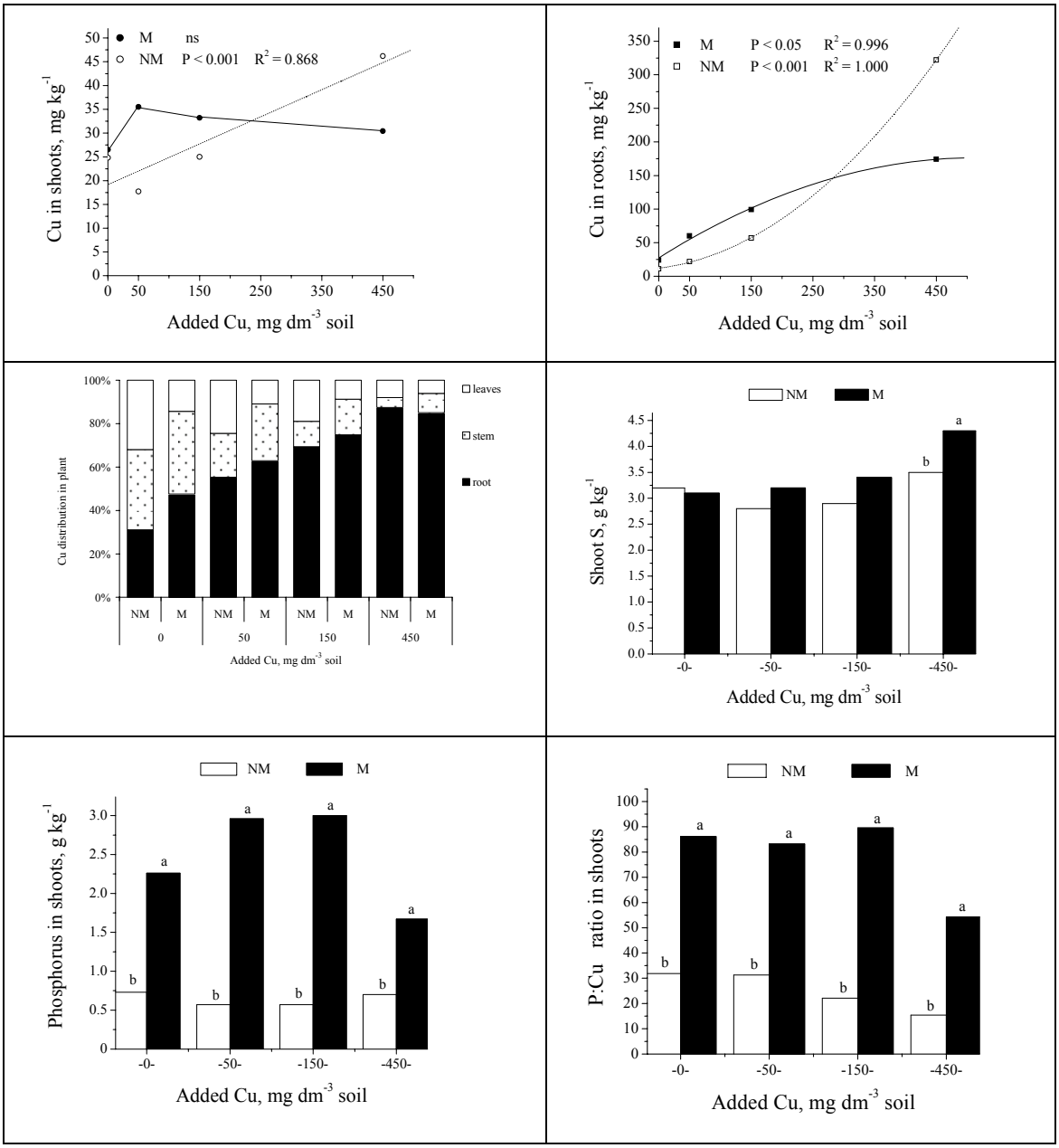
The association of coffee plants with AMF conferred higher biomass production in all Cu levels in soil, with 14 and 9 times higher values for shoots and roots than NM plants, revealing the great importance of mycorrhizae in coffee plants development. As with many other agronomic crops, AM growth promotion in coffee plants has been mainly attributed to the nutritional effects of the symbiosis (Siqueira et al., 1998). Improvements in plant mineral nutrition are mainly related to uptake by extra-radical hyphae and transport to the plant. Coffee roots were highly colonized by AMFs in plants growing in all Cu levels in soil, suggesting a certain tolerance of the fungal species to high soil Cu concentrations. On the

other hand Cu addition caused a reduction in plant growth the toxicity symptoms being more pronounced in NM plants which showed leaf chlorosis and necrosis in the highest dose of Cu added to soil. M coffee showed higher growth than NM coffee plants, mainly due to their better nutritional status. In the shoots, Cu concentrations were much lower than in the roots and lower in AMF inoculated than in non-inoculated plants. Cu uptake showed a similar distribution in M and NM plant tissues accumulating mainly in roots and increasing as levels increased in the soil. However, in NM roots the increase was higher than in M roots, with 322 $mg.kg^{-1}$ of dry weight, which represent 45% more Cu than in M roots in the highest doses applied. Cu concentration in M shoots had no influence of increasing concentrations of Cu in soil, whereas NM plants had a linear response, being that at the highest Cu level in soil NM coffee had 50% more Cu in shoots than M ones. As expected M plants absorbed significantly higher P concentrations in shoots and roots than NM plans, maintaining higher P:Cu than NM homologues in the highest level of Cu applied to soil. M plants showed an increase in S concentration in tissues with increasing levels of Cu in soil, and this may be related to an increasing demand of this element in conditions of excesses Cu in soil, suggesting a higher sulphur metabolism possibly related to thiol-binding peptides or cysteine rich compounds involved in metal detoxification (Nocito et al., 2002). The results indicate that mycorrhiza benefits to coffee plants under conditions of excess Cu in soil by diminishing Cu concentrations in plant tissues, possibly through retention of the metal in the extra-radical mycelium and limiting its translocation to the shoots. In conclusion, although the growth of coffee seedlings was limited in soil with high Cu concentrations AMF inoculation improved tolerance of seedlings to excess Cu. The capacity of M plants to maintain better P nutrition increasing P:Cu ratios may contribute to improve tolerance under excess Cu conditions.

Table 2. Shoot (SDW) and root (RFW) dry weight and mycorrhizal colonization (Myc. Col.) of mycorrhizal (M) and non-mycorrhizal (NM) *Coffea arabica* plants in response to Cu addition to soil.

Added Cu soil	**SDW** g		**RDW** g		**Myc. Col.** %	
$mg.dm^{-3}$	NM	M	NM	M	NM	M
0	0.32 b	5.5 a	0.15 b	1.81 a	0	44
50	0.38 b	6.3 a	0.16 b	1.45 a	0	28
150	0.31 b	5.5 a	0.15 b	2.02 a	0	20
450	0.32 b	2.1 a	0.14 a	0.53 a	0	37
Regression	ns	x^2	ns	x^2	-	ns
R^2	-	0.973	-	0.878	-	-

Figura 1. Cu concentrations, Cu distribution, P concentrations and P: Cu ratio in mycorrhizal (M) and non-mycorrhizal (NM) *Coffea arabica* plants in response to Cu addition to soil.



REFERENCES

Lepp N.W., Dickinson N.M., Ormand K.L., 1984 Distribution of fungicide-derived copper in soils, litter, and vegetation of different aged stands of coffee (*Coffea arabica* L.) in Kenya. Plant Soil 77:263-270

Nocito F.F., Pirovano L., Cocucci M., Sacchi A.G., 2002 Cadmium-induced sulfate uptake in maize roots. Plant Physiol. 129, 1872-1879.

Siqueira J.O., Saggin-Junior O.J., Flores-Aylas W.W., Guimarães P.T.G., 1998 Arbuscular mycorrhizal inoculation and superphosphate application influence plant development and yield of coffee in Brazil. Mycorrhiza 7:293-300.

Arboreal Leguminous Windbreak as Bank of Beneficial Mites for Coffee Plants

P. REBELLES REIS [1], M.S. ZACARIAS[2], T. MANSOUR BOTELHO DE CARVALHO[3]

[1]EPAMIG/EcoCentro, P.O. Box 176, Zip Code 37200-000, Lavras, MG, Brazil, CNPq researcher
[2]Embrapa Café/EcoCentro, Lavras, MG, Brazil
[3]UFLA/EPAMIG/EcoCentro, Lavras, MG, Brazil

SUMMARY

Three families of phytophagous mites are of economic importance on coffee (*Coffea* spp.) plantations: Tetranychidae, Tenuipalpidae and Tarsonemidae, with the Phytoseiidae likely to be the main predators of these mites. Several studies indicate that the abundance and diversity of entomophagous arthropods present in a particular crop are closely related to the nature of the surrounding vegetation. In this study, leguminous arboreous species for multiple use, used as windbreak (alley cropping system, hedgerows), were evaluated in relation to phytossanitary aspects of coffee crop. The arboreous leguminous species used as windbreak in the experiments were: Pigeon pea (*Cajanus cajan* Millsp.), Bracatinga (*Mimosa scabrella* Benth.)**,** Leucaena [*Leucaena leucocephala* (Lam.) De Wit] and Acacia (*Acacia mangium* Willd.), planted perpendicularly to the predominant winds orientation. Leaves were collected from both windbreak and coffee plants each year after the rainy and dry seasons. Wash method was used to remove the mites from the collected leaves. Coffee plants under the influence of Leucaena revealed the highest number of specimens of mites, almost near twice of the total number found in plants under the influence of the remaining alley cropping. The mite families found in coffee plants and in the alley cropping are quite the same, which should be considered beneficial since the eventual use of chemicals for pest control in coffee areas, with resulting reduction in mite population, can have in windbreak a source for replacement, mainly of beneficial ones.

INTRODUCTION

Three families of phytophagous mites are of economic importance on coffee (*Coffea* spp.) plantations: Tetranychidae, Tenuipalpidae and Tarsonemidae, with the Phytoseiidae likely to be the main predators of these mites (Reis and Zacarias, 2007). Several studies indicate that the abundance and diversity of entomophagous arthropods present in a particular crop are closely related to the nature of the surrounding vegetation (Altieri, 1994). In this study, leguminous arboreous species for multiple use, used as windbreak (alley cropping system, hedgerows), were evaluated in relation to phytossanitary aspects of coffee crop.

MATERIAL AND METHODS

This experiment was conducted at the "São Sebastião do Paraíso" Experimental Station, of EPAMIG, in Minas Gerais State, Brazil, from 2003 to 2006. The arboreous leguminous species used as windbreak in the experiments were: Pigeon pea (*Cajanus cajan* Millsp.), Bracatinga (*Mimosa scabrella* Benth.)**,** Leucaena [*Leucaena leucocephala* (Lam.) De Wit] and Acacia (*Acacia mangium* Willd.), planted perpendicularly to the predominant winds orientation. Leaves were collected from both windbreak and coffee plants each year after the

rainy and dry seasons. Wash method was used to remove the mites from the collected leaves (Zacarias et al., 2004).

RESULTS AND DISCUSSION

Results obtained from 2003 to 2006 demonstrate that the percent of mites found in coffee plants under the influence of the hedgerows was 30% in Leucaena, 19% in Acacia, 18% in Bracatinga, 16% in Pigeon pea and, 17% in Control, distributed among several mite families, Phytoseiidae among those with predatory habits, mainly in coffee crop under the influence of Leucaena. There was less mite specimens in the alley cropping (3,616) (Table 1) than in coffee plants (8,645) (Table 2).

Table 1. Total number of mites, according to family, found in alleys of different leguminous species after seven samplings. São Sebastião do Paraíso, Minas Gerais, Brazil, March and December of 2003, 2004, 2005 and 2006.

Families	Number of mites/family/leguminous species				Total of mites/family
	Acacia	Leucaena	Bracatinga	Pigeon pea	
Tydeidae (G)[1]	293	757	28	11	1,089
Tenuipalpidae (F)	175	186	557	47	965
Tetranychidae (F)	431	3	2	370	806
Phytoseiidae (P)	93	32	26	200	351
Eriophyidae (F)	1	2	54	208	265
Tarsonemidae (F)[2]	14	29	1	57	101
Winterschmidtiidae (G)	8	3	2	11	24
Stigmaeidae (P)	1	2	1	3	7
Ascidae (P)	0	0	1	3	4
Cunaxidae (P)	1	1	0	0	2
Acaridae (G)	0	0	0	1	1
Oribatida (Suborder) (G)	0	0	0	1	1
Total	1,017	1,015	672	912	3,616
Number of families	9	9	9	11	12
Hn (Shannon-Weiner) Diversity	1.36	0.81	0.67	1.52	1.63
e (Pielou) Uniformity	0.62	0.37	0.31	0.63	0.66
d (Pielou) Richness	1.30	1.30	1.38	1.61	1.46

[1] *Classification by eating habits: G = generalist; F = phytophagous and P=predator.*
[2] *In spite of presenting a lot of generalist species, this family was considered phytophagous for the present work.*

In plants under no influence of alley cropping (Control), 1,496 mite specimens were collected. Considering the alley cropping in general, the Tydeidae ranked first with 1,089 specimens, followed by Tenuipalpidae (967), Tetranychidae (806), Phytoseiidae (351), Eriophyidae (265), Tarsonemidae (110), Winterschmidtiidae (24), Stigmaeidae (7), and Ascidae (4) (Table 1).

Table 2. Total number of specimens of mites, according to family, found on coffee plants under the influence of different types of leguminous alleys after seven samplings. São Sebastião do Paraíso, Minas Gerais, Brazil, March and December of 2003, 2004, 2005, and March, 2006.

Families	Number of mites /family/coffee plant					Total of mites/ family
	Acacia	Leucaena	Bracatinga	Pigeon pea	Control	
Winterschmidtiidae (G)[1]	87	501	254	430	556	1,828
Tydeidae (G)	326	781	300	153	120	1,680
Tarsonemidae (F)[2]	515	384	256	193	308	1,656
Tenuipalpidae (F)	311	293	342	280	259	1,485
Phytoseiidae (P)	310	371	261	247	190	1,379
Tetranychidae (F)	75	185	99	99	42	500
Stigmaeidae (P)	6	21	6	10	9	52
Ascidae (P)	2	6	7	7	4	26
Bdellidae (P)	2	4	3	0	3	12
Eriophyidae (F)	1	3	1	3	1	9
Acaridae (G)	0	2	1	0	2	5
Oribatida (Suborder) (G)	0	2	0	1	0	3
Eupodidae (G)	0	2	0	0	1	3
Cunaxidae (P)	0	0	2	0	0	2
Hypopus (Suborder Acaridida) (G)	0	0	1	0	0	1
Pigmephoridae (G)	0	1	0	0	0	1
Cheyletidae (P)	1	0	0	0	0	1
Pyroglyphidae (G)	0	0	0	1	0	1
Anystidae (P)	0	0	0	0	1	1
Total	1,636	2,556	1,533	1,424	1,496	8,645
Number of families	12	14	13	11	13	20
Hn (Shannon-Weiner) Diversity	1.67	1.78	1.81	1.77	1.64	1.81
e (Pielou) Uniformity	0.67	0.67	0.71	0.74	0.64	0.60
d (Pielou) Richness	1.62	1.78	1.77	1.51	1.78	2.21

[1] Classification by eating habits: G = generalist; F = phytophagous and P=predator.
[2] In spite of presenting a lot of generalist species, this family was considered phytophagous for the present work.

In coffee plants, Winterschmidtiidae (1,828) had the largest number of mites, followed by Tydeidae (1,679), Tarsonemidae (1,656), Tenuipalpidae (1,485) and Phytoseiidae (1,379). Specimens of Tetranychidae (500) were less abundant than other families previously cited, and the number of specimens of Stigmaeidae (52), Ascidae (26), Bdellidae (12) and Eriophyidae (9) was considered very low (Table 2).

Coffee plants under the influence of Leucaena revealed the highest number of specimens of mites (2,556), almost near twice of the total number found in plants under the influence of the remaining alley cropping as follows: Acacia (1,636), Bracatinga (1,533) and Pigeon pea (1,424). However, this doesn't mean a problem, because the relationship between the total number of predatory and phytophagous specimens is the highest among all (0.465).

CONCLUSIONS

The mite families found in coffee plants and in the alley cropping are quite the same, which should be considered beneficial since the eventual use of chemicals for pest control in coffee areas, with resulting reduction in mite population, can have in windbreak a source for replacement, mainly of beneficial ones.

ACKNOWLEDGMENTS

To the “Fundação de Amparo à Pesquisa do Estado de Minas Gerais - Fapemig” and “Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café CBP&D/Café” for the financial support and, to CNPq for the scholarship.

REFERENCES

Altieri M.A. The influence of adjacent habitats on insect populations in crop fields. In: Altieri, M.A. Biodiversity and pest management in agroecosystems. New York: Food Products Press, p.109-129, 1994.

Reis P.R. and Zacarias M.S. Ácaros em cafeeiro. Belo Horizonte: EPAMIG, 2007. 76p. (EPAMIG, Boletim Técnico, 81).

Zacarias M.S., Reis P.R., Silva D.C. Comparación entre métodos de coleta de ácaros para estudios de diversidad del filoplan. In: Simpósio Latinoamericano y del Caribe – “La Biodiversidad Acarina: Utilización, Protección y Conservación ”, 1., La Habana, Cuba, 2004. Resúmenes... La Habana: INISAV, 2004. p. 73. (CD-ROM).

Farmers' Empowerment for Sustainable Coffee Productivity and Quality: a Case Study of Kagera Region, Tanzania

N.M. NG'HOMA, D.L. KILAMBO, J.M. TERI, P.M.L. KAIZA

Tanzania Coffee Research Institute (TaCRI), Lyamungu, P.O. Box 3004, Moshi, Tanzania.
Tel. +255 027 2756868, Fax +255 027 2756773
E-mail: nyabisin@yahoo.com - tacriced@kicheko.com - www.tacri.org

SUMMARY

Since 1970s efforts have been placed on improving the impact of agricultural research and extension. The focus was on greater involvement of farmers in technology development and transfer process. Several participatory approaches such as Farming Systems Research and extension were developed over time such as teaching, supplying inputs, and facilitate farmer participation. In late 1980s farmers began to be seen as partners in research and extension and the key players in technology development and transfer and therefore client oriented research (CoR). But the impact was not realised. Since the inception of TaCRI, emphasis have been in the use of participatory extension approaches using farmer groups to impart appropriate knowledge and skills and therefore empowering farmers for sustainable coffee recovery. Farmers in Kagera were also involved in this approach, and this paper reviews the potential of empowering farmers through discovery learning approaches for sustainable dissemination of appropriate technologies for rejuvenation of coffee industry in the region. Effect of farmers' empowerment was assessed in 11 farmer groups in 11 villages existing since 2004. Assessment was conducted in terms of productivity and quality improvement particularly in the top coffee bean size screen 18.

INTRODUCTION

Agricultural extension services provide a link between researchers, policy makers and farmers. Until recently extension services have been organized and operated on the assumption that farmers are passive, illiterate and therefore ignorant, and are unable to innovate or integrate new practices into their agricultural production systems (CTA, 1997). Recently there has been wind of change. Farmers are encouraged to participate in planning, implementing and evaluating their development activities (Rolling and Wagemakers, 1998). Farmers are encouraged to become drivers of efforts to improve their economic and social well-being.

For agricultural extension agents this change implies that instead of continuing to be agents for concepts or technologies imposed to farmers, they need to be catalysts, helping farmers to achieve the goals they have defined for themselves (Enserink and Kaitaba, 1996). Since its inception TaCRI has put emphasis on Farmers Participatory Approaches (Temu *et al.,* 2006), as a way of enabling farmers to plan, implement and solve productivity and quality improvement in the major coffee zone including Kagera. This implies farmers' empowerment.

This report outlines the potential of using Participatory Extension Approaches which empowers coffee farmers with the knowledge and skills to apply good agricultural practices to improve coffee productivity and quality 11 farmer groups in Kagera region.

MATERIALS AND METHODS

The study was conducted across coffee agro-ecosystems of Bukoba, Karagwe, Misenyi and Muleba districts. Eleven villages were under pilot study of the effect of farmers' empowerment on productivity and quality improvement. To assess productivity, a plot of 160 coffee trees was divided into two equal half. A plot of 80 trees receiving improved coffee practices such as pruning, farm yard manure (40 kg per tree) and inorganic fertilizer 120 gm split into 3 times per season, weeding, mulching and proper intercropping with banana. The other half of 80 coffee trees of Robusta was under farmers management i.e. low frequently weeding, no FYM and inorganic fertilizer application, no mulch and improper or no pruning at all. Each plot formed a group of 30 farmers and each village 3 study plots. The group meet once per month; TaCRI Extension Agronomist and Village Extension agent assist them in application of treatment and collecting production per unit tree and proper drying. The groups are organized themselves under the chairman and the secretary. Coffee harvested from these treatments was processed according to the recommended practices of hard coffees (Robinson, 1964). During harvesting only ripe cherries were picked. Cherries were dried on mats/ racks for 14 or 21 days depending on weather. Dry cherries were hulled using motorized hullers. Thereafter, hulled coffees were winnowed; sorted and weighed. Grading of the coffee bean sizes were based on screen sizes in the order of the best quality from 18, 16, 14, 12, and inferior grade designated as UG. Mean yields per tree were collected and analysed using Excel and MstatC software packages.

RESULTS AND DISCUSSION

Figure 1 present mean results of productivity across the 33 farmer groups under the study. Results showed significant differences (P = 0.05) of productivity between plots received improved technologies and farmers practices. On average plots of improved technologies scored 3.9 Kg per tree of clean coffee and from farmers practices 0.32 kg per tree. It is estimated that 990 farmers benefited directly from this training, and each one of the 990 has imparted knowledge to three farmers per season therefore about 11480 farmers have benefited from this training over four years (2004 to 2007)! This shows that farmers' empowerment using Participatory approaches can assist extension workers and TaCRI researchers to disseminate knowledge and skills in proper coffee husbandry with a very short time.

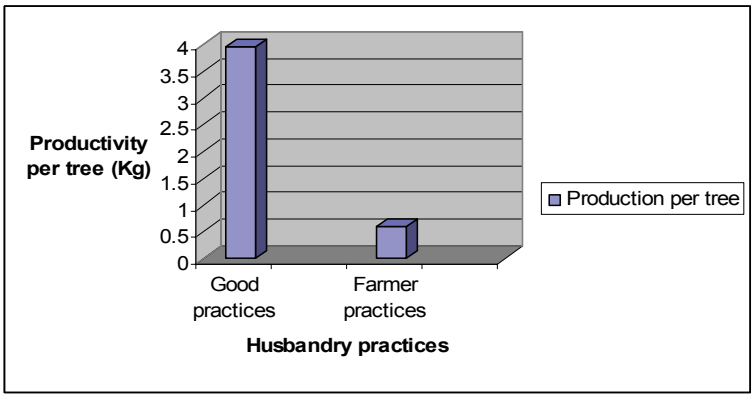


Figure 1. Clean coffee produced on plots receiving good practices compared to plots receiving farmer's current practices in Kagera.

Production of coffee marketed from the farms of the 33 farmer groups under the study have improved. For example, Tweyambe farmer group one among the 33 farmer groups, increased coffee productions from six tonnes in 2003 to 68 tonnes in 2007; an increase of 91.2% (Figure

2). Under the current price of 1.605 USD Kg of clean coffee, this is worth 9,630 US $ in 2003 to an increase of wealth equivalent to 109,140 USD in 2007! This is because of farmers' empowerment through participatory approach! This resulted in increased income and profit.

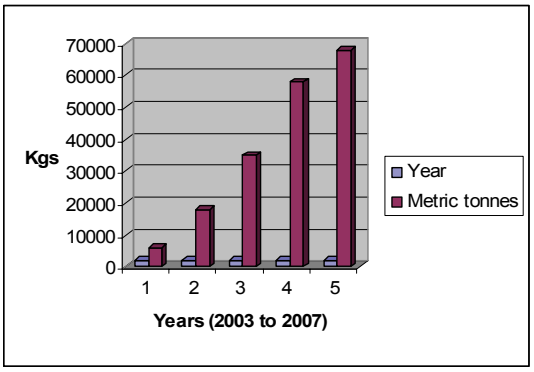


Figure 2. Quantities of coffee sold by Tweyambe farmer group directly at the Moshi Coffee Exchange, 2003-2007.

Average bean quality of the samples collected and analysed from the 33 plots are presented in Figures 3 and 4. There is great improvement of the bean sizes as while from good practices 38.1 % of the samples were retained in screen 18 the top quality class (Figure 3), in farmers practices only 16.4 % using the same screen (Figure 4).

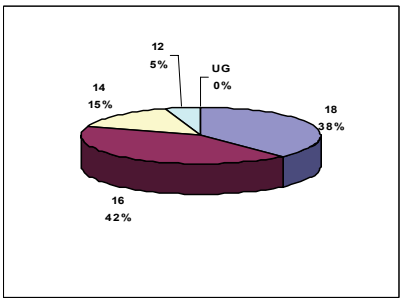


Figure 3: Percentage of coffee grades from good practices.

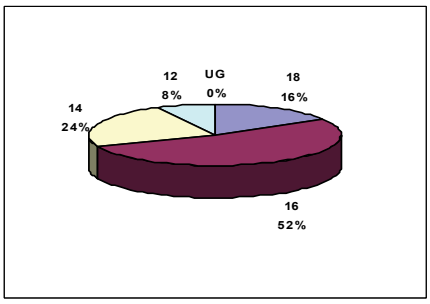


Figure 4: Percentage of coffee grades from farmers practices.

Participatory approach which emphasis farmers learning by doing and sharing experiences assist to empower farmers to actively identify and solve their known problems (Enserink and Kaitaba, 1996)). This has proved to be true in this pilot study that farmers empowerment has assisted to improve productivity, quality and ultimately incomes within four years.

CONCLUSION AND RECOMMENDATIONS

This study has shown that it is possible to improve productivity and quality of Robusta coffee in Kagera region. To achieve this, farmers should be empowered in terms of production skills and knowledge in good practices of coffee husbandry. For TaCRI this is the big challenge to upscale the experiences gained from this work, to cover more coffee growing areas in Tanzania.

ACKNOWLEDGEMENT

This work was financed from FMO 94-99 STABEX CONTRACT No. DLO/COF99/01/14 Project No. TA STA COF99 01. We are grateful to the EU and Tanzania Government for this support.

REFERENCES

CTA (1997) Extension services: Masters or servants, Spore Newsletter No. 68.

Enserink H.J., and Kaitaba E. (1996) Farming System Zonation Lake Zone. A Report of a Mission Commissioned by the Farming Systems Research Programme Lake Zone Agricultural Research and Training Institute. LZARI/FSR-Programme. 42 pp

Robinson J.B.D. (Eds) (1964) A hand book on Arabica coffee in Tanganyika. Published by the Tanganyika Coffee Board.pp85.

Rolling N.M. and Wagermarkers M.A.E. (1998) Facilitating sustainable agriculture. In: Participatory Approaches and Scaling-up.pp1-12 (Eds Elske van de Fliert, Rini Asmunati & Warsito Tantowijoyo).

Temu M.H., Nzallawahe T.S., Teri J.M. and Mdemu S.Y. (2006) The potential of participatory extension approaches in coffee rejuvenation-Experience from Northern Tanzania. In: Proceedings of 21st International Conference on Coffee Science (ASIC) 2006, 11-15 Sept. 2006, Montpellier, France.

Irrigated Coffee Flowering and Grain Maturation Uniformity as Affected by Controlled Water Stress in the Brazilian Cerrado Region

A.F. GUERRA*, G.C. RODRIGUES, O.C. ROCHA, C. SANZONOWICZ, C. JERKE, A.C. MERA, A. CORDEIRO

Brazilian Agricultural Research Corporation (Embrapa Cerrados), C. Postal 08223, Planaltina, Federal District, Brazil. *E-mail: guerra@cpac.embrapa.br

SUMMARY

The objective of this research was to establish the period and intensity of water stress in coffee trees (*Coffea arabica* L.) to cause buds development synchronization and a high uniformity in flowering and fruits maturation. The research was carried out in the experimental field of the Brazilian Agricultural Research Corporation (Embrapa Cerrados) located at 15° 35´33´´ S, 47° 30´´ W, 1077 asl, in Planaltina, DF, Brazil. Coffee trees were submitted to five irrigation regimes: irrigation all over the year when soil water potential reached -0.5 MPa (IR1); irrigation suppression from June 24 until leaf water potential reached -1.2 MPa (IR2) and -2.2 MPa (IR3); supplemental irrigation after blossoming be induced by rain (IR4) which resulted in mean leaf water potential of -3.4 MPa and without irrigation (IR5) when leaf water potential reached values below -4.0 MPa. Soil water content was measured by using profile probes and leaf water potential at predawn with a Sholander-type pressure pump. The results shows that suppression irrigation from June 24 until leaf water potential reach -2.2 MPa in September 4 was the best treatment for buds development synchronization. In this treatment flowering uniformity reached values highers than 85% and production of berry fruits at harvesting was up to 83% optimizing the potential for producing high quality coffees. The higher yield reach values around 78 bags of 60 kilograms per hectare of processed coffee. It occurred in treatments submitted to mild (IR2) and adequate water stress (IR3). Significant reduction in yield was observed in the treatments irrigated all over the year and when plants were subject to intensive water stress. Adequate water stress promote high yields because guarantee an intensive and uniform flowering period in the beginning of September, when maximum and minimum temperatures are suitable for this process, producing viable pin-heads. Also, the reduction in the percentage of greens fruits at harvest, which did not complete the filling process, contributed to the higher yields observed in the IR3.

INTRODUCTION

The increase of irrigated coffee production in the Cerrado region determines the necessity of adequate technologies to support the necessary improvements in the existing production systems. Actually, Cerrado region is responsible for approximately 40% of national coffee production. However, it is necessary increases yield and coffee quality to maintain the activity sustainable and competitive. In this context irrigated coffee production systems allows water application management to supply crop water requirements during dry season and the application of a controlled water stress just before flowering period to synchronize buds development and obtain high percentage of berry fruits increasing yield and coffee quality.

Several research works such as [1, 2] have been indicating the necessity of a water stress period to improve flowering uniformity. [3] defined a best period and magnitude of water stress to synchronize buds development and obtain high level of flowering and maturation uniformity.

However, the objective of this paper was to present an efficient coffee irrigation management strategy to maximize yield and grain quality.

MATERIAL AND METHODS

The research was carried out in the experimental field of the Brazilian Agricultural Research Corporation (Embrapa Cerrados) located at 15° 35´33´´ S, 47° 30´´ W, 1077 asl, in Planaltina, DF, Brazil. The experimental area is irrigated by a center pivot of eight hectares and a two hectares area without irrigation. The irrigated area was split in four quarters of two hectares to test four irrigation regimes. The soil of experimental area was classified as a Clayey Dark Red Latosol. Coffee trees were submitted to five irrigation regimes: irrigation all over the year when soil water potential reached -0.5 MPa (IR1); irrigation suppression from June 24 until leaf water potential reached -1.2 MPa (IR2) and -2.2 MPa (IR3); supplemental irrigation after blossoming be induced by rain (IR4) which resulted in mean leaf water potential of _-3.4 MPa and without irrigation (IR5) when leaf water potential reached values below -4.0 MPa. Soil water content was measured by using profile probes and leaf water potential at predawn with a Sholander-type pressure pump. Leaf water potential readings were made between 3:00 and 5:00 a.m.

The coffee trees, (*Coffea arabica* L.) cv. Catuaí Rubi MG1192, were implanted at field conditions in February of 2001. Coffee trees were spaced 2.80 meters between lines and 0.5 m between plants. Water was applied always coffee trees extracted around 50% of available water in the upper 0.40 m soil profile. Soil water content was measured in the soil profile of one meter depth by using a Profile Probe Delta-T. The amount of water applied by irrigation was calculated to raise soil water content of the upper 0.40 m profile to field capacity condition (-0.008MPa).

Experimental plots were harvested manually at once and ten samples of 100 fruits were taken to measure the percentage of green, berry and dry fruits. The plots production was dried in cemented patio until grain humidity reached 12% (WB) to estimate gross grain yield. Coffee grains were pealed to obtain processed coffee yield and coffee grains sieve and type classification.

RESULTS AND DISCUSSION

In all irrigated plots leaf water potential was about -0.3 MPa in June 25 (Figure 1). In IR2 and IR3 leaf water potential reached -1.2 and -2.2 MPa after 53 and 72 days of irrigation suppression, respectively. IR4 and IR5 presented leaf water potential of -3.4 and less than -4.0 MPa in October 3 just before first significant rain occurred. The very low leaf water potential in IR5 was caused by the end of rainy season on the beginning of May determining a very long period without water supply.

The higher yield reached values around 78 bags of 60 kilograms per hectare of processed coffee (Figure 2). It occurred in treatments submitted to mild (IR2) and adequate water stress (IR3). Significant reduction in yield was observed in the treatments irrigated all over the year (IR1 - 61 bags.ha^{-1}) and when plants were subject to intensive water stress (IR4 – 58 bags.ha^{-1}; IR5 – 20 bags.ha^{-1}). Adequate water stress promote high yields because guarantee an

intensive and uniform flowering period in the beginning of September, when maximum and minimum temperatures are suitable for this process, producing viable pin-heads. Also, the reduction in the percentage of greens fruits at harvest, which did not complete the filling process, contributed to the higher yields observed in the IR3.

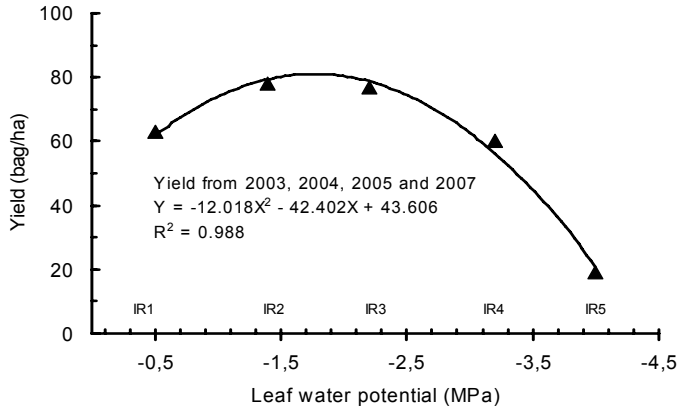


Figure 1. Mean leaf water potential of coffee trees for the five irrigation regimes.

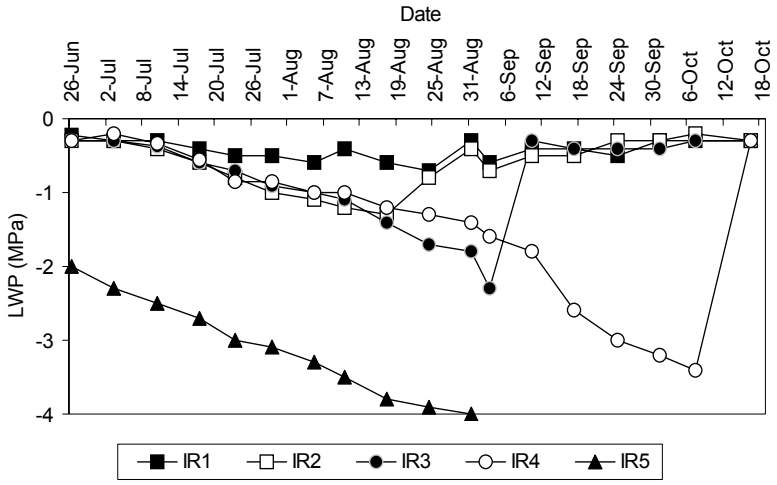


Figure 2. Mean of four year processed coffee yield as affected by five irrigation regimes.

The results showed that suppression irrigation from June 24 until leaf water potential reach -2.2 MPa in September 4 was the best treatment for buds development synchronization. In this treatment flowering uniformity reached values higher than 85% and production of berry fruits at harvesting was up to 83% optimizing the potential for producing high quality coffees (Figure 3). Intensive water stress (IR4), when flowering was induced by rain, resulted in the synchronization of bud development similar to the IR3 treatment. However, under this condition, we observed that flower buds or pin-heads (small green grains) dropped due to high temperatures (> 34 °C) which normally occurs in October, at the beginning of rainy season.

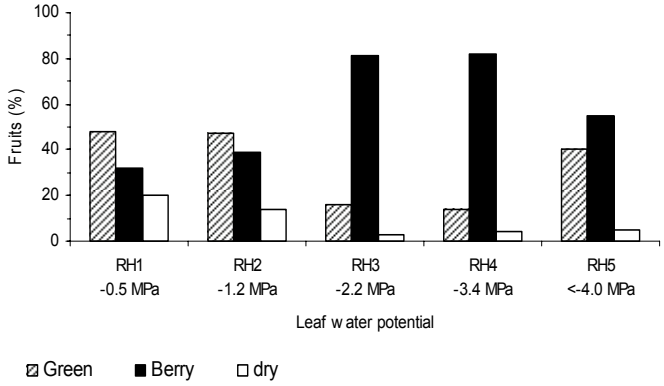


Figure 3. Mean percentage of green, berry and dry fruits as a affected by five irrigation regimes.

Similar to the treatment irrigated all over the year (IR1) mild water stress (IR2) was not sufficient to induce the a high level of bud development synchronization, and coffee trees presented in average three flowering events. In these treatments the percentage of berry fruits did not reach 45%. High production of quality grains, 33% of water and energy saving by suppression irrigation for about 72 days in dry season, and reduction in harvesting costs by allowing the operation at once are some of the several improvements in the irrigated coffee production system of the Cerrado region promoted by this technology.

CONCLUSIONS

1. Irrigation suppression from June 24 to September 4 is reliable for coffee buds development synchronization resulting in high flowering and grain maturation uniformity;
2. The use of controlled water stress aid to reach sustainability of the irrigated coffee systems and reduce pressure on the water and energy use during the dry season when occurs the maximum demand for annual crops irrigation systems production in the Cerrado region;
3. Irrigation management strategy using controlled water stress increases yield and grain quality and reduce irrigation costs.

REFERENCES

Crisosto C.H., Grantz D.A. and Meinze F.C. Effect of water deficit on flower opening in coffee (Coffea arabica L.). Tree Physiology 10:127-139. (1992).

Drinnan J.E. and Menzel C.M. (1994). Synchronisation of anthesis and enhancement of vegetative growth in coffee (Coffea arabica L.) following water stress during flower initiation. Journal of Horticultural Science 69: 841-849.

Guerra A.F., Rocha O.C., Rodrigues G.C., Sanzonowicz C., Ribeiro Filho G.C., Toledo P.M.R. dos., Ribeiro L.F. Sistema de produção de café irrigado: Um novo enfoque. ITEM, Irrigação e Tecnologia Moderna, , Brasília, n. 73 p. 52-61, 2007.

Improvement of Coffee Production System by Using Controlled Water Stress and Phosphorous Application in the Cerrado and South of Minas Gerais State Regions

A.F. GUERRA[1*], O.C. ROCHA[1], G.C. RODRIGUES[1], C. SANZONOWICZ[1], G.C. RIBEIRO FILHO[2], A.C. MERA[1], C. JERKE[1], A. CORDEIRO[1]

[1]Brazilian Agricultural Research Corporation (Embrapa Cerrados), C. Postal 08223, Planaltina, Federal District, Brazil. [*]E-mail: guerra@cpac.embrapa.br
[2]Guy Carvalho Consultant

SUMMARY

The objective of this paper was to show the improvement of the coffee production systems in two different regions caused by the application of water stress for buds development synchronization and the increasing of phosphorus dose to guarantee plant growing and buds formation in all years reducing biennial yield pattern. The total validation areas of about 3 thousands hectares composed by several farms were selected in two important coffee production regions with different climatic characteristics, Cerrado and south of Minas Gerais state. In the Cerrado region irrigation was suppressed from June 24 to September 4 and in south of Minas Gerais state from June first to September 4. In all situations fertilizers were supplied as recommended except phosphorus dose which was increased from 80 to 300 kg.ha^{-1} of P_2O_5 to provide energy for plant growing and buds formation. In the Cerrado region where dry season is very well defined no rain event occurred during suppression irrigation period resulted in unique flowering period. The results showed up to 88% of flowering uniformity and 82% of berry fruits at harvesting time. Mean yield in three consecutives harvest reached values higher than 60 bags of processed coffee per hectare. In the south of Minas Gerais state region suppression of irrigation did not result in unique flowering event due to occurrence of a rain event along suppression irrigation period. In this region the number of flowering events was reduced from 5 to 2 and an increase from 35 to 69% of berry grain. Mean yield in the last two consecutives harvesting reached values higher than 50 bags of processed coffee per hectare. In non irrigated coffee phosphorus application increased yield of two consecutives harvesting from about 35 to over 50 bags.ha^{-1} of processed coffee. In non irrigated coffee production system the nutritional equilibrium and the maintenance of plants in good sanitary conditions were the tools for improving coffee crop performance in dry spell periods which are of common occurrence in the south of Minas Gerais state.

INTRODUCTION

The sustainability of irrigated coffee system production should be supported by three factors: high yield, high grain quality and yield costs reduction. The results of the research developed in the Embrapa Cerrados by Guerra et al. (2007) allowed to fit the technology of controlled water stress in coffee trees for flowering and grain maturation uniformity, to develop a water management program which is available for free use in Embrapa`s Cerrados homepage. Also, it indicated the necessity to adjust phosphorus supply to reduce the effect of biennial pattern in coffee production. As research was being developed it was conducted several validation units in commercial production areas located in west part of Bahia state – Cerrado region and south of Minas Gerais state to ensure the benefits of developed technologies.

In spite of conflicting results from Drinman and Menzel (1994), Guerra et al. (2007), Soares et al. (2001) on the necessity of a water stress period for flowering and maturation uniformity the implementation of irrigation suppression in the right time and adequate intensity as recommended by Guerra et al. (2007), allowed demonstrate that a moderate water stress must be applied in order to optimize the coffee crop production capacity. In addition any single technology is not enough to solve all problems in irrigated coffee system production. However to reach yield stability it is necessary to achieve a nutritional equilibrium by an increase of phosphorus dose as suggested by Guerra et al. (2007). In this way plants can express their potential yield and it becomes possible to take all coffee produced increasing the value of produced coffee.

However, the objective of this paper is to present the results from technology validation units conducted in different regions on the improvement of irrigated coffee production systems.

MATERIALS AND METHOD

The total validation areas of about 3 thousands hectares composed by several farms were selected in two important coffee production regions with different climatic characteristics, west of Bahia state – Cerrado region and south of Minas Gerais state. In the Cerrado region irrigation was suppressed from June 24 to September 4 and in south of Minas Gerais state from June first to September 4. Irrigation management was carried out using as a criterion the program for coffee irrigation scheduling developed by Embrapa Cerrados and available for free use in Embrapa's homepage. A depth of 40 mm of water was applied at the end of water stress period to guarantee full blossoming. In all situations nitrogen, potassium, and micronutrients were supplied as recommended by Guimarães et al. (1999). However the phosphorus dose was increased from 80 to 300 $kg.ha^{-1}$ of P_2O_5 as recommended by Guerra et al. (2007) to provide energy for plant growing and buds formation even when coffee trees are supporting high amount of grains. The annual dose was split in two applications. Two thirds of annual dose was applied just before irrigation returns after suppression of irrigation period or before rainy season in plantation without irrigation in south of Minas Gerais state and 1/3 in December before coffee plants start fruit filling process.

Percentage of flowering, fruits maturation, yield, grains sieves and type classification and coffee drinking quality were evaluated to establish the improvement of the introduced technologies on the different coffee production systems.

RESULTS AND DISCUSSION

In the Cerrado region, where dry season is very well defined, no rain event occurred during suppression irrigation period resulting in unique flowering period about 14 days after return irrigation in September 4. The results of water stress at farms level showed up to 88% of flowering uniformity and 82% of berry fruits at harvesting time confirming the experimental results from Guerra et al. (2007). Mean yield in three last consecutives harvest reached values around 60 bags of processed coffee per hectare (Figure 1). The increase in yield resulted from several factors such as: increase in plant growing and buds formation due to adequate water management and nutritional equilibrium with increase in phosphorus annual dose, better grain filling process and reduction in harvest losses. Also, compensatory growing following water stress helped to improve growing to increase productivity levels.

In addition, the improvements of the coffee system production resulted in a reduction from 20 to 10% of the no well formed beans, increase in coffee drinking quality, reduction in 40% of

the harvest costs by allowing harvest to be accomplished at once and reduction in 42% of water and energy used in irrigation.

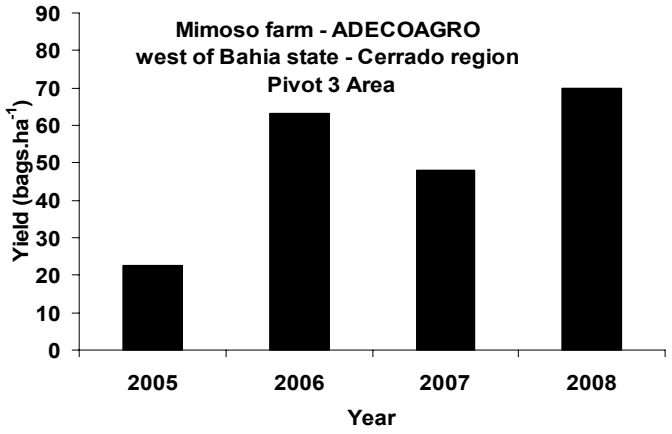


Figure 1. Increase in coffee mean yield resulted from adequate water stress and increase in phosphorus dose in Mimoso farm - west of Bahia state.

In the south of Minas Gerais state region suppression of irrigation did not result in unique flowering event due to occurrence of a rain event along suppression irrigation period. In this region it was possible to reduce the number of flowering events from 5 to 2. This resulted in increase from 35 to 68% of berry fruits at harvesting time. Mean yield in two consecutives harvesting reached values higher than 50 bags of processed coffee per hectare. Increase in phosphorus annual dose promoted plant growing and buds formation similar to that encountered in the Cerrado region. This result was not expected due to lower temperatures pattern in south of Minas Gerais state when compared to the Cerrado region.

Similar to the results from the Cerrado region improvement of the irrigated coffee system production caused reduction from 19 to 10% in no well formed beans, increase in drinking quality and 48% reduction in water and energy used in irrigation.

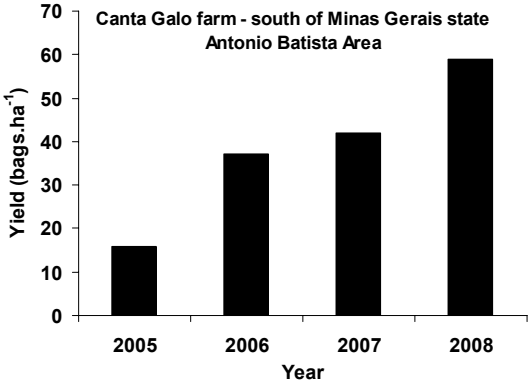


Figure 2. Increase in mean yield of no irrigated coffee as a resulted of changes in phosphorus annual dose from 80 to 300 kg.ha^{-1} of P_2O_5.

In non irrigated coffee the nutritional equilibrium caused by change in phosphorus dose increased processed coffee mean yield of two consecutives harvesting from about 35 to over 50 bags of processed coffee per hectare. In Figure 2 are presented the results from Antonio Batista production area of the Canta Galo farm in south of Minas Gerais state where mean yield increased systematically from17 bags of processed coffee per hectare in 2005 to 59

bags.ha^{-1} in 2008. In this coffee production system the nutritional equilibrium and the maintenance of adequate crop sanitary conditions were the tools for improving coffee crop performance in dry spell periods which are of common occurrence in the South of Minas Gerais state.

CONCLUSIONS

1. In the Cerrado region where dry period is very well defined controlled water stress resulted in high flowering and fruits maturation uniformity;
2. In the south of Minas Gerais state were occasional rain in the dry period occurs controlled water stress resulted in a reduction in the number of flowering events;
3. Increase in phosphorus dose resulted in adequate growing for all regions and systems production even in plants with high amounts of grain reducing the biennial production pattern.

REFERENCES

Drinman J.E. and Menzel C.M. (1994). Synchronisation of anthesis and enhancement of vegetative growth in coffee (Coffea arabica L.) following water stress during flower initiation. Journal of Horticultural Science 69: 841-849.

Guerra A.F., Rocha O.C., Rodrigues G.C., Sanzonowicz C., Ribeiro Filho G.C., Toledo P.M.R. Dos., Ribeiro L.F. Sistema de produção de café irrigado: Um novo enfoque. ITEM, Irrigação e Tecnologia Moderna, , Brasília, n. 73 p. 52-61, 2007.

Guimarães P.T.G., Garcia A.W.R., Alvarez V., Vh., Prezotti L.C., Viana A.S., Miguel A.E., Malavolta, E., Corrêa J.B., Lopes A.S., Nogueira F.D., Monteiro A.V.C. Cafeeiro. In: Ribeiro, A.C.; Guimarães, P.T.G.; Alvares, V.; Vh. (Ed.). Recomendações para uso de corretivos e fertilizantes em Minas Gerais – 5ª Aproximação, Viçosa: Comissão de Fertilidade do Solo do Estado de Minas Gerais, 1999. p.289-302.

Soares A.R., Rena A.B., Mantovani E.C., Soares A.A., Batista R.O. Estudo do efeito do déficit hídrico sobre a quebra da dormência na floração de um cultivar de café arábica irrigado por gotejamento. In: Simpósio de Pesquisa dos Cafés do Brasil (2.: 2001: Vitoria, ES) Resumos do II Simpósio de Pesquisa dos Cafés do Brasil. Brasília: Embrapa Café, 2001. p. 48-49.

Water Deficit in Arabica Coffee Trees as Affected by Irrigation Regimes in the Cerrado Region

O.C. ROCHA, A.F. GUERRA, G.C. RODRIGUES, C. SANZONOWICZ, A.C. MERA, C. JERKE, A. CORDEIRO

Brazilian Agricultural Research Corporation (Embrapa Cerrados), C. Postal 08223, Planaltina, Federal District, Brazil. E-mail: omar@cpac.embrapa.br

SUMMARY

Water application management on coffee crop may affect root growth and development and, consequently, the pattern of water extraction on the soil profile. Recent results on irrigation strategies for coffee (*Coffea arabica* L.) production in the Cerrados region has indicated that a period of around 70 days of irrigation suppression cause a high level of buds development synchronization resulting in uniform flowering and fruits maturation. The best period for irrigation suppression is from the end of June to the beginning of September. The economy of applied water and energy of around 33% resulted by this temporary irrigation suppression. However the impact of the reduction in applied water on the coffee water extraction from soil profile deserves further studies to explain the real intensity of the water deficit on the coffee trees. The research was carried out in the experimental field of the Brazilian Agricultural Research Corporation (Embrapa Cerrados) located in Planaltina, DF, Brazil. Coffee trees were submitted to two irrigation regimes: irrigation all over the year when soil water potential reached -0.05 MPa (IR1); irrigation suppression in the dry season until leaf water potential measured at predawn with Sholander pressure pump reached values around -2.0 MPa (IR2). Soil water content was measured by using soil probes installed at 0.1, 0.3, 0.5 and 1.0 m from soil surface to allow the calculation of soil water extraction on the soil profile. Daily soil water content was measured in two different periods. The results show that in both years the accumulated evapotranspiration during the irrigations suppression periods, calculated by Penman-Montheith method, reached values of 231 and 284 in 2003 and 2007, respectively. In 2003 the accumulated water extractions during irrigation suppression period were 443 and 218 mm in IR1 and IR2, respectively. Similar results were encountered during irrigation suppression period in 2007 when accumulated water extraction resulted in 370 and 135 mm for IR1 and IR2 respectively, demonstrating that in fact plants subject to long irrigation suppression period can still draw about 30% of water demand using soil water reserves.

INTRODUCTION

The Brazilian Cerrados region presents adequate conditions for the development of irrigated coffee and already contributes with 40% of national production. Recent results on irrigation strategies for coffee (*Coffea arabica* L.) production in the Cerrado region has indicated that a period of around 70 days of irrigation suppression cause a high level of buds development synchronization resulting in uniform flowering and fruits maturation (Guerra et al., 2007; Rocha et al., 2007).

The objective of this research was to evaluate the magnitude of the coffee real water deficit due to irrigation suppression for buds synchronization and compare this value with the water deficit estimated by evapotranspirometric demand estimated by Penman-Monteith method.

MATERIAL AND METHODS

The research was carried out in the experimental field of the Brazilian Agricultural Research Corporation (Embrapa Cerrados), located in Planaltina, DF, Brazil. Coffee trees, c.v. catuaí rubi, irrigated by center pivot were implanted in January of 2001 and submitted to two irrigation regimes: irrigation all over the year when soil water potential reached -0,05 MPa (IR1) and irrigation suppression in the dry season until leaf water potential measured at predawn reached values around -2,0 MPa (IR2). The readings of leaf water potential were made with a pressure pump between 3:00 and 5:30 a.m. (Scholander et al., 1964).

Soil water content was measured by using soil probes (Theta probe, model ML2) installed at 0.1, 0.3, 0.5 and 1.0 m depth. Daily soil water content was measured at 8:00 a.m. in two different periods (Figure 1 and 2).

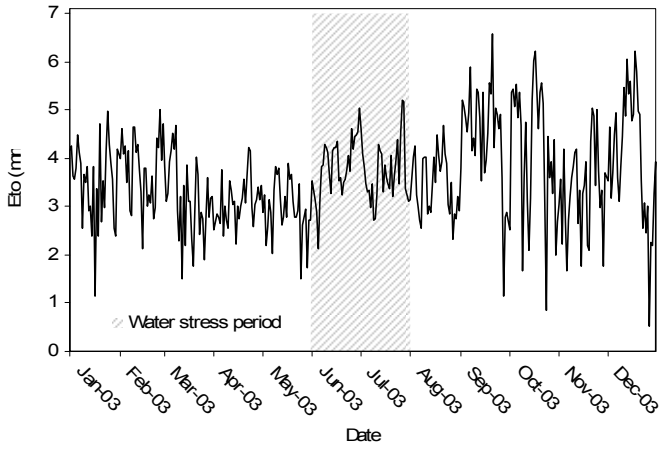


Figure 1. Evapotanspirometric demand in 2003 estimated by Penman-Monteith combination equation in Planaltina, Federal District, Brazil.

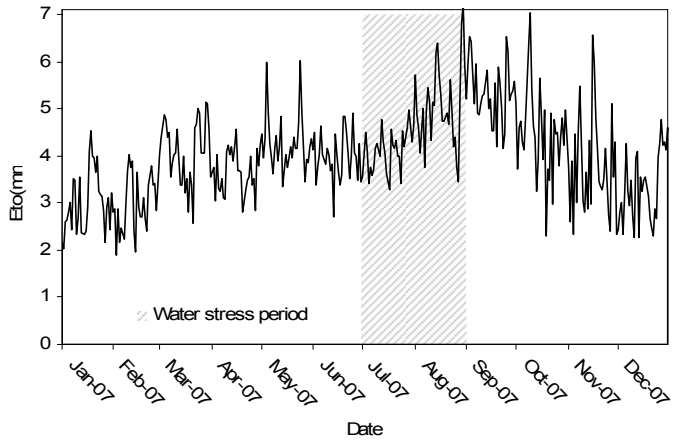


Figure 2. Evapotanspirometric demand in 2007 estimated by Penman-Monteith combination equation in Planaltina, Federal District, Brazil.

The measurements were taken during irrigation suppression in 2003 after two irrigation suppression periods and, in 2007 after six irrigation suppression periods. In 2003 the water suppression interval ranged from June 1 to July 31. In 2007 this interval ranged from July 1 to August 31. Water deficit for each period was estimated by using coffee soil water extraction and evapotanspirometric demand estimated by Penman-Monteith combination equation.

RESULTS AND DISCUSSION

The evapotranspirometric demand estimated by Penman-Monteith combination equation, during the irrigations suppression periods, were the 231 and 284 mm in 2003 and 2007, respectively (Figure 3). During irrigation suppression period coffee soil water extraction in 2003 were 297 and 98 mm in IR1 and IR2, respectively. Lower coffee water extraction were encountered during irrigation suppression period in 2007 when accumulated water extraction resulted in 294.8 and 85.7 mm for IR1 and IR2, respectively (Figure 3). It occurred because coffee trees presented a lower leaf area index due to pruning in August of 2006.

Calculating the water deficit from the difference between the evapotranspiration estimated by Penman-Monteith method and the depth of water extracted from soil profile by coffee trees in the two irrigation suppression periods it can be verified that water deficit were 133 and 198 mm in 2003 and 2007, respectively. The lower water deficit in 2003 may be related to the differences in climatic conditions in the two períods of the irrigation suppression.

However, when IR1 is compared with IR2 in relation to the amount of water extracted from soil profile, it may be observed that IR2 extracted 199 and 209 mm less water in 2003 and 2007, respectively. It demonstrate a similar water deficit in the two studied periods.

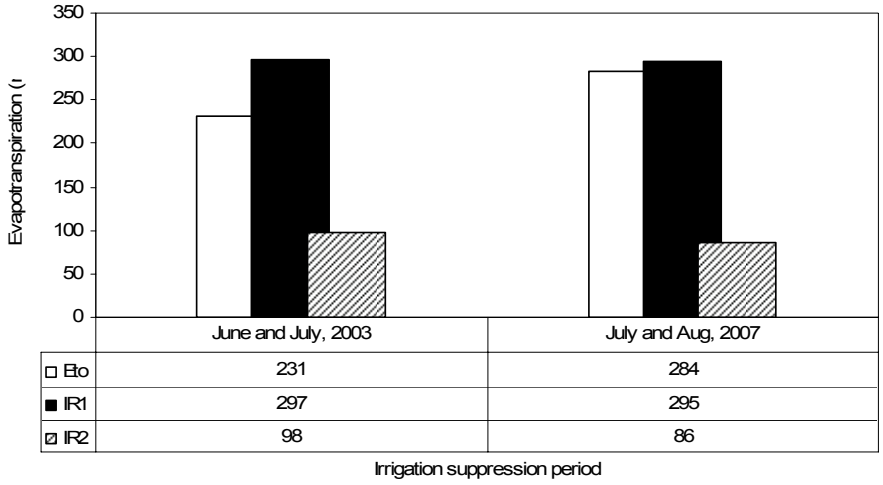


Figure 3. Comparison between evapotranspiration calculated by soil water extraction and estimated evapotranspiration from Penman-Moteith method in two irrigation regimes in the Cerrado Region.

CONCLUSION

1. Coffee trees subject to irrigation suppression period can still extract about 30% of the hole water extracted in fully irrigated crop;
2. The water deficit during the period of irrigation suppression was lower than the one indicated by climatological method.

REFERENCE

Guerra A.F., Rocha O.C., Rodrigues G.C., Sanzonowicz C. Manejo da irrigação do cafeeiro, com uso do estresse hídrico controlado, para uniformização de florada. In: ZAMBOLIM, L.(ed.). Boas práticas agrícolas na produção de café. 1. ed. Viçosa: UFV, DFP, 2007d. p. 83-115.

Rocha O.C., Guerra A.F., Rodrigues G.C., Sanzonowicz C., Silva F.A.M., Ribeiro L.F., Toledo P.M.R. Manejo estratégico de irrigação para a cultura do café (*Coffea arabica* L.) no Cerrado

brasileiro. In: Simpósio de Pesquisas dos Cafés do Brasil, 5, 2007, Águas de Lindóia. Anais. 2007. p. 70-76.

Scholander P.F., Hammel H.T., Hemingsen E.A. and Bradstreet E.D. 1964. Hydrostatic pressure and osmotic potencials in leaves of mangroves and some other plants. Proceedings of National Academy Science 51: 119-125.

Temporary Shading of Coffee (*Coffea arabica*) Plantations with Pigeonpea *(Cajanus cajan)* in Southern Brazil

H. MORAIS, P.H. CARAMORI, R.G. HUGO, A. ANDROCIOLI FILHO

Instituto Agronômico do Paraná, Rod. Celso Garcia Cid, Km 375,
86047-902 Londrina, PR, Brazil

SUMMARY

Temporary shading of coffee plantations with pigeonpea is a technique idealized by a coffee farmer in the North of Paraná state, Brazil, in the beginning of the decade of 1980. Since then it has been investigated at IAPAR and nowadays it is used by many coffee farmers due to its advantages. One of the benefits of this technique is the protection of coffee plants against frost. During the winter the dossel of pigeonpea intercepts a significant fraction of the longwave radiation emitted at night by the soil and plants. Studies have shown that minimum air and leaf temperatures of the young coffee plants during winter nights were between 0 and 2.3 °C warmer inside the areas shaded with pigeonpea. Dense shading of coffee plants with two years also reduced frost damages, decreased transpiration and increased superficial soil moisture, but dense shading led to a reduction on photosynthesis and number of nodes per branch, causing a decrease of 80% in coffee yield. Appropriated management of shade through pruning can bring excellent results during the reproductive period, especially under stresses of water and high temperature. Results with a three years old coffee crop showed that under conditions of temperatures above normal and water deficit during grain filling, shaded plants had a coffee production about 20% higher than plants grown under full sun. Besides, coffee grains of the shaded plots had higher grain size and weight. These results demonstrate the high potential of pigeonpea for consorciation with coffee in the North of Paraná.

INTRODUCTION

Coffee cropping is a strategic agricultural activity in the Brazilian economy as it is a major source of foreign exchange and jobs. One of the peaks of coffee production in Brazil occurred in the decades of 1970 and 1980, due to factors such as new cultivars with genetic improved characteristics, implantation of crops with high density planting, use of fertilizers and spray for pests and diseases, and intensification of agricultural mechanization. However, the indiscriminate use of agrochemicals and inadequate management caused disequilibrium in many plantations and in the environment, raising the cost of coffee production and contaminating/degrading land and water resources.

In this scenario, researchers and farmers were involved in the search for alternatives to recover soil fertility, reduce costs and preserve the environment. Agroforestry Systems (AFS) is a sustainable model of cropping, which responded positively to the challenges of the new demands of the coffee crop. Its success was mainly due to easy adaptation to shade conditions of the arabic coffee, originated in the understoreys of forests in Ethiopia and Sudan.

Among the species used in the AFS, pigeonpea has shown a great potential. This consorciation was idealized by a coffee farmer in the North of Paraná state, at the beginning of the decade of 1980. Results obtained reveal its contribution to biomass production, nutrient recycling and atmospheric nitrogen fixation, production of grains and seeds, use as wind

breaks and erosion control (Boehringer and Caldwell, 1989). Once associated with the coffee crop, in addition to all these benefits, contributes to better establishment of the coffee seedlings and to minimize damage caused by severe frosts (Caramori et al., 1999).

This system offers other advantages such as: weed reduction; less occurrence of pests and diseases, especially those caused by bacteria and coffee leaf miner; prevention of physical damages and injuries due to wind frosts; possibility of use for animal and human feeding; soil and water conservation. In this paper are presented some results of researches carried in the North of Paraná with consorciation of coffee and pigeonpea.

MATERIAL AND METHODS

A field experiment was carried out in Londrina (23° 23' S, 51° 11' W), in the North of Paraná State, Brazil (Caramori et al., 1999), in a uniform area with slope of about 2% completely surrounded by coffee plantations. Coffee seedlings of the cultivar IAPAR 59, with six pairs of leaves, were planted spaced 1.5 m by 0.8 m, during the first week of January 1998. The area was split into two blocks of 60 m by 50 m. One of them was kept unshaded and in the other, one line of pigeonpea cultivar IAPAR PPP-1264 was manually sown between the coffee rows, at the density of 6 plants per meter. In the beginning of June, the pigeonpea plants were about 3.5 m tall and covering the coffee plants almost completely. Microclimate underneath the dossel of pigeonpea was monitored with thermocouples of cooper-constantan attached to the lower side of two uppermost fully-expanded leaves of coffee plants.

Another field work was conducted in Londrina, Brazil (Morais, 2003), to assess the effects of consorciation with pigeonpea on microclimate, physiology and productivity of coffee, compared to an open-grown plot. The experiment was conducted from December 2000 to August 2002, with the coffee cultivar IPR 99, planted in December 1998, in the spacing of 2.5 x 0.70 m. The experimental area was 6,000 m^2, divided in two sub-areas containing open-grown and consorciated coffee. Pigeonpea was sown between the coffee rows in the density of 3 plants per linear meter in December 2000, reaching its maximum growth in May 2001 (4 m height), when it covered the coffee plants completely. In September 2001 pigeonpea was pruned at 60 cm height, and resumed its growth again, reaching its maximum height in April 2002.

The effects of pigeonpea management on coffee production in conditions of stress of high temperature and water deficit were assessed in a field experiment carried in Abatiá, North of Paraná, Brazil (Cruz et al., 2002). The experiment was installed in July 2001 and consisted of three treatments of four repetitions in randomized blocks. The following treatments were evaluated: pigeonpea without management; pigeonpea with side pruning (managed); and coffee in monoculture. The assessment of coffee production was made in July 2002.

RESULTS AND DISCUSSION

Observed leaf temperature daily amplitudes were much higher in the plots exposed to full sun (Caramori et al., 1999). Differences ranged from 0.3 to 5.8 °C for maximum and from 0 to 2.3 °C for minimum. During typical conditions of radiative frosts in Londrina the minimum leaf temperature in the shaded plot was between 2.0 °C and 2.3 °C higher. The effect on maximum temperature was normally more pronounced, due to the impact of solar radiation interception during the daytime (Caramori et al., 1999). At night the dossel of pigeonpea intercepts a significant fraction of the longwave radiation emitted by the soil and plants. This will decrease the rate of night cooling, contributing to minimize damages of radiative frosts. Average maximum air temperature reduction of 5.4 °C and average minimum air temperature

increase of 1.5 °C within a coffee plantation shaded by 205 trees/ha of *Inga jinicuil* were also reported in Mexico (Barradas and Fanjul, 1986). In Brazil, observed minimum coffee leaf temperatures were between 2 and 4 °C higher in an area shaded with 250 trees ha^{-1} of *Mimosa scabrella*, during nights with radiative frost (Caramoriet al., 1996). Differences of 2.5 to 3.5 °C for maximum leaf temperature and about 0.5 °C for minimum leaf temperature were reported in shaded maize (Allen et al., 1997).

Coffee shade reduced the levels of global solar radiation, net radiation and photosynthetically active radiation, decreased leaf, air and soil temperatures during the day and reduced the rate of air and leaf night temperature drop, especially during frost episodes (Morais, 2003). Arborization changed the energy balance of the system, reducing the risk of damage caused by temperature extremes, reduced transpiration and increased surface soil moisture, but dense shading led to a reduction on photosynthesis and number of nodes per branch, causing a decrease of 80% in coffee yield. There are evidences that excessive shading also affects coffee physiology, reducing the development of reproductive structures (Fahl and Carelli, 1994).

Production of dry coffee was similar in the open-grown and in consorciation with pigeonpea. However, each bag of dry coffee with 40kg resulted in 15.4 and 19.4 of clean coffee for the open-grown and shaded treatments, respectively, with an average production per plot of 32.59 kg in the open-grown and 40.74 in the shade (Cruz et al., 2002). The lower production of coffee in the open can be explained when considering the agroclimatic conditions of the period. In the months of January to July 2002, the average air temperature remained 1 to 2 °C above normal, and in the period of February to May 2002 there was a severe soil water deficit. This situation of high demand for water and nutrients not met by the coffee plants due to water deficit, resulted in fruits with smaller size and weight, reducing the final production of coffee in the plots under full sun.

REFERENCES

Allen S.J., Roberts J.M., Smith D.M. et al. Modelling the modification of crop microclimate by spaced tree canopies. In: Agroforestry Modelling and Research Co-ordination Annual Report, 1996- 1997. Edinburgh: Institute of Terrestrial Ecology, 1997. p. 61-70, (ODA Forestry Research Programme, Project R5652).

Barradas V.L., Fanjul L. Microclimatic chacterization of shaded and open-grown coffee (*Coffea arabica* L.) plantations in Mexico. Agricultural and Forest Meteorology, n.38, p.101-112, 1986.

Boehringer A., Caldwell R. *Cajanus cajan* (L) Millsp. as a potential agroforestry component in the Eastern of Zambia. Agroforestry Systems, n.9, p.127-140, 1989.

Caramori P.H., Androcioli Filho A., Leal A.C. Coffee shade wit *Mimosa scabrella* Benth. For frost protection in southern Brazil. Agroforestry Systems, n.33, p.205-214, 1996.

Caramori P.H., Leal A.C., Morais H. Temporary shading of young coffee plantations with pigeonpea (*Cajanus cajan*) for frost protection in southern Brazil. Revista Brasileira de Agrometeorologia, v.7, p.195-200, 1999.

Cruz R.F.R., Hugo R.G., Caramori P.H., Morais H. Efeito da proteção de cafezal com guandu na primeira colheita após o plantio, em condições de alta temperatura e deficiência hídrica In: 28º Congresso Brasileiro De Pesquisas Cafeeiras, 2002, Caxambu, MG. Anais... Caxambu: MAPA-PROCAFÉ, 2002. p.392–393.

Fahl J.I., Carelli M.L.C. Influência do sombreamento nas características fisiológicas envolvidas no crescimento de espécies de coffea. In: Simpósio Internacional Sobre Café Adensado, 1994, Londrina, PR. Anais... Londrina: IAPAR, 1994, p.289-290.

Morais H. Efeitos do sombreamento de cafeeiros (*Coffea arabica* L.) com guandu (*Cajanus cajan*) no norte do Paraná. 2003. 118 f. Tese (Mestrado) - UEL, Londrina, 2003.

Technological Change and Development of Coffee Plantation: a Case Study of Kodagu District

K.B. KIRAN

Assistant Professor, Department of Humanities, Social Sciences and Management, National Institute of Technology Karnataka, Surathkal, Mangalore, India

SUMMARY

The importance of technological advance to economic growth has become an accepted fact. Technological change and efficiency improvement are important sources of production growth in any economy. Coffee industry in Kodagu District registered a remarkable progress during the period from 1956-57 to 2003-04, the area under coffee increased steadily by three-fold from 24,321 ha to 82,554 ha. The productivity has also doubled from 659 kg/ha to 1125 kg/ha during the same period. The compound growth rate for area, yield and production of coffee for the period 1956-57 to 2003-04 was worked out as 2.89 per cent, 1.17 per cent and 4.2 per cent respectively, per annum indicating that both the expansion of area and improvement in yield have contributed to the growth in production. The analysis of decomposition technique to study the relative contribution of area and yield improvements to output growth indicated that the relative shares of area and yield to the production of coffee during the sixties, seventies and eighties were, by and large, similar in nature. The area effect was very strong in these decades. This analysis, however, highlights that during the decades mentioned above, both the yield effect and interaction effect were negative, while the area effect was phenomenal. Whereas in nineties, both the area and yield effect contributed almost equally to the production growth while, in the beginning of this century, the area effect was strong compared to yield effect. Interaction effect was weak for both the decades. It is clear from the study that the production growth in coffee in the seventies and the eighties has taken a different path with negative yield effect.

INTRODUCTION

The importance of technological advance to economic growth has become an accepted fact. Technological change and efficiency improvement are important sources of production growth in any economy. Technological change is one of the most crucial factors determining the pattern and pace of agricultural growth.

TRENDS IN AREA, PRODUCTION AND PRODUCTIVITY OF COFFEE IN KODAGU (1956 TO 2004)

Principally Kodagu grows two varieties of coffee, namely Arabica and Robusta. The bulk of the area and production is that of Arabica in North Kodagu, whereas, it is Robusta in the case of South Kodagu. According to the 1972-75 census of the coffee orchards in India, Kodagu District had the largest number of coffee orchards producing 1/3rd of the country's production. In 1972-75, the area under coffee in Kodagu District was 42,320 hectares[1]. The area under coffee increased to 60,289 hectares in 1980-81 and further to 75,098 hectares in 1992-93;

[1]Suryanatha U. Kamath (ed), 1993, Gazetteer of India, Karnataka State, Kodagu District. A Government of Karnataka publication, Bangalore, p. 244.

which formed 27 per cent of the total planted area under coffee in India. The production has shot up from 41,085 tonnes (1985-86) to 64,000 tonnes (1992-93) or 40 per cent to the India's total production of 1,61,500 tonnes. In this context a study of the growth rates in area, production and productivity assumes importance.

Karnataka holds the key to coffee production in India. Although India accounts for only about four per cent of world production, Karnataka contributes about 70 per cent of India's production. It is gratifying to note that the area under coffee and its production have been increasing over the past thirty years. There are approximately 1.78 lakh coffee holdings in India, of which 98% come under small holder category (10 ha and less). These small holdings occupy 65% of the total area under coffee and contribute around 60% of the country's production. The remaining 2% of the holdings come under large grower sector (above 10 ha) and occupy 35% of the area and contribute 40% to the total production. Kodagu can be compared to some other coffee-growing areas. While in Kerala small plantations are very dominant, in Chikmagalur, second highest coffee growing district of Karnataka, the number of very large estates is higher than in Kodagu. The percentage of small plantations (< 10 ha) to the total area under coffee in Kodagu, Wyanad (largest coffee growing district of Kerala) and Chikmagalur is 62%, 85% and 30% respectively. Besides, the tree cover in Kodagu plantations shows a much higher diversity than in those of Chikmagalur where silver oak and dadap are the only shade trees in most plantations. The distribution of coffee area among small, medium and large holdings, to a large extent, achieves a more equitable sharing of income.

Coffee industry in Kodagu District registered a remarkable progress during the last century. During the period from 1956-57 to 2003-04, the area under coffee increased steadily by three-fold from 24,321 ha to 82,554 ha. The productivity has also doubled from 659 kg/ha to 1125 kg/ha during the same period. India stands in third position in terms of productivity next only to Vietnam (2000 kg/ha) and Costa Rica (1500 kg/ha). Productivity is far ahead of major coffee producers viz. Brazil (738 kg/ha) Colombia (810 kg/ ha) and Indonesia (539 kg/ha). As a result, coffee production has reached the level of 1,00,000 tonnes from a small 16,037 tonnes some fifty years ago with a remarkable increase of six folds.

To make the production analysis more incisive and to apportion the effects of area and productivity (yield/ha) on production, growth rates for area, production and productivity were worked out separately (Table 1.1). It could be noted from the table 1.1 and figures 1.1, 1.2 and 1.3 that the compound growth rate for area, yield and production of Coorg coffee for the period 1956-57 to 2003-04 was worked out as 2.89 per cent, 1.17 per cent and 4.2 per cent respectively, per annum indicating that both the expansion of area and improvement in yield have contributed to the growth in production.

The decade wise growth rates indicated that the higher growth in production (8.83percent) during 1950s is equally through the area growth (4.75 per cent) and productivity growth as the adoption of high yielding improved varieties/ selections of coffee have taken place on large scale in the early 50s. In later decades (1960s to 1990s), increase in area and productivity have contributed to the growth of coffee production except in the 1970s where the productivity growth rates were negative is mainly due to price fluctuations and very low prices during that period.

The most important point that should be driven home here is, Robusta trends are outstripping Arabica in terms of area and production. During the last decade, it is interesting to note that Robusta production had surpassed Arabica production at a faster rate. The tremendous increase in area as well as production of Robusta could be attributed to several factors.

Foremost among them are, Robusta demands lesser maintenance and care than does Arabica and that there has been an element of price responsiveness to Robusta area. Further responsiveness to irrigation during blossom and backing showers has improved productivity of Robusta to a greater extent. As India is considered to be the producer of 'mild Arabica', a proper policy mix to maintain adequate proportion of area of Arabica and Robusta is important.

The analysis of decomposition technique to study the relative contribution of area and yield improvements to output growth indicated that the relative shares of area and yield to the production of Coffee during the sixties, seventies and eighties were, by and large, similar in nature. The area effect was very strong in these decades. This analysis, however, highlights that during the decades mentioned above, both the yield effect and interaction effect were negative, while the area effect was phenomenal (Table 1.2). Whereas in nineties, both the area and yield effect contributed almost equally to the production growth while, in the beginning of this century, the area effect was strong compared to yield effect. Interaction effect was weak for both the decades. It is clear from the study that the production growth in coffee in the seventies and the eighties has taken a different path with negative yield effect.

MATERIALS AND METHODS

The data was obtained from the various issues of 'Coffee Statistics' published by the Coffee Board of India.

The compound growth model used was an exponential function of the form:

$$Y=AB^{x}$$

Where, Y = Variable for which the growth rate was calculated

A = Constant
B = 1 +r, r being the growth rate
X = Time period in years

The estimated form of the function was the following type;

$$\text{Log } Y = \log A + X \log B$$

where, log A and log B are obtained by ordinary least square method.

Further, the relative contribution of area and yield improvements to growth of output is studied with the help of the Minhas and Vaidyanathan (1965) decomposition technique for last five decades using the following formulae (Das, 1989).

$$Pn - Po = Ao\,(Yn - Yo) + Yo\,(An - Ao) + (An - Ao)\,(Yn - Yo)$$

where, Pn = Level of output in the terminal year n
Po = Level of output in the year 0
An = Area under the crop in the year n
Ao = Area under the crop in the year 0
Yn = Productivity per unit area in the year n
Yo = Productivity per unit area in the year 0

Research studies conducted on the experiment stations and demonstration fields have shown that it is possible to obtain an average yield of 2000 kg/hectare[2]. Also, many progressive planters in the district have succeeded in this direction. The use of new technology consisting of HYV plants, fertilizers, pesticides and sprinkler irrigation is gaining in popularity in the large estates and explains the much higher yield of their plantations. Though, at present, improved coffee production technology is available to the growers, the average yield in small-holder plantations are far below the demonstrated levels of yield or the yield rates realized by large growers' sector. Generally, the yields obtained by the small growers have been in the range of 500 to 850 kg/hectare. Thus, there exists a wide gap between the yields obtained by the growers and the yield that can be realized by adopting the improved technology. Kodagu should also aim for this yield. It will not only help the individual better his economic status, but will have a favourable impact on the country's coffee production.

In the sample area the tendency is more towards Robusta cultivation than Arabica coffee. The reasons for this leap forward in area and production of Robusta are very many. The foremost among them is the comparatively lesser maintenance care that is needed by Robusta than Arabica. Secondly there had been an element of price responsiveness especially during 1970s to Robusta and the preference to Robusta to Arabica for instant coffee manufacturing and blends has been an added factor for its increasing demand.

Table 1.1 Trends in area, production and productivity of Coffee in Kodagu (1956 to 2004).

Year	Planted area (ha)			Production (Tonnes)			Productivity (kg/ha)		
	Arab.	Rob.	Total	Arab.	Rob.	Total	Arab.	Rob.	Overall
1956-57	12132	12189	**24321**	11070	4967	**16037**	912	407	**659**
1960-61	13342	14310	**27652**	13570	10735	**24305**	1017	750	**879**
1970-71	16790	16031	**32821**	21725	20820	**42545**	1294	1299	**1296**
1980-81	22051	25506	**47557**	17808	20102	**37910**	808	788	**797**
1990-91	27480	46792	**74272**	28000	42000	**70000**	1019	898	**942**
2000-01	26100	55250	**81350**	27600	80100	**107700**	1057	1450	**1324**
2001-02	26100	56200	**82300**	28800	73800	**102600**	1103	1313	**1247**
2002-03	26100	56250	**82350**	23950	68625	**92575**	918	1220	**1124**
2003-04	25904	56650	**82554**	24750	68150	**92900**	955	1203	**1125**
Growth Rate %									
Year	**Planted Area (ha)**			**Production (Tonnes)**			**Productivity (kg/ha)**		
	Arab	**Rob**	**Total**	**Arab**	**Rob**	**Total**	**Arab**	**Rob**	**Total**
56-57 to 59-60	4.72	4.86	**4.79**	1.58	21.84	**8.83**	-2.99	16.25	**3.87**
60-61 to 69-70	2.59	0.75	**1.67**	2.68	0.95	**2.08**	0.09	0.2	**0.41**
70-71 to 79-80	2.96	5.3	**4.2**	1.92	6.56	**4.15**	-1.01	1.19	**-0.01**
80-81 to 89-90	1.56	5.63	**3.89**	0.52	4.32	**2.62**	-1.01	-1.24	**-1.22**
90-91 to99-00	-0.61	1.75	**0.92**	-0.25	5.25	**3.31**	0.36	3.44	**2.38**
00-01 to 03-04	-0.23	0.76	**0.45**	-4.99	-5.42	**-5.32**	-4.76	-6.14	**-5.75**
56-57 to 03-04	**1.72**	**3.71**	**2.89**	**2.24**	**5.71**	**4.2**	**0.62**	**1.93**	**1.27**

[2]Nithya Shree D. A. and Siddaramaiah B. S., May 1993, 'An Analysis of Yield Gap in Coffee', Indian Coffee, Vol. 57, No. 5, Coffee Board, Bangalore, P. 9.

Table 1.2 Percentage contributions of area, yield and their interaction to the growth of Coffee production in Kodagu (1956-2004).

Period	Area effect (%)	Yield effect (%)	Interaction effect (%)	Total (%)
56-57 to 59-60	56	38.65	5.35	100
60-61 to 69-70	198.6	-70.56	-28.04	100
70-71 to 79-80	223.99	-88.91	-35.08	100
80-81 to 89-90	261.18	-103.51	-57.67	100
90-91 to99-00	15.53	78.55	5.92	100
00-01 to 03-04	110.75	-9.13	-1.62	100
56-57 to 03-04	**49.94**	**14.75**	**35.31**	**100**

CONCLUSION

The previous research studies have brought to focus education of farmers and the possession of irrigation facility had positive and significant relationship with the knowledge of improved practices of coffee plantation. Similarly extension participation and extension contact of farmers had significant and positive relationship with the knowledge as well as adoption of improved cultivation of coffee. These findings indicate that farmers, who are educated, possessed sprinkler irrigation facility, maintained regular contact with extension agency and participated in extension activities, had more knowledge of improved practices and adopted these practices to a greater extent.

Coffee growers (small farmers) have medium to high level of knowledge and adoption of improved cultivation practices. Many practices have not been adopted fully by the farmers. In respect of many improved practices, farmers lack complete knowledge and this is not a desirable situation. Farmers must possess very sound knowledge of the practices and adopt them fully to obtain best yields. This calls for intensive educational efforts by the Coffee Board. Suitable learning opportunities have to be created through discussion, meetings, field demonstrations, field days, field visits and other suitable extension literature in the form of folders, booklets must be distributed to the farmers.

REFERENCES

Bavappa K.V.A., 1987. Role of coffee and tea plantation in altering farming system. Proc. Symposium on Alternate Farming System. Indian Society of Agronomy, New Delhi.

Bisaliah S., 1978. Decomposition analysis of employment change new production technology in Punjab Agriculture. Indian Journal of Agricultural Economics 32(4): 193-201pp.

Das Prafulla K., 1989, Production trends, market instability and remedial measures in beverage crops. J. Plantn Crops 16 (Supplement): 119-131.

Kiran K.B., 2007, Technological change and development of coffee plantation: a case study. Ph*.D., Thesis* submitted to Mangalore University.

Minhas B.S. and Vaidyanathan A., 1965, Growth of crop output in India: 1951-54 to 1958-61, An analysis by components. J. Indian Soc. Agric. Stat. 29(2): 230-252.

Thirtle C.G., 1985. Technological change and the productivity slowdown in field crops. Southern Journal of Agricultural Economics. 17(2): 33-42Pp.

Venkataramanan L.S. and Prahladachar M., 1980, 'Growth Rates and Cropping Pattern Changes in Agriculture in Six States: 1950-1975', Indian Journal of Agricultural Economics, 35(2): 71-84.

Determining the Critical Period of Weed Competition in Young Clonal Robusta Coffee in Ghana

F.K. OPPONG*, K. OPOKU-AMEYAW AND F. OWUSU ANSAH

Cocoa Research Institute of Ghana, P. O. Box 8, Tafo-Akim, Ghana.
*E-mail: foppong@crig.org

SUMMARY

Field trials were set up in Ghana to determine the critical period of weed competition in young Robusta coffee plants during establishment. Nine-month old clonal coffee plants were transplanted at 1.5 m x 1.5 m with each plot containing nine plants. Manual clean weeding treatments for different durations were imposed on the plots. The treatments consisted of clean-weeding the sub-plots at 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48 weeks after which weeds were allowed to grow until 52 weeks after transplanting. Alternatively, weeds were allowed to grow in the sub-plots for the same durations after which clean-weeding was done until 52 weeks after transplanting. Keeping the plots weed-free for 52 weeks or allowing weeds to grow in the plots for 52 weeks constituted the control treatments. The trial was repeated for three consecutive years. The pooled 3 years growth data showed that keeping the plots weed-free during the initial 12 weeks after transplanting and not weeding again until 52 weeks after transplanting retarded the growth of the coffee plants. Plant growth was however not adversely affected when the plots were not weeded during the initial 12 weeks after transplanting but subsequently clean-weeded until 52 weeks after transplanting. The critical period of weed competition of Robusta coffee during establishment was found to be 12-28 weeks after transplanting. Tolerance of the young Robusta coffee plants to weed competition during the initial 12 weeks after transplanting implied that a weeding interval of 12 weeks would not adversely affect the growth of young Robusta coffee in Ghana.

INTRODUCTION

Robusta coffee thrives well in area with fertile soils and relatively high and well distributed rainfall with adequate sunlight. These edaphic and climatic conditions also favour the rapid growth of weeds which compete with the coffee plants for these resources. Effective and timely control of weeds in coffee farms therefore plays a major role in the growth and productivity of coffee. In Ghana, several weed control strategies have been recommended for managing weeds during the early stages of field establishment and subsequent growth of coffee plants. These include manual weeding of coffee farms at least four times per annum (Ampofo and Osei Bonsu, 1988), applying herbicides such as glufosinate ammonium, glyphosate or sulfosate (Oppong et al., 1998; 1999; 2006a) or intercropping with suitable food crops (Opoku Ameyaw et al., 1999; 2003). Indeed in most coffee growing areas in Ghana, weed resurgence occurs 6-8 weeks after weeding making it difficult for farmers to cope with the high cost of labour for weeding. Although herbicide application has been demonstrated as a cost-effective option (Oppong et al., 1999) for coffee farmers in Ghana, emerging market trends towards preference for coffee produced with minimal use of agrochemicals calls for alternative strategies to reduce the cost of weed control without compromising on the quality of green coffee.

Controlling weeds within specific periods in the growth cycle of crops has been shown to reduce competition between crop plants or crop plants and weeds (Nieto et al., 1968; Zimdahl, 1988; Swanton and Wiese, 1991 and Knezevic et al., 2002). Generally, the presence of weeds before or after the critical period of weed competition has little effect on the growth, development and yield of crops. This paper reports on studies to determine the critical period of weed competition in young clonal Robusta coffee plants with the aim of reducing the frequency and cost of weeding during initial field establishment.

MATERIALS AND METHODS

The trial was established at the Cocoa Research Institute of Ghana, Tafo. The weed species on the plot were recorded before preparing the plot for planting. Nine-month old uniform coffee clones were transplanted at 1.5 x 1.5 m in June 2002 in plots, each containing nine plants with 2.0 m separating the individual plots. Two sets of manual weeding treatments were imposed on the plots. The first set of treatments consisted of maintaining the plots weed-free for durations of 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44 or 48 weeks, after which weeds were allowed to grow until 52 weeks after transplanting. In the second set of treatments, weeds were allowed to grow for durations of 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44 or 48 weeks, after which the plots were maintained weed-free until 52 weeks after transplanting. The control treatments consisted of keeping the plots weed-free for the entire 52 weeks or allowing weeds to grow in the plots throughout the 52-week period. The trial was replicated three times.

Plant girth at 10 cm from the soil level, height, number of leaves and laterals were recorded at quarterly intervals. Dry weights of the plants were determined at 52 weeks after transplanting by oven drying at 80 C for 48 hours. The experiment was repeated for two more growing seasons and the pooled data were analysed using the Genstat Statiscal software version 11.

RESULTS AND DISCUSSION

The results on girth increments, number of leaves and laterals as well as dry weight showed that keeping the plots weed-free during the initial 12 weeks after planting and not weeding again thereafter adversely affected the growth of the young coffee plants. Alternatively, leaving the young plants unweeded during the initial 12 weeks after planting and keeping the plots free of weeds until one year after planting had no adverse effects on the plants. Significantly higher ($P \leq 0.05$) girth increment, number of leaves and laterals as well as dry weight were recorded at 4 and 8 weeks for coffee plants kept initially in weedy conditions than those which were maintained weed-free for the same duration initially but not weeded thereafter (Table 1). With the exception of plant height, similar results were recorded for all the growth parameters at 12 weeks after transplanting but the differences were not significant. Generally, there were no significant growth differences between the weed-free and weedy treatments at 16 or 20 weeks after transplanting. However, maintaining the plots weed-free for 24 weeks and beyond resulted in significantly better ($P \leq 0.05$) growth than when the coffee plants were left unweeded for the same durations before clean-weeding the plots (Table 1). Plant height did not follow the same trend as the other growth parameters. This may be due to the fact that competition for light generally results in increased plant height in tree crops culminating in the masking of treatment effects. Competition between crop plants and weeds for soil nutrients, water and light would only manifest when the weed population threshold is exceeded. This may explain the delay in the onset of competition during the initial 12 weeks after transplanting. The results obtained indicate that the critical period of weed competition

for young transplanted clonal Robusta coffee during the first year of establishment is between 12 and 24 weeks.

Table 1. Mean girth and height increments, leaf number, number of laterals and dry weight per plant of young clonal Robusta coffee, 52 weeks after transplanting as influenced by varying durations of weed-free and weedy conditions (3 years pooled data).

Treatment	Girth increment (mm)		Height increment (cm)		No. of leaves		No. of lateral		Dry weight (g)	
Duration (weeks)	Weed-free	Weedy	Weed-free	Weedy	Weed-free	Weedy	Weed-free	Weedy	Weed-free	Weedy
4	5.2	8.1	38.2	53.5	81.2	169.0	11.4	18.9	54.7	172.7
8	6.1	8.7	53.8	56.3	96.8	172.4	13.9	19.5	75.9	145.8
12	7.0	7.9	64.2	49.3	124.5	146.7	16.5	16.9	121.6	137.2
16	7.5	7.0	59.8	47.6	141.2	144.3	18.0	15.7	167.8	109.2
20	6.9	6.3	57.8	43.1	134.1	135.4	18.2	14.6	151.5	90.6
24	8.1	6.3	59.6	46.4	153.7	114.8	18.6	14.2	170.5	115.3
28	8.2	5.5	62.4	40.9	159.6	102.3	19.0	13.1	160.4	73.2
32	8.6	4.4	59.6	34.8	164.2	76.3	18.9	9.2	240.1	64.7
36	8.8	5.9	56.1	43.3	201.7	93.7	20.8	12.3	206.2	111.3
40	7.3	4.4	47.2	36.0	151.0	80.8	16.1	9.9	124.5	54.3
44	7.4	4.9	48.9	43.7	155.5	99.9	17.7	12.3	119.3	78.6
48	8.9	4.5	63.3	41.9	189.9	76.2	20.6	10.1	235.1	61.4
52	7.7	4.8	55.9	45.9	142.9	85.0	17.5	10.2	144.7	88.9
LSD 5%	1.69		13.25		45.8		4.1		67.2	
% CV	15.2		15.9		21.4		15.9		65.3	

Oppong et al. (2006b) made similar observations in young cocoa under ecological conditions similar to that of the present studies. Since the young coffee plants competed well with the weeds during the initial 12 weeks after transplanting, it would be reasonable to suggest that a weeding cycle of 3 months intervals or clean-weeding 3 times per annum, taking into consideration the annual 2-3 months dry period, would not be detrimental to the growth and development of young Robusta coffee plants. This implies that some savings on cost of weeding could be made when the frequency of weeding is reduced to 3 times per annum instead of the previously recommended minimum frequency of 4 times per annum for coffee in Ghana (Ampofo and Osei-Bonsu, 1988).

CONCLUSION

Transplanted clonal Robusta coffee plants tolerated weed completion during the initial 3 months after transplanting. The results obtained indicated that the critical period of weed competition for young Robusta coffee is 12-24 weeks. A weeding cycle of 3 month intervals would therefore not adversely affect the growth of young Robusta coffee plants.

ACKNOWLEDGEMENTS

The authors are grateful to the staff of the Agronomy/ Soil Science Division of Cocoa Research Institute of Ghana for providing technical assistance and to Executive Director for permission to publish this paper. The lead author is grateful to CTA for sponsorship to 22nd ASIC conference.

REFERENCES

Ampofo S.T. and Osei-Bonsu K. (1988). A Practical Guide for Coffee Extension Workers. 24pp.

Knezevic S.Z., Evans S.P. Blankenship E.E., Van Acker R.C. and Lindquist J.L. (2002). Critical period of weed control: the concept and data analysis. *Weed Science* 50: 773-786.

Nieto H.J., Brondo M.A. and Gonzalez J.T. (1968). Critical periods of crop growth cycle for competition from weeds. *Pans*, (c) 14: 159-166.

Opoku-Ameyaw K., Oppong F.K., Ofori-Frimpong K., Amoah F.M. and Osei-Bonsu K. (2003). Intercropping robusta coffee with some edible crops in Ghana: Agronomic performance and economic returns. *Ghana Journal of Agricultural Science* 36, 13-21.

Opoku-Ameyaw K., Oppong F.K., Amoah F.M. and Osei-Bonsu K. (1999). Preliminary investigations into the use of intercropping for weed management in young coffee in Ghana. *Proceedings of the 18^{tth} International Scientific Colloquium on Coffee,* Helsinki, Finland, 1999: 441-444.

Oppong F.K., Osei-Bonsu K., Amoah F.M. And Opoku-Ameyaw K. (1998). Evaluation of Basta (glufosinate ammonium) for weed control in coffee. *Journal of Ghana Science Association Volume 1 No. 1:* 60-68.

Oppong F.K., Osei-Bonsu K and Amoah F.M. (1999). The efficacy of low volume Application of Roundup (glyphosate) on weed suppression in some plantation crops in Ghana. *Journal of Ghana Science Association (Special Edition) Volume 2 No. 3:* 40-48.

Oppong F.K., Sarfo J.E., Osei-Bonsu K. and Amoah F.M. (2006a). Sulfosate effects on weed suppression, cocoa pod set and yield of cocoa and coffee. *Ghana Journal of Agricultural Science* Vol. 39, No. 2, 115-122.

Oppong F.K., Opoku-Ameyaw K., Ofori-Frimpong K and Owusu-Ansah F. (2006b). Investigations into the critical period of weed competition in young *Theobroma cacao* L. *Proc. 15th Int. Cocoa Res. Conf.,* San Jose, Costa Rica, 9-14 October 2006; Vol. 1: 281-290.

Swanton C.J. and Weise S.F. (1991). Integrated weed management: the rationale and approach. *Weed Technology*, 5:648-656.

Search for Suitable Coffee Planting Materials for Coffee Farmers in Ghana: Effects of Improved Robusta Coffee Clones on Soil Properties

K. OFORI-FRIMPONG, A.A. AFRIFA, S. ACQUAYE AND E. ANIM-KWAPONG

Cocoa Research Institute of Ghana, P. O. Box 8, New Tafo Akim, Ghana

SUMMARY

As part of the coffee breeding programme towards the selection of suitable planting materials for coffee farmers in Ghana, the effects of coffee clones of different growth forms (large and compact) on soil properties were monitored over a five year period at three locations which are representatives of the main ecological zones where coffee is grown in Ghana. Clonal effects on soil pH across the locations were not significant though coffee with large growth forms decreased soil pH. Soil organic carbon contents were similar, relative to their initial values. Total N and exchangeable K contents of soils significantly ($p < 0.05$) decreased at all locations and for both coffee with large and compact growth forms more especially in Tafo (suitable site). The NPK contents of berries of coffee with different growth forms were not significantly different. It is concluded that the demand for N and K of coffee could be higher than P irrespective of the growth form of the clones and the location where they are cultivated.

INTRODUCTION

Coffee is an important export commodity crop in Ghana's economy. The crop is however grown under peasant level with very little inputs. Currently, breeding programmes on coffee which include the selection of improved robusta clones for high yielding and better adaptability in different environments are in progress to provide suitable planting materials to the farmers. In general, the yield performance of crops determines their effect on soil factors. Krishnar and Iyengar (1975) reported that the nutrients required for the production of 1000 kg clean coffee over a unit area of Arabica coffee is 70 kg N, 8 kg P_2O_5 and 85 kg K_2O. The nutrient requirements as well as the effects of the improved robusta clones on soil properties are not known. This study reports on the changes in the properties of some coffee growing soils and the nutrient off take by improved robusta coffee in three locations which are representatives of the main ecological zones where coffee is grown in Ghana as guiding baseline for planning fertilizer requirements of coffee clones in Ghana.

MATERIALS AND METHODS

The study is part of a trial started in 1996 with the aim of assessing the yield performance of some improved robusta coffee clones under different ecological conditions.

Experimental sites

Three locations in Ghana namely Tafo, Fumso and Bechem were selected for the trial. These three locations fall within different ecological zones where coffee is grown in Ghana and could be described as suitable (Tafo), moderately suitable (Fumso) and marginally suitable

(Bechem) in terms of rainfall availability and soil factors. The soils at Tafo, Fumso and Bechem are classified as Rhodi-lixic ferralsol (WRB, 1998) developed over horublende granodiorite. Some chemical properties of the soils at the study sites are presented in Table 1.

Table 1. Some chemical properties of soils at the study sites.

Soil property	Location			
	Tafo	Fumso	Bechem	Mean
pH (1:2.5H_2O)	7.04	5.14	6.56	6.25
Organic Carbon (%)	1.93	1.21	1.35	1.49
Total N (%)	0.320	0.183	0.192	0.232
Available P (mg/kg)	36.90	8.60	9.90	18.49
Exchangeable K ($cmol^{(+)}$/kg)	5.823	4.550	3.999	4.790

Coffee clones

A total of 18 improved robusta coffee clones made up of 11 large and 7 compact growth forms which are differentiated by the lengths of their laterals and planted at 2 m x 3 m apart in a randomized complete block design were used.

Soil sampling and analysis

Surface soils (0-15 cm depth) under the clones were sampled in December 2002. The soils were air-dried, crushed and passed through 2 mm sieve. The soil samples were then prepared for chemical analysis of pH, C,N, P and K using standard methods.

Plant sampling and analysis

Monthly samples of harvested coffee berries were taken from each location from September to December from 1998 to 2002. Sub samples of the harvested coffee berries were bulked together, sun dried and pulp and parchment separated from the beans and further dried to constant weights. The pulp and parchment and bean were ground separately and analysed for NPK using standard methods.

RESULTS

Table 2 and 3 present results of effects of coffee cultivation on soil properties and berries nutrient contents.

Table 2. Effects of cultivation of improved robusta coffee on some selected soil properties (0-15 cm depth).

Clones	pH	C %	N %	Avail.P mg / kg	K C mol/ $^{(+)}$ / kg
A 115	5.95	1.1	0.82	10.317	0.280
107	6.01	1.13	0.070	20.500	0.260
E 90	6.66	1.01	0.073	18.930	0.303
A 129	6.00	1.25	0.083	15.633	0.310
197	6.14	1.08	0.079	16.780	0.280
E 138	5.81	1.04	0.077	20.110	0.267
181	6.35	1.12	0.087	16.530	0.280
B 170	6.15	1.11	0.076	19.630	0.280
E 174	5.75	1.29	0.076	17.497	0.283
B 191	5.96	1.03	0.075	14.240	0.297
149	6.13	0.98	0.077	19.857	0.277
B36	5.70	0.99	0.073	13.543	0.250
A 101	6.01	1.10	0.088	12.650	0.347
E 152	6.02	1.12	0.077	20.713	0.327
E 139	5.89	1.10	0.114	26.297	0.250
B 96	6.05	1.08	0.108	21.147	0.347
375	6.28	1.33	0.116	16.137	0.280
126	5.74	1.06	0.083	17.92	0.310
Mean	6.03 ± 0.4	1.11 ± 0.01	0.084 ± 0.02	17.691 ± 2.6	0.290 ± 0.02
Locations					
Tafo	5.32	1.15	0.073	12.686	0.353
Fumso	5.99	1.14	0.090	18.869	0.272
Bechem	6.76	1.21	0.089	20.000	0.247
	6.02±0.4	1.11 ± 0.01	0.084 ± 0.02	17.185 ± 2.3	0.291 ± 0.03
Growth form					
Large	5.98	1.10	0.087	18.2	0.294
Compact	6.09	1.11	0.084	17.0	0.297
Mean	6.05 ± 0.06	1.10 ± 0.07	0.086 ± 0.02	17.6 ±0.06	0.296 ± 0.001

Table 3. NPK contents of coffee berries (kg/ha) over five year period.

Location	Average Yield (kg/ha)	Large Growth Form		
		N	P	K
Tafo	2143.2	88.4	3.6	107.9
Fumso	1929.1	79.6	3.2	97.1
Bechem	704.6	29.1	1.2	35.5
Mean	1592.3	65.7	2.7	80.2
	Compact Growth Form			
Tafo	1954.7	82.6	3.1	93.9
Fumso	1713.6	72.4	2.7	82.3
Bechem	679.6	28.7	1.1	32.7
Mean	1499.3	61.2	2.3	69.6

- Clonal effects on soil pH at Tafo was significant ($p < 0.05$) with a decrease in the initial soil pH by 1.01 units. Soil pH were not significantly different across the locations. Coffee with large growth forms decreased the soil pH relative to the initial value.
- Soil organic carbon contents did not significantly change though clones B36 (large) and 149 (compact) recorded lower carbon contents than the initial value at all locations.
- Total N content of soils decreased significantly ($p < 0.05$) under the clones at all locations and for the growth forms relative to the initial value. The decrease was more pronounced in Tafo than in the other environments.
- Available P contents increased significantly ($p < 0.05$) in Fumso and Behcem but decreased significantly ($p < 0.05$) in Tafo relative to the initial value. Growth forms did not significantly affect the available P contents across the locations.
- Clonal, locational and growth forms effects were significant on the exchangeable K contents of soils. Exchangeable K in the soils significantly ($p < 0.05$) decreased with cultivation.
- NPK contents of coffee berries in the large growth form were not significantly different from those of the compact growth forms at all locations (Table 3).

CONCLUSIONS

- Available P content of soils from the suitable site decreased significantly but increased in soils at moderately suitable and marginally suitable sites.
- Both total N and exchangeable K contents decreased appreciably at all locations and reflected in their higher amounts in the coffee berries.
- The demand for N and K could be higher than P irrespective of the growth form of the coffee clones and the locations where they are cultivated.

ACKNOWLEDGEMENTS

The authors acknowledge the technical support of field and laboratory staff of CRIG and grateful to the Executive Director, CRIG, Tafo for the permission to publish the paper.

REFERENCES

Krishnar N.W.R. and Iyengar B.R.V. (1975). Leaf analysis as a diagnostic tool in coffee. India coffee, 39(6), 175-180.

WRB (1998). World Reference Base for Soil Resources Reports No. 84, FAO, Rome.

Evaluation of Some Robusta Coffee (*Coffea canephora* Pierre ex A. Froehner) Clones for High Density Planting in Ghana

G.J. ANIM-KWAPONG*, E. ANIM-KWAPONG, F.K. OPPONG

Cocoa Research Institute of Ghana, P.O. BOX 8, New Tafo-Akim,Ghana.
*E-mail: gkwapong@crig.org

SUMMARY

Increasing productivity is a main objective in Robusta coffee improvement in Ghana. A field trial was established in 1998 to evaluate the potential of ten Robusta coffee clones for high density planting, at the experimental field of the Afosu sub-station of the Cocoa Research Institute of Ghana. The clones were evaluated at three planting densities (1667, 2222 and 2667 trees ha^{-1}) in a split plot design with three replicates, for yield and five vegetative traits namely: stem diameter, crown diameter, orthotropic internodes length, plagiotropic internodes length and number of bearing nodes per plagiotropic branch. The main factors of clone and planting density as well as their interaction had significant ($P < 0.001$) effect on five years cumulative clean coffee yield and all the vegetative traits. The highest yield was obtained from the highest planting density with clones 197, A129, B191, 181, and A115 having better yields (averaging 1289.5 kg ha^{-1} $year^{-1}$) compared to clones E152, B96, E138, E139 and B36 which recorded 827.5 kg ha^{-1} $year^{-1}$. These clones (197, A129, B191, 181, and A115) also had compact growth habit and recorded lower values for all the vegetative traits compared to the other five clones (E152, B96, E138, E139 and B36). The findings suggest that productivity in Robusta coffee could be increased through the selection of plants with compact growth habit suitable for high density planting.

INTRODUCTION

A critical management option with potential for improving crop performance is plant density and arrangement (Cooper and Hammer 1996). To increase productivity, crops must either capture more light, water and nutrients or use them more efficiently. When resources are not limiting, densely planted monocultures usually provide the most efficient capture systems (Ong et al., 1996). Since Robusta coffee is strictly self-incompatible it is essential to cultivate a number of cross-compatible clones in the field to ensure cross pollination and good yields. However, it must be emphasized that since there are complex interactions between the spatial arrangement of plants, the nature of resources, the episodic availability of resources and the plants' physiological and morphological response to levels of resource supply (Park et al., 2003; Schwinning and Weiner, 1998), consideration must be given to planting materials (genotypes/clones) of similar potential vigour (tree of uniform size and conformation) to reduce asymmetrical competition among plants which occurs when large individuals utilize a disproportionately large share of the available resources to the detriment of the growth of smaller neighbours. This brings in to question the practice of evaluating planting materials at a single planting density and hence the need to test new materials under a wide range of planting densities. The aim of this paper is therefore to identify Robusta coffee clones suitable for high density planting and ultimately, to match specific planting material type to specific planting density that can be considered as optimum. Planting coffee at the optimum spacing will give the greatest economic return of Robusta coffee yield per unit area.

MATERIALS AND METHODS

The study was initiated in 1998 at the Afosu substation of the Cocoa Research Institute of Ghana (CRIG). Ten Robusta coffee clones (197, A129, B191, 181, A115, E152, B96, E138, E139 and B36) were evaluated at three planting densities for growth and yield over five years under shade of *Gliricidia sepium* planted at 9 m x 12 m. Inter-row spacing was 3m and the intra-row spacing levels were 1.5 m, 2 m and 3 m given a planting density of 2222 plants ha^{-1}, 1667 plants ha^{-1} and 1111 plants ha^{-1} respectively. The trial was planted in a split plot design with three replicates. The main plot was the planting density and the subplot was the clones which were planted in line plots of 12 plants per clone per line plot. The plants were initially cultivated on one to two stems and height controlled at 1.7 m.

Clean coffee yield, some yield components and vegetative traits namely: stem diameter (measured at 15 cm from the base of the stem), crown diameter (span), plagiotropic internodes length and number of bearing nodes per plagiotropic branch were recorded during the first cycle of growth. Trees were coppiced after six years of growth. Two years after coppicing (second cycle), orthotropic internodes length was determined on free growing trees with three stems. Data on five year cumulative clean coffee yield, stem diameter, crown diameter, plagiotropic internodes length and number of bearing nodes per plagiotropic branch at five years and orthotropic internodes length were subjected to analysis of variance (ANOVA, General Linear Models) using the MINITAB release 12 statistical software. Treatment means were compared using the Standard Error of the Difference between Means (SED). Correlations among the measured parameters were also carried out. Clustering of coffee clones was done using mean values of all the measured parameters of the clones with the complete linkage method measured in squared Euclidean distance using the MINITAB release 12 statistical software.

RESULTS AND DISCUSSIONS

Highly significant ($p < 0.001$) interactions between clone and spacing were observed for all the parameters measured. However, data is presented on only the five years cumulative clean coffee yield in Table 1. Clone A129 performed best at 3m x 1.5m (2222 tree ha^{-1}). Generally, the yield of this clone increased with increasing plant density. Similar trend was observed for clones 197, B191, 181 and A115. In contrast, the yields of clones E152, B96, E139, E138 and B36 declined with increasing density. Though clones E152, B96, E139, E138 and B36 performed best at 3 m x 3m the average yield of 5580 kg ha^{-1} at this spacing was relatively lower than that of clones 197, A129, B191, 181 and A115 at the close spacing of 3 m x 1.5 m which was 6447.6 kg ha^{-1} (Table 1). This results show that specific clones could be matched to appropriate spacing (planting density) for optimum yields.

Table 1. Effect of coffee clone and spacing (planting density) on five years cumulative clean yield at Afosu.

SPACING				
Clean coffee yield (kg ha^{-1})				
CLONES	3m x 3m	3m x 2m	3 m x 1.5 m	CLONE MEAN
197	4403	5515	6978	5632
A129	4494	5180	7975	5883
B191	4354	4998	6228	5193
181	4536	6114	6408	5686
A115	3277	4081	4649	4002
E152	6420	5487	5270	5726
B96	6031	5362	4613	5335
E139	7203	5504	5179	5962
E138	4042	3942	3092	3692
B36	4206	3856	2534	3532
SPACING MEAN	4897	5004	5292	

s.e. of difference for comparing two spacing means = 77.7 (4 df)
s.e. of difference for comparing two clones means = 141.8 (54 df)
s.e. of difference for comparing two values in body of table = 245.7 (54 df)

Hierarchical cluster analysis grouped the clones into two clusters (Figure 1). Clones in cluster 1 (197, A129, B191, 181 and A115) were relatively compact with mean stem diameter (4.6 cm), crown diameter (158.7 cm), length of plagiotropic internodes (4.7 cm) and length of orthotropic internode (6.5 cm) compared to 5.3cm, 185.4cm, 5.5cm and 8.1cm respectively for clones in cluster 2 (E152, B96, E139, E138 and B36). Montagnon et al. (2001) associated the magnitude of stem diameter and length of orthotropic (main stem) internodes with level of vigour and aggression in Robusta coffee. On this basis therefore, clones in cluster 2 could be considered as the more vigorous of the two groups. Montagnon et al. (2001) further indicated that vigorous clones were more aggressive than others and that aggressive clones would undergo their own aggressiveness when grown alone in plantations. This observation plausibly explains the results shown in Table 1 which indicates that the vigorous clones are better suited for wide spacing planting where competition effect could be less intense.

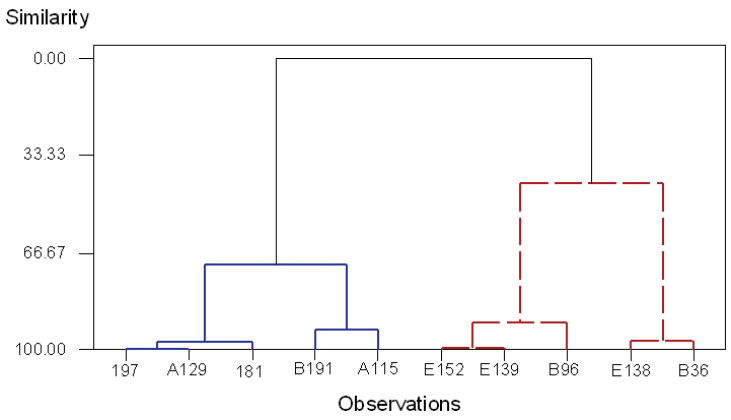


Figure 1. Dendrogram showing the hierarchical clustering of the ten clones used in the present study.

Among significant correlations, stem diameter showed positive correlation with crown diameter ($r = 0.955$; $p < 0.001$) indicating that a clone with bigger stem has a corresponding larger crown diameter (span). Length of orthotropic internodes showed significant negative correlation with number of bearing nodes ($r = -0.788$; $p < 0.001$) and the correlation of number of bearing nodes and yield was significantly positive ($r = 0.479$; $p < 0.007$).

CONCLUSION

The grouping of clones based on similarity of morphological characters and yield as done in this study using hierarchical cluster analysis is necessary for avoiding asymmetrical competition among clones of varying vigour and aggressiveness. This will ensure that coffee clones are planted at an appropriate density using appropriate planting material types to achieve optimum yield. Generally, the large clones in cluster 2 should be planted at 3 m x 3 m and clones in cluster 1 planted together at 3 m x 1.5 m for best results.

ACKNOWLEDGEMENT

This paper is published with the permission of the Executive Director, CRIG.

REFERENCES

Cooper M. and Hammer G.L. (1996). Synthesis of strategies for crop improvement. In: M, Cooper and G. L. Hammer (eds) Plant Adaptation and Crop Improvement. CAB International. Wallingford.

Montagnon C., Flori A. and Cilas C. (2001). A new method to assess competition in coffee clonal trials with single-tree plots in Cote d'Ivoire. Agronomy Journal 93:227-231.

Ong C.K., Black C.R., Marshall F.M. and Corlette J.E. (1996) Principles of resource capture and utilization of light and water. In: Tree-Crops Interactions (Ong C.K. and P. Huxley), pp 73-158. CAB International.

Park S.E., Benjamin L.R. and Watkinson A.R. (2003). The theory and application of plant competition models: an agronomic perspective. Annals of Botany 92: 741-748.

Schwinning S. and Weiner J. (1998). Mechanisms determining the degree of size- symmetry in competition among plants. Oecologia 113: 447-455.

Characteristics of *Coffea canephora* and *Coffea arabica* Production in West Region of Sao Paulo State, Brazil

F.M.M. BLISKA[1], C.L.R. VEGRO[2], S.P. PEREIRA[1], G.S. GIOMO[1]

[1]Instituto Agronômico – IAC, Av. Barão de Itapura, 1481, Campinas, SP, Brazil, 13.020-902
[2]Instituto de Economia Agrícola – IEA, Av. Miguel Stéfano, 3900, São Paulo, SP, Brazil, 04.301-903

SUMMARY

There is a long tradition of arabica coffee cultivation in the Brazilian State of Sao Paulo and it still maintains a relevant contribution to agricultural revenue. The process of its substitution by other cultures now in course should be moderated by robusta coffee cultivation in areas considered marginal for arabica. Based on this supposition and due the large demand for this product, socio-economic information and costs of the two cultivations were compared for Alta Paulista region. The hypothesis of agronomical and economical feasibility of robusta farming is confirmed by this study, and resources and efforts are recommended in order to make this culture an economic alternative for that region.

INTRODUCTION

Sao Paulo State is the third Brazilian coffee producer, with 10% of the volume of national coffee production (Conab, 2008). However, coffee farming is not very important for Sao Paulo agricultural sector, due the great diversification of the State economy. However, its importance is significant if we consider the overall agribusiness, since several roasters, two soluble industries and the largest and most important Brazilian port of coffee exportation are located there. Additionally, Sao Paulo is the greatest national coffee drink consumer.

Coffea arabica L., arabica coffee, is traditionally cultivated in Sao Paulo. However, mainly the West Region (Nova Alta Paulista, Northwest and Araraquarense) shows some limitations related to arabica cultivation, due the high temperatures, soil eroded and nutritionally unbalanced, occurrence of nematodes and long periods without rain. Since high temperatures and dry periods hamper arabica crop, an alternative to West Region could be the progressive introduction of *Coffea canephora* P., robusta coffee, based on technical researches, since it shows rust resistance and significant tolerance to higher temperatures and nematode infestation (Vegro, et al 1996).

Robusta coffee is more productive than arabica, however it is sensitive to very low temperatures and intense winds that can occur throughout the year in that region, then it is necessary to use windbreaks in order to minimize the culture damage. Long dry periods also reduce the productivity of non-irrigated crops, compared to arabica. Moreover, robusta is more attacked by coffee berry borer than arabica, and crop mechanization is very difficult, specially the harvest. Some advantages in robusta cultivation: production costs below the average for arabica, cutting propagation, possible creation of clonal gardens, and possible use as rootstock for arabica.

Nowadays it is possible to discuss robusta quality, particularly the conilon prepared by parchment. Some characteristics desired by the industry are: the highest level of soluble

solids, caffeine and chlorogenic acid; greater capacity to support a more intensive roasting without undesirable bitterness; higher complex sugar content and less lipids, that favours the persistence and creamy consistency of espresso coffee. In the composition of blends, the addition of small percentage of robusta, without defects, does not change the final beverage and can even correct some acidity excess of arabica (Ribeyre, 2007).

Since the mid-nineties, Sao Paulo industry suggests that this State should begin the commercial production of robusta coffee – especially in the West Region, to use it both in soluble industry and in blends with arabica in the roasting and milling industry. The reduction of coffee imports from other states would reduce the industrial production cost of coffee, mainly, according to logistics optimization. However, as noted by Bliska et al (2007), we must carefully examine the technical feasibility and cost of deploying a program to encourage the commercial cultivation of robusta coffee in that State, even the current strong interest of Sao Paulo coffee farmers, due the high market prices and the relative scarcity of supply observed in roasting and milling industry, solubilization and export.

OBJECTIVES

This work aims to compare the effective operational robusta production cost, Apoatã variety, growing in West of Sao Paulo State, with the average of that cost to arabica crop in the same region, that is considered marginal area for arabica specially due climatic characteristics. The agronomic and socio-economic characterizations of *C. canephora* and *C. arabica* in the West Region of Sao Paulo State aimed, mainly, to subsidize programs for public and private policy, to transform coffee crop into a profitable and sustainable regional economic activity. These characterizations can also help us concerning the technical feasibility of commercial cultivation of robusta in that region.

METHODOLOGY

A survey of agronomic and socio-economic indicators was held between April and September 2006, through application of structured questionnaire, prepared by the team. The questionnaire was applied to scientific researchers, extension and technical expertise in coffee as well as coffee producers and directors of a regional cooperative.

RESULTS

Alta Paulista is the main coffee production area in the West Region of Sao Paulo State. Results indicate that, in that region, the land structure is based in small coffee farms, with family labour and low education level, manual crop management and intermediary technological level for both crops, *C. arabica* and *C. canephora* (Table 1). These characteristics probably reflect the start of coffee production in that region: by settlers who migrated from Garça-Marília, Ribeirao Preto-Franca and South of Minas Gerais State, that bought, in general, small areas. Results also indicated that the low productivity of arabica coffee in that region is related to nematodes and soil conditions, physical and chemically deteriorated, eroded, without replenishment of nutrients and organic-matter. An intense effort to recover such land would be a way to minimize these problems in order to facilitate the further development of the coffee crop in the West Region of Sao Paulo State.

Table 1. Summary of coffee indicators, *C. canephora* and *C. arabica*, West Region of Sao Paulo State, 2006.

Indicator	Coffee specie	
	C. canephora	*C. arabica*
Average annual volume of coffee (bags 60kg – processed)	*100*[*]	320 mil
Total area of coffee plantation (ha)	5	15.600
Number of coffee farmers	8	3000
Medium size of farms (ha)	15	15
Medium size of coffee crops (ha)	0,6	4,5
Percentage of crops renewal (%)	0	5
Percentage of crops expansion (%)	0	-2
Average yield – four years (bags/ha)	35 (irrigated)	17 (non irrigated)
Predominant crop system (pl/ha)	≤ 800	≤ 3000
Predominant cultivar	Apoatã IAC 2258	Mundo Novo
Production system	Manual	Manual
Harvest	Manual	Manual
Cherry preparation[**]	Pulped coffee	Natural
% Irrigated area	70	5
Farm management system	Family	Family
Technological level	Medium	Medium
Workforce	Family labour	Family labour
Education level of workforce	Low	Low
Storage place	Farm	Cooperative
Place of coffee processed storage	Farm	Cooperative
Time for coffee commercialization	≤ 3 months	3 a 6 months
Estimated production cost: inputs + workers services (R$/bags)	114,79[***]	234,83
Average price sept./06 (bag of 60 kg – processed coffee)	160,00	220,00
Production purpose	Rootstocks /Seedlings	Roaster/Milling

** Volume for the industry, resulting from the disposal of seed production.*
*** "Natural" is used to specify the coffee obtained by dry system of processing.*
**** Were not considered the costs of irrigation.*
Source: Instituto Agronômico – IAC, 2006.

However, in that region, robusta crop is specific for seed production, to obtain rootstocks seedlings, resistant to rootworm nematodes *Meloidogyne exigua* and *M. incognita*. This way, Apoatã IAC 2258 – robusta cultivar – is used as the rootstock that enable arabica production in West Region, despite the higher cost of seedlings for producers, but with significant advantage: without pesticides to nematodes control.

One of the most significant indicators for regional coffee crop is the average production cost: inputs and agricultural operating costs. Due to the low productivity observed, results indicated very high cost to arabica (R$234.83/bag) and lower for robusta (US$114.79/bag). Since robusta cultivation is specific to seeds production, cultivation only for grain market may have lower cost than that, mainly due the significant investment to obtain pulped coffee for seeds. These results corroborate information obtained in interviews, that arabica crop, with populations of less than 2,500 plants per hectare, resulting in losses, especially when producers receive less than R$250.00/bag.

Considering robusta production cost and it market price, we observe an interesting advantage. However, it is necessary to examine this situation very carefully, because regional experience with robusta cultivation is based only on a specific cultivar, aimed seeds production. Also, there are no conclusive studies on the technical feasibility of commercial cultivation of other robusta and conilon cultivars in that region. Furthermore, the total area cultivated with robusta coffee in Alta Paulista is around 5 ha, 70% of them irrigated, led by eight producers, which together produce annually, on average, only 800 kg of seeds and 100 bags of coffee, resulting from the seed production disposal.

CONCLUSIONS

Now days, robusta cultivation in Sao Paulo State, in marginal areas for arabica coffee, especially those constrained by high temperatures, could be an interesting alternative for rural producer, mainly for small ones. However, the necessary irrigation, wind influence and low temperatures in some periods of the year, considering the genetic material currently available, could require additional investment: for instance, windbreaks and irrigation.

Therefore, the empirical research could be very important for robusta implementation, through the development of new cultivars from seeds or clonal gardens, more productive and resistant to pests and diseases, with acceptable beverage. Additionally, it is important to develop routines for the appropriate farm management, such as fertilization, pruning, irrigation, harvesting and post-harvest. Simultaneously, could be a government policy, to rural credit and commercialisation, for instance, targeted to those producers, since that most of them do not have financial resources to investments, have low educational level, and use family labour. Therefore, in that context there is a long way to be followed.

Considering that sugar cane burning will be prohibited after 2012, robusta coffee cultivation could be highly desirable for strategic agricultural development of Sao Paulo State. Sloped areas, where it is not possible to use machines in the sugar cane harvest, could be used to robusta cultivation, contributing to diversification in some regions where the sugar-alcohol sector is most important today.

REFERENCES

Bliska F.M.M. et al. Importância do café robusta coma alternativa à geração de emprego e renda no Estado de São Paulo,. In: Bliska, F.M.M. and Guerreiro Filho, O. Prospecção

de demanda na cadeia produtiva do café no Estado de São Paulo. Instituto Agronômico de Campinas, Campinas, SP, 2007. 53-58p

Cortez J.G. O conillon, este desconhecido. Jornal do Café, Associação Brasileira da Indústria do Café, RJ, ano III, jan.99, 4-9p.

Os Frutos Da Pesquisa. Revista Panorama Rural, ABIMAQ, SP, ago. 2007, 32-35p.

Ribeyre F. Reconhecendo a qualidade do café robusta. In: SALVA, T.J.G.; et al. Cafés de Qualidade: aspectos tecnológicos, científicos e comerciais. Instituto Agronômico de Campinas, 2007. 371-387p.

Tsunechiro A. et al. Valor da produção agropecuária do Estado de São Paulo em 2007: uma estimativa preliminar. Informações Econômicas, SP, v.37, n.10, out. 2007. 60-69p.

Vegro C.L.R. et al. Café Robusta: uma alternativa para a cafeicultura paulista. Informações Econômicas, SP, v.26,n.7, jul. 1996

Application of Urea with Urease Inhibitor in Young Coffee Plants

A.L. ALVARENGA GARCIA[1*], A.W.R. GARCIA[1], L. PADILHA[2]

[1]Fundação Procafé, Varginha, MG. [*]E-mail: garcialmg@gmail.com
[2]Embrapa Café, Brasilia, DF

SUMMARY

The nitrogen is the most required nutrient by the coffee culture and its application is usually done in the form of urea. Although easy to handle, significant losses of N to atmosphere may occurs due to the transformation of urea in ammonium, as a consequence of urease action, an enzyme produced by soil microorganisms. This work aimed to evaluate NBPT efficiency, an urease inhibitor, in the reduction of N losses by volatilization. The work was carried out in a greenhouse using a factorial design comprised of three N doses in the form of urea, in the presence and absence of NBPT, applied in two times, and with two ways of irrigation: before and after fertilizer application. Three independent treatments were added: a control without N, and 7.2g of N in just one application with and without NBPT. Dry weight and mobilized N were evaluated four months after the treatments. It was found that the addition of NBPT to urea significantly increased dry weight and mobilized N per plant, and that the response was proportional to the N applied. No response was observed to the time of irrigation. In the treatments where N was applied in just one dose, plant death was observed in two days in absence of NBPT, or in six days, when NBPT was used, corroborating the idea that NBPT can delay N volatilization.

INTRODUCTION

Among the essentials chemical elements to the coffee tree, the nitrogen has a larger demand than the others, due the quantities required and exported by the plant. This macronutrient induces direct response in the coffee bean production. With 45% to 46% of N-amidic, the urea is the nitrogen fertilizer more concentrated, and it is cheaper to transport and storage. The easiness of application combined to the low cost makes the urea the most widely nitrogen source used in the agriculture. The urea is the result of the reaction from CO_2 with NH_3 under high temperature and pressure. Significant losses due to very fast urea nitrification can be occurred when this fertilizer is applied over the straw in high temperature and humidity excess conditions. On the other hand, when the humidity is low, the urea remains in the soil for a few days.

The urease is an enzyme produced by soil microorganisms and it has an action over the urea. When the molecule of urea is broken by the urease the N-urea is released and losses can be occurred by ammonia volatilization.

Losses by ammonia volatilization from N-urea fertilizer can be reaching 30% in conventional soil tillage (urea applied over the soil surface) and until 70% when urea is applied over the straw (direct planting – no tillage) in a Red Yellow Latosol or in a Dark Red Latosol (Cabezas et al., 2000). Soils with high organic matter content have greater urease activity and, consequently, higher N losses rates. These N losses are influenced by the soil characteristics and environmental factors.

Investments have been made towards the development of new physical and chemical technologies to reduce the N losses. Among the new products, NBPT [N-(n-Butyl) Tiophosphoric triamide] is used to inactivate the urease temporarily and to avoid the molecular break in urea. This product is mixed to urea fertilizer. The effect of NBPT over the urease is correlated to the NBPT concentration in the urea mix and increasing levels of NBPT raising urease inhibition timing. In agreement with manufacturer significant N loss will not happen in a period of time during seven to 14 days.

In this way, this work was conducted to evaluate the urease inhibitor performance NBPT in the development of young coffee plants.

MATERIAL AND METHODS

This work was begun at December 2005 and was carried out in Varginha Experimental Farm-Fundação Procafé, Varginha, MG.

The experiment was conducted in the greenhouse. The used experimental design was completely randomized with three replication in a 3 x 2 x 2 factorial scheme involving three N levels (0.9, 1.8 and 3.6 g N/ pot) in two split applications; two N sources (urea and urea + NBPT) and two irrigations forms (before or after each urea application). This kind of irrigation was made with the aim to simulate field conditions which occur after the rain, or when there is no water available in the soil at the moment of fertilization. The control did not receive N fertilizer and two additional treatments were considered: 7,2 g of single urea application, with or without NBPT.

Young coffee plants were growing in pot (9L) and micro and macronutrients were supplied in agreement with recommendations presented at 5ª Aproximação (1999). The seedlings were transfered to the pot with three leaves pair. The first split of nitrogen fertilizer were applied 20 days after the transfer, and second split, 60 days after the first. Four months after the second fertilizer application the plants of each plot was taken from the pots, washed and evaluated to the following characteristics: total dry weight, mineral analysis of the whole plant and mobilized nitrogen (N x% MS).

Each experimental plot was formed by four pots with three plants. We used the Scott Knott test to compare the averages, with 5% of level significance.

RESULTS AND DISCUSSION

Significant differences ($p \geq 0.05$) were found to N levels and N source but, there wasn't significant interactions between the three factors (level N x source N x irrigation form). No effect of irrigation form was found in any evaluated parameter.

The increment in the urea level allowed linear increase of nitrogen fixed in the coffee plant (Figure 1). The control without N supply showed severe N deficiency, with leaves chlorosis and short plants. These characteristics also were observed, to a smaller degree, when 1.8 grams of N/ pot was applied. The increase in the N level supplied allowed increment in dry matter accumulation, although no difference was noted between 1.8 and 3.6 g N / pot levels.

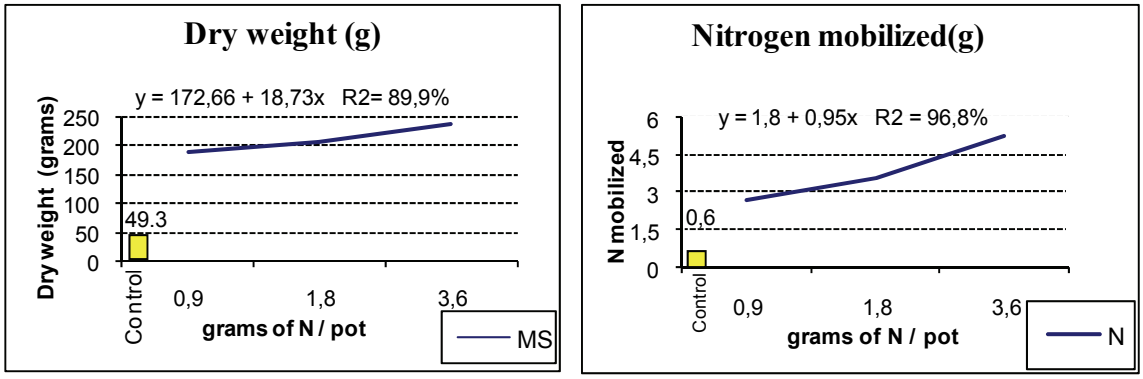


Figure 1. Dry matter and %N fixed in coffee plant six months after the transfer to the pot. Were applied 0.9, 1.8 and 3.6 g N/pot. Varginha, 2006.

Increments of dry matter and N fixed by coffee plants in treatments that received the urea+NBPT, showed significant gains, compared to treatments that received only urea (Table 1). Probably these gains are due to the reduction of N losses by ammonia volatilization, in function of the slow release, allowing a better use of nitrogen.

Table 1. Dry matter and nitrogen fixed by coffee plants whose N supply was done with urea or urea+NBPT. Varginha, 2006.

N source	Dry matter (g)	N dag / Kg (%)	N fixed (g)
Urea	198,7 a	1,72 a	3,43 a
Urea + NBPT	234,0 b	2,05 b	4,54 b

Averages followed the same letter do not differ among them, Scott-Knott test, 5% significance.

For the different sources and fertilizer levels the use of irrigation did not influence significantly the nitrogen used by plants. This is probably because of water remaining in pot, two days after irrigation, be enough to dissolve the fertilizer applied in coverage.

Table 2. Dry matter and nitrogen fixed by coffee plants irrigated. Varginha, 2006.

Irrigation form	Dry weigth(g)	N dag / Kg (%)	N mobilized (g)
Before N supply	223,6 a	1,83 a	4,03 a
After N supply	209,6 a	1,94 a	4,04 a

Averages followed the same letter do not differ among them, Scott-Knott test, 5% significance.

Plants died in 24 h when 7.2 g of single urea were applied, but when NBPT was mixed in this urea, coffee plant death happened six days after application indicating the gradual and slow nitrogen releasing due to NBPT.

CONCLUSION

In a greenhouse, the inhibitor of urease NBPT allows a better use of the N-urea, by the coffee plants.

REFERENCES

Lopes A.S. (1999) Recomendações de calagem e adubação no sistema de plantio direto. Recomendações para o uso de corretivos e fertilizantes em Minas Gerais – 5ª Aproximação, p.93-98.

Cabezas L., Yamada (2000). Como melhorar a eficiência da adubação nitrogenada do milho. Informações agronômicas, n 91, p.1-5.

Scott A.J., Knott M. (1974). A cluster analysis method for grouping means in the analysis of variance. Biometrics, vol. 30**,** p. 507-512.

Weed Control on Coffee Crop by Glyphosate Applied Through Controlled Droplet Application System (Micron-Herbi)

E.N. DE ALCÂNTARA[1], R.L. DA CUNHA[2]

Minas Gerais State Research Institution/South of Minas Technological Center
[1] Weed Science researcher, D.Sc from EPAMIG (MG state research institution), PO. BOX 176, 37200.000 − Lavras, MG, Brazil. E-mail: elifas@epamig.ufla.br
[2] Coffee Science researcher, D.Sc. from EPAMIG (MG state research institution), PO. BOX. 176, 37200.000 − Lavras, MG, Brazil. E-mail: rlc@epamig.ufla.br

SUMMARY

Different glyphosate concentrations applied on weeds by controlled droplet application (C.D.A.) equipment were assessed through out the weed control potential in two weed growth stages on coffee crop. Glyphosate was applied at 2.5, 5.0, 7.5, 10.0 and 15.0% concentration in 15 L ha^{-1} on weeds at 15 to 20 cm and 30 to 45 cm height on weed interrows of coffee plants. Glyphosate 2.5% applied on weeds at two growth stages did not control buttonweed (*Spermacoce latifólia* Aubl.), common purslane (*Portulaca oleracea* L.), goosegrass [*Eleusine indica* (L) Gaertn] and pigweed (*Amaranthus viridis* L.). Weeds from 15 to 20 cm tall were controlled by glyphosate sprayed at 5.0%, except buttonweed. When weeds reached 30 to 45 cm tall, only glyphosate at 7.5% or above controlled weeds. Glyphosate at 5.0% on weeds at 30 to 45 cm height stage, did not control, buttonweed, pigweed and wild poinsettia (*Euphorbia heterophylla* L.). Glyphosate concentration raises presented quadratic response on weed control, reaching higher control at 96.6% and 91.0% respectively, from 12.7 and 12.9 % of glyphosate on 15 to 20 and 30 to 45 cm weeds plant height.

INTRODUCTION

Weeds that grow in coffee crops should be controlled to decrease the weed coffee competition. Coffee losses due to weeds were estimated by Oliveira et al. (1979) to 43% and from 55 to 77% by Blanco et al. in 1982. There are several weed control methods in coffee, among them the chemical control presents economical and operational advantages (Guimarães and Mendes, 1997; Marochi, 1993). Several equipments are used for herbicides application, the micron-herbi device s a pulverization sytem producing “micronized” droplets with controlled size, through centrifugal force generated by rotative nozzles, and it is little studied and practically ignored by the coffee grower great majority.

The micron-herbi spraying produces uniform drops size of approximately 250 microns, improving the tank mixture spraying and avoiding the flowing out the target.

On the other hand, the flat fan type nozzles, used in conventional spraying, produces variable drop sizes from 10 to 600µ where the small drops get away from the leaves and great ones slip out (Monsanto s.d.) and also, uses big spraying volumes, and greater rates to counterbalance what is lost by derive during applications. Several researches have shown that it is possible to reduce the volume of the pre and post herbicides applied rate without affecting the weed control of some invader weed species (Addala et al., 1978; Ayres and Merritt, 1978; Kivlin and Doll, 1988; Wilson and Taylor, 1978).

Wiese, cited by Hatsuta (1982), verified that it is possible to reduce the water volume and glyphosate and paraquat rates for micronized spraying to control pigweed (*Amaranthus* sp) at 15 and 35 cm height. Taking in account that weed growing in Brazil differs from those observed in temperate weather countries this research was made to evaluate the different effects of glyphosate concentrations on weeds when applied with controlled size drops at weeds at two growth stage in coffee plantations.

MATERIALS AND METHODS

The experiment was conducted at EPAMIG experimental station in district of the of São Sebastião do Paraiso town, for two consecutive years in 1994 and 1995 on coffee crop with Catuai, cultivar IAC 99, planted at 4.0 by 1.0 meter row space totalizing 2,500 plants/ha.

The experiment was located at the Southwest of Minas Gerais, in dystrophic purple Oxisol area, with loamy texture, and soft landscape, at 820 meters of altitude, Cwb type climate by Köppen classification. The area presents annual medium precipitation of 1470.4 mm concentrated from October to March, and annual medium temperature of 20,8 °C, with originally vegetation of tropical transitional forest changing to savannah.

The experiment was established in randomized blocks design with three replications with plot of 64 squared meters. Each plot had eight coffee plants limited by two adjacent rows. The central area was teken as useful area eliminating 1 m at each end.

The treatments were herbicide Glyphosate, applications at concentrations of 2.5, 5.0, 7.5, 10.0 and 15.0 %, on the weed growth stage from 15 to 20 cm and 30 to 45 cm height, in this research denominated stage 1 and stage 2, respectively.

For comparison effect a hand weeded check was used. The herbicide application was made with micron-herbi equipment, using 15 L volume of carrier per ha. The treatment applications were made in the morning with medium temperature of 22 °C and 78% of relative humidity. The weed control evaluations were accomplished 15 days after each spraying. A data variance analysis was performed, averages were compared by Scott-Knott (Ferreira, 2000) test and regressions for the glyphosate concentrations were made at the 5% probability level.

RESULTS AND DISCUSSION

Nineteen percent of weeds in experimental area were buttonweed (*Spermacoce latifolia* Aubl.), 10% of common purslane (*Portulaca oleracea* L.), 8% tropical spiderwort (*Commelina bengalensis*), 15% of goosegrass (*Eleusine indicates* (L.) Gaertn), 8% smooth pigweed (*Amaranthus viridis* L.), 9% of Brazil pusley (*Richardia brasiliensis* Gomes), and 5% wild of poinsettia *Euphobia heterophilla* L), 7% of hairy beggarticks (*Bidens pilosa* L.), 4% of bermudagrass [*Cinodon dactylon* (L.) Pers], 12% of alexandergrass [*Brachiaria plantaginea* (Link) Hitchc] and 8% of a kind of crabgrass *Digitaria horizontalis* (L.) Scop.], and the last two were found in cycle end.

As shown in Table 1, we observed that the control on weed population was not satisfactory at 2.5% glyphosate concentration. But starting from 5% glyphosate concentration weeds at 15 to 20 cm height were affected, without presenting significant differences from weeded check. At the 7.5% and 10.0% glyphosate concentrations, weeds were affected at the two growth stages; high susceptibility to the herbicide was oberved at 15.0% concentration in stage 1. There was no buttonweed, control. According to Lorenzi 2006, a weed is considered susceptible when its

control goes from 85 to 95% of the weed population and it is highly susceptible when the control is above 95%.

Table 1. Percentage of weed control in function of the glyphosate concentration sprayed by micro-herbi, in two growth stage. São Sebastião do Paraiso, MG.

% of glyphoste concentration	Glyphosate volume l/ha	Weeds at 15 to 20 cm growth stage	Weeds at 30 to 45 cm growth stage	Weeded Check
		Weed control percentage (%)		
2.5	0.375	75.00 b[1]	68.33 b	100.00 a
5.0	0.750	90.00 a	70.00 b	100.00 a
7.5	1.125	91.67 a	88.33 a	100.00 a
10.0	1.50	92.67 a	90.00 a	100.00 a
15.0	2.25	96.67 a	90.00 a	100.00 a

[1]*Means within a column followed by same letters do not differ at Scott Knott test at 5%.*

It was verified that concentrations from 12.7% to 12.9%, gave better control above of which there would be no increase in weed control at both growth stages 1 and 2 respectively. The maximum weed control estimates on these herbicide concentrations were 96.6% and 91.1% according to regression equations of $\ddot{y}_1 = 66.52 + 4.78x - 0.19x^2$ e $\ddot{y}_2 = 51.72 + 6.15x - 0.24x^2$, respectively (Figure 1).

Button weed (*Spermacoce latifolia* Aubl.), common purslane, and goose grass were not controlled at 2.5% Glyphosate concentration, independently of the plant stage. Pigweed and wild poinsettia were not controlled at 5% Glyphosate concentration in the two growth stages. However, Wiese, cited by Hatsuta, (1982) observed pigweed control from 96 to 99%, when glyphosate was applied rates varying from 0.13 to 0.54 kg per ha and using a water volume of 9.35 L /ha using micronized spraying in the plant stage from 15 to 35 cm of height, and the conventional spraying, using the same rates, had only 50% control of 50%, and 243.6 L of water in the tank. Starting from the concentration 7.5% all the weeds plants presented control superior to 88%, being statistically similar to the weeded check, in the two growth stadiums, except for button weed *Spermacoce latifolia* that was not controlled. These species were, however, as observed by Rodrigues and Almeida, 1998, as susceptible to the glyphosate; and according to Lorenzi, 2006), these plants susceptible to glyphosate in early application, but in practice this weed has shown tolerance to the glyphosate. Another explanation for this result is the umbrella effect that protected plantules recently emerged in spraying occasion not being reached by herbicide.

CONCLUSIONS

The best weed control stage with Glyphosate, applied by micron-herb, was from 15 to 20 cm of height.

The smallest Glyphosate concentration, applied by micron-herb, giving effective weed control was 5%, and 7.5% in both growth stages. Glyphosate applied by micron-herb were superior to 90% control when the commercial product concentration on carrier was above 12% in both growth stages.

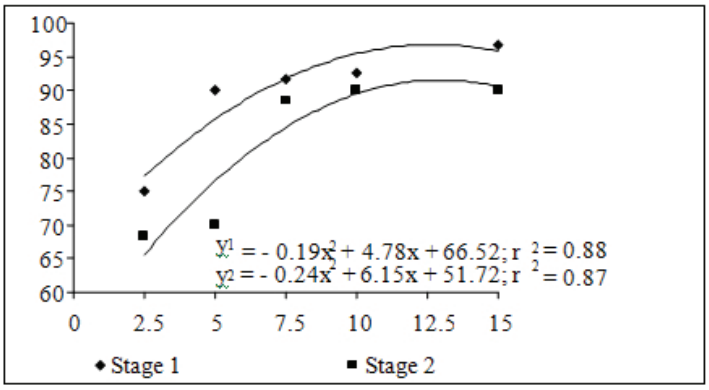


Figure 1. Effect of glyphosate rates applied by Micron-herbi sprayer on weed control at 15 to 20 cm (■) and 30 to 45 cm (◆) growth stages, at 15 days after herbicide application.

ACKNOWLEDGMENTS

The Authors are thanks to FAPEMIG and Brazilian Consortion for Research and Development Financial support.

REFERENCES

Addala M.S.A., Hance, R.J., Drennan D.S. Effects of application method on the performance of some soil-applied herbicides. I. Field studies. Weed Research, Oxford, v. 24, n.2, p. 105-113, April 1978.

Ayres P. and Merritt C.R. Field experiments with controlled drop applications of herbicides for the control of dicotyledonous weeds. Weed Research, Oxford, v. 18, n. 4, p. 209-214, August, 1978.

Blanco H.G., Oliveira D.P., Pupo E.I.H. Período de competição de uma comunidade natural de mato em uma cultura de café em formação. O Biológico, 48 (1): 28 9-20.1982.

Lorenzi H. Manual de identificação e controle de plantas daninhas: plantio direto e convencional. 6 ed. Nova Odessa: Instituto Plantarum, 2006, 361 p.

HATSUTA. Pulverização micronizada – resultados de pesquisa. Boletim informativo, série 82, n. 1, ago. 1982. 9 p.

Ferreira D.F. Análises estatísticas por meio do Sisvar para Windows 4.0 In: Reunião annual da região brasileira da sociedade internacional de biometria, 45.; 2000. São Carlos. Anais… São Carlos, SP. UFSCAR, 2000.p.255-258.

Guimarães R.J. and Mendes A.N.G. Manejo da lavoura cafeeira. Lavras: UFLA/FAEPE, 1997, 49p.

Kivlin P.T. and Doll J.D. Quackgrass (*Agropyron repens*) control with SC-0224 and Glyphosate. Weed Technology, v. 2, n. 2, p. 147-152, April 1988.

Marochi A.I. Tecnologia de aplicação de defensivos agrícolas. In: Simpósio internacional sobre semeadura direta em sistemas sustentáveis, 1993, Castro. Anais... Castro: Fundação ABC, 1993. P.208-227.

MONSANTO. Gotas menores produzem maior eficiência herbicida: Agricultura e Produtividade. São Paulo: Monsanto. Ano2, n.5, out., nov., dez, s.d.

Oliveira J.A., Matiello J.B., Carvalho F. Estudo do efeito da época de controle das plantas daninhas sobre a produção do café. In: Congresso brasileiro de pesquisas cafeeiras, 7, 1979, Araxá, MG. Resumos...Rio de Janeiro: IBC/GERCA, 1979, p.350-352.

Rodrigues B.N. and Almeida F. S. Guia de herbicidas. 4 ed. Londrina, 1998. 648p.

Wilson B.J. and Taylor W.A. Field trials with controlled drop application on barban and difenzoquat for the control of wild oats (*Avenua fatua* L.) in spring barley. Weed Research, v. 18, n. p. 215-221, August, 1978.

Recycled Coffee Wastes as Potential Replacements of Inorganic Fertilisers for Coffee Production

A. CHEMURA, R. MANDHLAZI AND C. MAHOYA

Coffee Research Institute, P.O. Box 61, Chipinge, Zimbabwe

SUMMARY

Coffee is usually produced under high input regimes using inorganic fertilisers. With the view of reducing costs of inputs in coffee production, coffee by-products and levels of inorganic fertilisers were evaluated for their effect on plant growth, yield and cup quality. Cost benefit analysis of using the by-products in combination with different fertiliser regimes was done. High NPK (80:40:80) levels did not provide significantly higher yields ($p > 0.05$) than the medium (40:20:20) and lower (20:10:20) NPK applications. The contribution of the organic by-products to yield were significant only in the first two years of harvesting ($p < 0.05$) where composted pulp alone resulted in the highest yields. Addition of a live mulch of Fine stem stylo reduced yields in the first four years but resulted in highest yields in the fifth year. Although overall results were not statistically significant, they suggested that a combination of composted pulp alone and medium fertiliser levels resulted in higher yields. The three levels of inorganic fertilisers and coffee by-products did not produce significant differences in leaf nutrients as well as coffee quality. Coffee pulp produced positive NPV values under low and medium fertiliser regimes but negative under high levels, with the addition of live mulch being the least in financial performance. Composted coffee pulp has the highest potential to partially substitute inorganic fertilisers in sustainable coffee production.

INTRODUCTION

There are significant quantities of by-products and wastes produced in the management of coffee plants in the field as well as during the processing of the crop. Some of the major wastes of wet processing of coffee are pulp, parchment and flocculent. One tonne of clean coffee beans produced, produces one tonne of pulp and parchment (Oliveira et al., 2008). There are no profitable uses for this residue and usually end up as environmental contaminants (Oliveira et al., 2008; Endris, 2007). General management practices such as pruning, produces vegetative litter as wastes. On the other hand, coffee is generally considered a high input crop. The inorganic fertilizers and chemicals used apart from being expensive, scarce and posing healthy risks, are point sources of environmental pollution (Korikanthimath and Hosmani, 1999; Mitchel, 1990; Kutywayo et al., 2007). Organic fertilisers or combining organic fertilisers and inorganic fertilisers have been shown to have much potential in the production of coffee (Mitchel, 1990; Kutywayo et al., 2007; Njoroge et al., 1990). With fluctuating world coffee prices, escalating cost of various inputs and the call for environmentally sustainable crop production methods, recycling locally available coffee wastes and by-products could minimise production costs, enrich the fertility status of the soil and protect the environment without affecting either quality or yield of the crop (Mitchel, 1990; Njoroge et al., 1990). This study was therefore carried out to evaluate the economic potential of using recycled coffee wastes and by-products together with a live legume mulch as organic sources of nutrients in coffee production.

MATERIALS AND METHODS

The study was carried out at the Coffee Research Institute, Chipinge, Zimbabwe in a field in which coffee plants were planted under a two tree cova system at a spacing of 2.4 m inter-row by 1.6 m intra-row spacing resulting in a population of 5212 plants/ha. Three inorganic fertiliser levels; low (20-10-20NPK), medium (40-20-40NPK) and high (80-40-80NPK) and five inorganic treatments and a positive control were applied: (1) composted pulp, (2) composted + parchment, (3) composted + parchment + flocculent, (4) control – inorganic fertiliser only, (5) composted + parchment + flocculent + pruned material, (6) composted + parchment + flocculent + pruned material + live mulch *(fine stem stylo)*. Dolomitic lime was applied before planting whereas Compound S (6:17:6NPK) at 650Kg/ha and Single Super Phosphate (90Kg/ha) were applied at planting. Where composted pulp and parchment were mixed, it was at a ratio of 1:1 and these were applied into covas starting six months after planting. Subsequent applications were done annually in April at a rate of 2.6t/ha up to the end of the trial. Flocculent was treated with lime to moderate the pH (1Kg lime in 1000 litres flocculent) after curing for three days and then applied at a rate of 20 litres per cova. Dead coffee branches were pruned in November and applied as mulch in the covas. The live mulch was planted between plant rows. Treatments without live mulch were mulched with Bana grass (*Pennisetum sp.*). Measurements of leaf nutrients and coffee yields were taken and analysed for variance. A net present value (NPV) analysis using a 12% discounting rate was done to determine the most economic coffee production option.

RESULTS AND DISCUSSION

Leaf Nutrient Composition

There were no significant differences in leaf nutrient composition ($p > 0.05$) between low, medium and high fertilizer regimes (Figure 1).

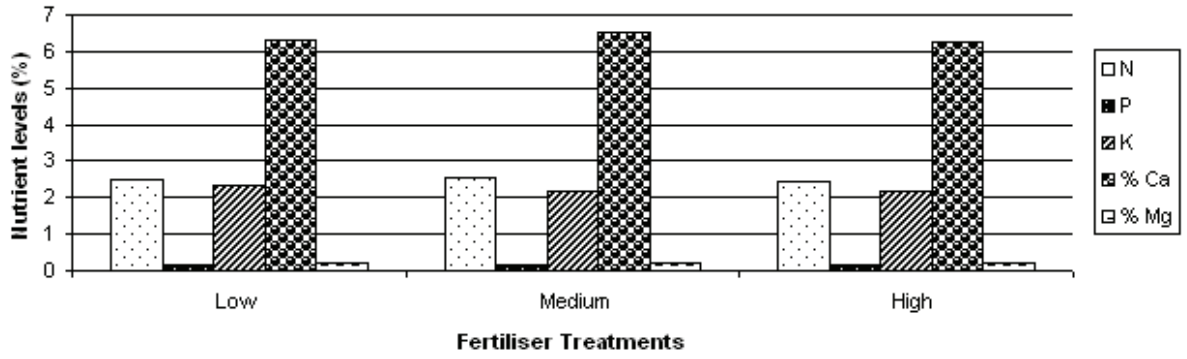


Figure 1. Effect of chemical fertilizer levels on mean major leaf nutrient composition.

Composted pulp applied alone resulted in higher N levels than where it was applied together with other materials although this was not significantly different from that of other treatments (Figure 2). In agreement with Njoroge (Njoroge, 2000), composted pulp proved to be a rich source of nutrients especially nitrogen.

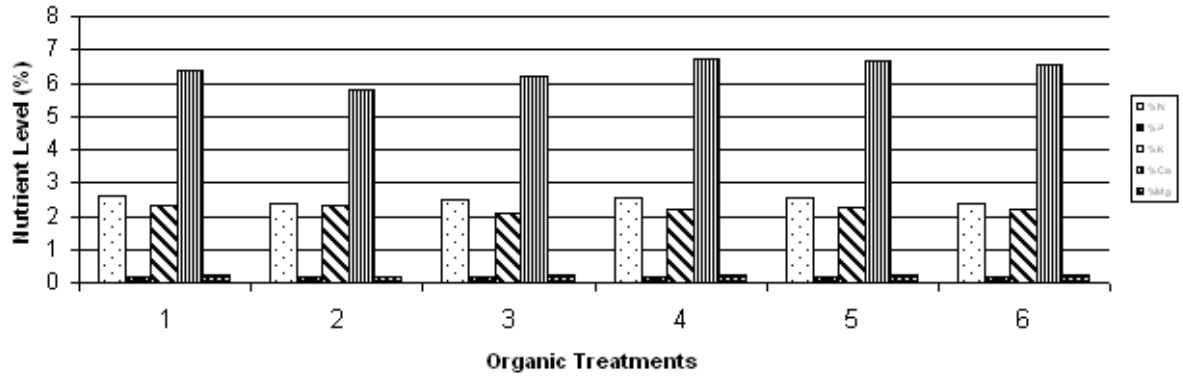


Figure 2. Effect of organic treatments on nutrients in the leaf over a 5 year period.

Yield Performance

There were no consistent yield advantages from increasing inorganic fertilizer levels (NPK ratios) from 20:10:20 to 80:40:80 over the study period (Table 1). Only the third year of harvesting showed a direct relationship between increasing NPK ratios and yields of coffee ($p < 0.05$). Biennial bearing might have contributed to the resultant response.

Table 1. Effect of fertilizer levels on clean coffee yields (Kg/ha) over a five-year period.

Level	Year 1	Year 2	Year 3	Year 4	Year 5	Mean
Low	869	2011	3493a*	2273	4479	1174
Medium	825	1742	3992a	2063	4758	1118
High	888	1820	4130a	2265	4854	1094
p	0.20	0.283	0.012	0.434	0.636	0.783

**Means followed by the same letter are not different after the Duncan's Multiple Range Test (DMRT).*

Recycled coffee wastes and by-products showed significant yield differences ($p < 0.05$) only in the first two years of harvesting. Composted pulp alone produced significantly higher yields (2245t/ha) followed by composted pulp plus parchment, pruned material and flocculent in the second year of harvesting (Table 2). Younger coffee showed greater yield response to organic treatments than older coffee in line with findings by other researchers (Kutywayo et al., 2007; Njoroge et al., 1990: Njoroge, 2000).

Table 2. Effect of recycled coffee wastes and by-products on coffee yield (Kg/ha) over a five-year period.

Treatment	Year 1	Year 2	Year 3	Year 4	Year 5	Mean
1	931ab*	2245a	3752	2287	4291	1103
2	849abc	1957a	3922	2196	4548	1206
3	805bc	1678a	3735	2457	4637	1250
4	1011a	1767a	3788	2102	4417	977
5	838abc	2105a	4317	2219	5051	1078
6	730c	1396a	3714	1939	5240	1158
p	<0.001	0.017	0.330	0.481	0.536	0.623

**Means followed by the same letter are not different after DMRT.*

The addition of live mulch to organic treatments as an intercrop consistently showed yield reducing effects in the first four years of harvesting and only resulted in higher yields in the fifth year, which however were not significantly different ($p > 0.05$) from the rest of the treatments (Table 2). This could be due to competition for nutrients and water between coffee and the live mulch. An interaction of medium inorganic fertilizer level (40:20:40NPK) and composted pulp produced higher yields than the other treatments.

Financial Performance

There was a positive net present value (NPV) percentage increase over the control at low fertilizer level when composted pulp only (15%) and composted pulp, parchment, flocculent and pruned materials (24.1%) were applied to the coffee plants (Table 3).

Table 3. Net present value values for recycled coffee wastes under different fertiliser levels.

Treatment	Low		Medium		High	
	NPV	NPV % over 4	NPV	NPV % over 4	NPV	NPV % over 4
1	12914.09	15.7	14528.85	4.7	12392.10	-11.9
2	9718.57	-12.9	10450.38	-24.7	13380.40	-4.9
3	9647.52	-13.6	11031.20	-20.5	11200.30	-20.4
4	11100.44	0.0	13874.40	0.0	14069.20	0.0
5	13849.95	24.1	13340.73	-3.8	15289.82	8.7
6	4440.62	-60.2	7198.90	-48.1	7394.49	-47.4

CONCLUSION

Composted pulp and a combination of parchment, composted pulp, flocculent and pruned materials gave higher coffee yields and financial returns when applied together with low fertilizer levels. They can therefore be used to partially substitute chemical fertilisers in coffee production.

REFERENCES

Oliveira L.S., Franca A.S., AlvesT.M., Rocha S.D.F. Evaluation of untreated coffee husks as potential biosorbents for treatment of dye contaminated waters, J. Hazard Mater.; 2008, 155, 507-512.

Endris S. Accelerated Composting of Coffee Processing By-products: an Organic Option for Soil Fertility Management in the Coffee Based Cropping System of Southwestern Ethiopia, Ethiopian Institute of Agricultural Research (EIAR), 2007, Jimma.

Korikanthimath V.S., Hosmani M.M. Organic recycling of coffee pulp in coffee based cropping systems, The Mysore J. Agri. Sci., 1999, XXXII No. 2: 714-719

Mitchel H.W. Effect of Manure and Fertiliser on Arabica Coffee in Kenya, Kenya Coffee Board, 1990, 1-4

Kutywayo D., Chingwara V., Chemura A., Mandhazi R., Masasi G. Integration of Inorganic and Organic Fertilisers in Coffee Production: Is it Worth the Effort? Proceedings of the Agricultural Science and Technology Symposium, 2007, AREX, Harare.

Njoroge J.M., Mwakha E. and Kimenia J.K. Effects of Planting Hole Size and Farm Yard Manure Rates on Establishment of High Density Arabica Coffee, Kenya Coffee, 1990, 52, 775-787

Njoroge J.M. Advances in Coffee Agronomy. In Naidu, Rn (2000) Proceedings of the International Scientific Symposium on Coffee, 4th December 2000, Bungalore, India: 2000, 104-118

Use of Precision Agriculture Techniques to Produce High Quality Coffee

D.M. QUEIROZ, F.A.C. PINTO, E.A. ALVES

Departamento de Engenharia Agrícola, Universidade Federal de Viçosa, Viçosa, MG, Brazil

SUMMARY

The objective of this work was to analyze the coffee quality by using the spatial correlation. The work was done in the harvesting seasons from 2004 to 2007 at Brauna Farm, located in Araponga, Minas Gerais state, Brazil. Coffee quality maps were generated. From the quality maps, the spatial autocorrelation analyses were performed by using the Moran Index. The results obtained indicated that the coffee quality has spatial dependence.

INTRODUCTION

The coffee quality is related to the interactions among the plants, the environment and the management practices on the fields. The conditions of weather, coffee field management, the way the product is harvested, pre-processed and stored define the final quality of the coffee. The number of factors that affect the quality and spatial variability of quality have made the optimization of coffee production difficult. According to Queiroz et al. (2004), the precision agriculture can help to optimize the coffee production because coffee is a crop that has high revenue per hectare and the coffee price is dependent on coffee quality. By using the precision agriculture techniques producers can identify the areas that potentially can produce coffee with better quality and to understand the factors that are associated with coffee quality. It is well known that the crop quality on the field can change from year to year and spatially due to soil, weather conditions and management of agricultural operations differences. The objective of this work was to analyze the coffee quality variability by using the spatial correlation.

MATERIAL AND METHODS

This work was performed from the 2004 and to the 2007 crop seasons. The farm chosen for performing this work was Braúna Farm, located close to Araponga city, Minas Gerais state, Brazil. This farm has a total area of 306 ha, the area cultivated with coffee Arabica is 86 ha. This area is divided in fields with 1 to 2 ha. From each field that was producing, coffee samples of cherry fruit were manually harvested. A total of 30 coffee trees were randomly chosen to be harvested per hectare. From each chosen tree, only four branches were selected to get the sample. At the end all the samples collected in a field were mixed together to form the field sample. The cherry coffee samples were unshelled, dried to a moisture content of 12% and stored in the Laboratory. Four months later, coffee quality of each sample was evaluated. From each sample, three cups of coffee were prepared and tested. The coffee quality was evaluated by its flavor, sweetness, acidity, body and taste. A scale that has a maximum value of 100 points was used to grade coffee quality. This procedure was developed by Brazilian Specialty Coffee Association (BSCA). According to this evaluation system, a coffee with a grade higher than 80 is considered to have a special quality, and is called special coffee. After doing the quality evaluation of coffee samples, a map of coffee quality was generated and then the spatial autocorrelation study was performed by using the Moran Index (Ebdon, 1988). After calculating this index, the null hypothesis by using the Z

statistical test, in this test the null hypothesis is represented by a randomly distribution of the quality grades. A strong spatial autocorrelation means that the quality grades for samples that were closer were highly correlated.

RESULTS AND DISCUSSION

In Figure 1, the histograms of coffee quality grades (2004-2007) are presented. Most of coffee fields produced good quality, in these cases a majority of the grades were higher than 70. The results showed that a low percentage of samples reached a grade equal or greater than 80 in the seasons of 2004 to 2007.

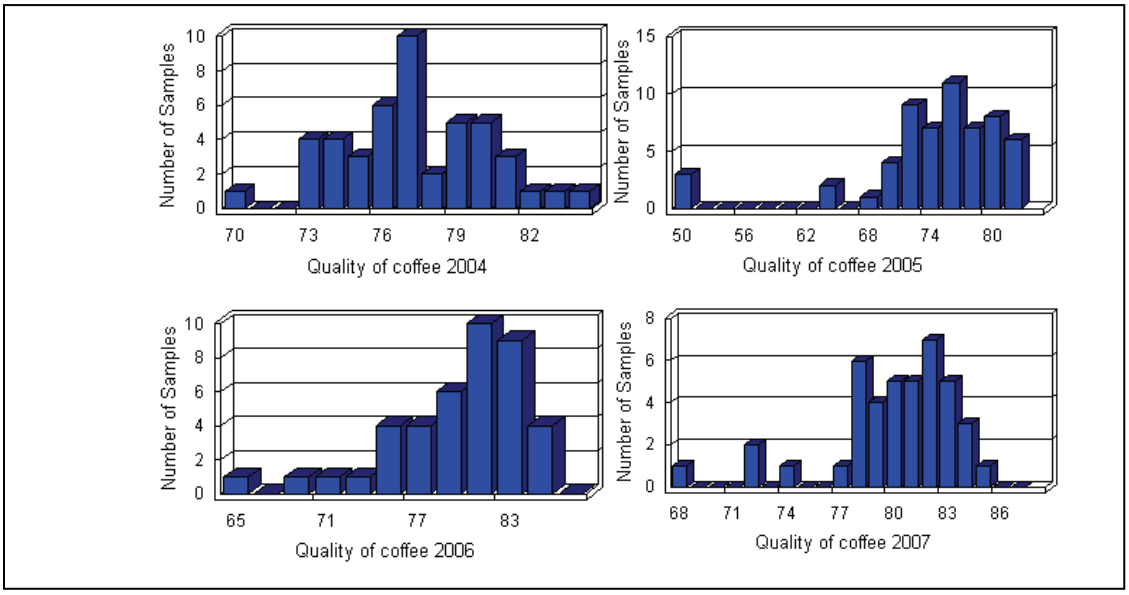


Figure 1. Histogram of the grades of the samples obtained in the cup tests.

In Figure 2 the coffee quality maps for the 2004 to 2007 harvesting seasons are presented. The farm was divided in three different regions because of the location of coffee fields. It was observed that there was an increase in the beverage quality of the coffee on the farm. This increase was both in number of plots with more than 80 grades and on the value of the grades of the samples. Visually, it can be seen that there is spatial variability of coffee quality. For the seasons of 2004, 2005, 2006 and 2007 was observed that 13%, 14%, 59% and 48% of samples got grades higher than 80 points on the farm. In those samples, 100%, 89%, 66% and 68% of the samples came from region 3 in the 2004, 2005, 2006 and 2007 harvesting seasons, respectively. In Table 1, are presented the calculated values of the Moran Index and the tests for spatial autocorrelation from 2004 to 2007 harvesting seasons. By performing the significance test, only region 3 presented a calculated Z value higher than the reference Z values, which rejects the null hypothesis that is the randomly distribution of the data for 2004 and 2005 seasons. Therefore, in the region 3 there is spatial dependence of the coffee quality. The average of the grade values in this region was higher than average to regions 1 e 2. This behavior can be caused by factor associated to the environment in this area and to the management practices and the coffee varieties that are grown in this area. Therefore, by performing the significance test, a calculated Z value higher than the reference Z values for the harvest seasons 2006 and 2007 do not rejects the null hypothesis that is the randomly distribution. For these seasons there was no striking autocorrelation of the quality in any area, or difference on the average quality between the three regions.

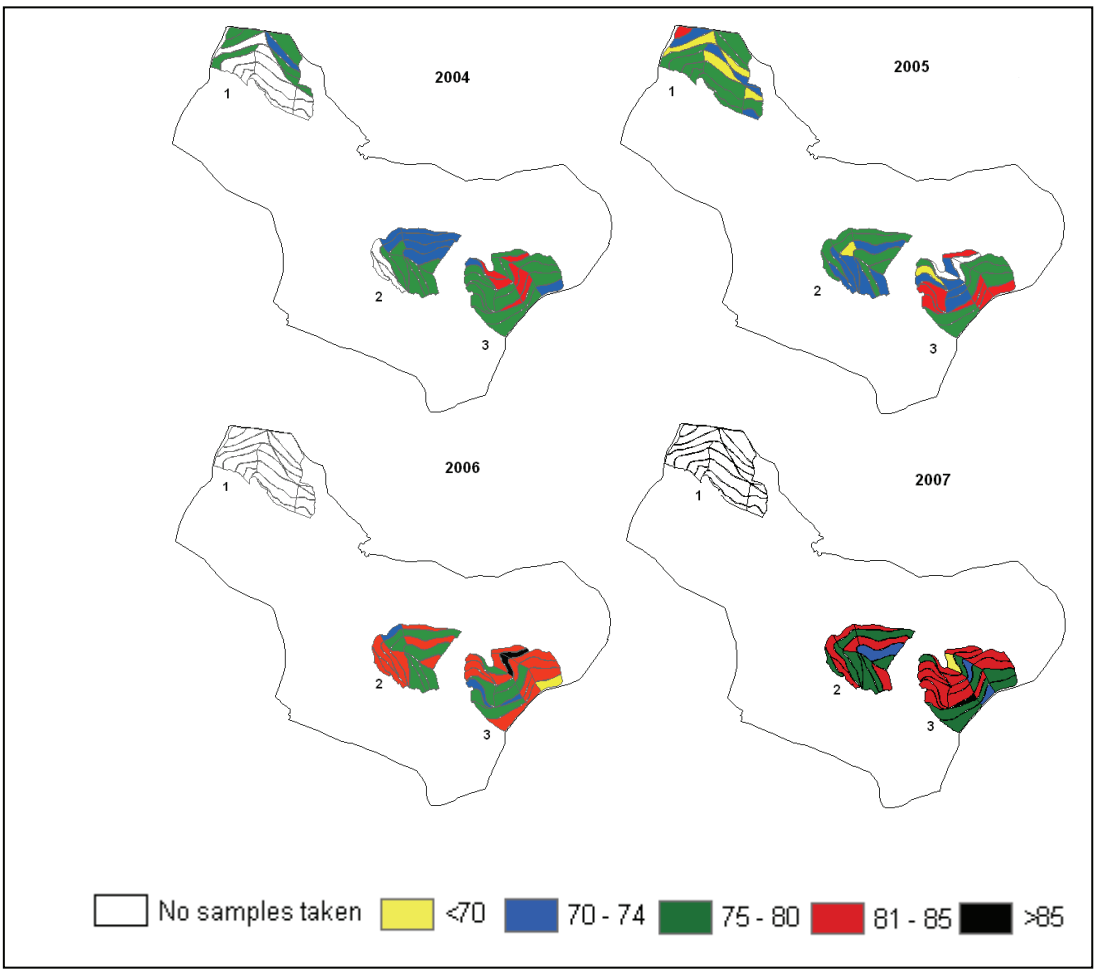


Figure 2. Quality grade maps based on the cup test for the 2004 to 2007 harvesting season

CONCLUSION

The obtained results have shown that there are some fields that have higher potential for producing coffee with better quality. There was spatial variability of coffee quality. Coffee quality changed from the year 2004 to 2007. However, there was a region in farm that produced better coffee both seasons. In one region the three regions of the farm it was shown a spatial dependence of coffee quality for the years of 2004 and 2005.

Table 1. Calculated values of the Moran index and the significance test of the coefficient of spatial autocorrelation for the 2004 to 2007 harvesting season.

2004

	Region 1	*Region 2*	*Region 3*
Moran Index (I)	-	0.26	0.21
Z calculated	-	1.54	1.66*
Number of coffee fields	-	13	26
Number of unions	-	17	42
Grade average	-	75.46	78.42
Standard deviation of the grades	-	2.76	2.99
2005			
Moran Index (I)	-0.16	0.07	0.34
Z calculated	-0.62	0.72	2.35**
Number of coffee fields	20	16	21
Number of unions	27	24	33
Grade average	71.05	75.31	78.10
Standard deviation of the grades	9.47	4.26	4.23
2006			
Moran Index (I)	-	0.08	0.10
Z calculated	-	0.76	0.96
Number of coffee fields	-	16	26
Number of unions	-	24	42
Grade average	-	80.13	80.31
Standard deviation of the grades	-	3.92	4.78
2007			
Moran Index (I)	-	0.18	0.16
Z calculated	-	0.75	1.03
Number of coffee fields	-	16	21
Number of unions	-	24	33
Grade average	-	79.31	80.33
Standard deviation of the grades	-	2.76	4.20

*** Significant at 5% probability level by the Z test.*

ACKNOWLEDGEMENTS

The authors of the work thank the FAPEMIG - Foundation for Research Support of the State of Minas Gerais, Brazil − for the financial support for accomplishment of this research.

REFERENCES

BSCA - Associação brasileira de cafés especiais. Regras de Competição Nacionais e Internacionais. Alfenas, [2004]. 5 p.

Queiroz D.M., Pinto F.A.C., Zandonadi R.S., Emerich I.N., Sena Junior D.G. Uso de Técnicas de Agricultura de Precisão para a Cafeicultura de Montanha. In: Zambolim L. (Ed.) Efeitos da Irrigação sobre a Qualidade e Produtividade do Café. Federal University of Vicosa, Vicosa, MG, Brazil, pages 77-108. 2004.

Arborized Coffee Crop in São Paulo State, Brazil: Microclimatic, Phenological and Agronomic Evaluations

J.R.M. PEZZOPANE[1*], M.J. PEDRO JÚNIOR[2], P.B. GALLO[3], M.B.P. DE CAMARGO[2], P.S. DE SOUZA[3], G.S. ROLIM[2]

[1]CEUNES, Universidade Federal do Espírito Santo, Rua Humberto de Almeida Franklin, 257, CEP 29933-415, São Mateus, ES, Brazil. [*]E-mail: josepezzopane@ceunes.ufes.br
[2]Centro de Ecofisiologia e Biofisica, Instituto Agronômico de Campinas, P.O. Box 28, CEP 13020-902, Campinas, SP, Brazil
[3]Pólo APTA Nordeste Paulista, P.O. Box 58, CEP 13730-972, Mococa – SP

SUMMARY

Microclimatic, phenological and agronomic evaluations were taken in coffee crop (*Coffea arabica* L.) grown in two different conditions: shaded and unshaded, in Garça and Mococa regions, São Paulo State, Brazil, from 1999 to 2006. It was evaluated the coffee crop shaded by dwarf coconut palm (*Cocos nucifera* L.) at Garça, and the coffee crop shaded by banana *(Musa sp.*) 'Prata Anã', and shaded by grevílea trees (*Grevillea robusta*), both at Mococa. The microclimatic measurements (solar radiation, net radiation, wind speed, air temperature and air moisture) were taken for the different crops conditions. The grain yield and phenological data and their variability for different positions in shaded coffee crops were taken, from 2002 to 2006. The results showed differences between the two cropping systems. The coffee shaded plants received the incoming solar radiation reduced by 21% to 42%, these values were influenced by the cultivation system evaluated and by the season of the year. The air temperature and air moisture, the average and daily extremes values had a quite different variation inside the shaded system used and the position of the shaded crop. Regarding the minimum air temperatures the largest differences among the unshaded and shaded crop cultivation were obtained in the coffee crop and dwarf coconut tree in Garça and, for the maximum air temperatures in the coffee crop shaded with grevílea in Mococa. Regarding the humidity of the air, the shaded crop of coffee with grevílea presented reduction of the vapor pressure deficit between 10 and 14 hours. In all the studied shaded systems significant reduction was verified in the incidence of the winds in comparison to the unshaded coffee crop, with monthly average values of reduction always 30% higher. Regarding aspects related to the yield, the experiments had shown until 2006 harvesting, similar productions between shaded and unshaded coffee crop, with slightly superior yield in the coffee crop shaded with grevílea.

INTRODUCTION

Association of coffee and shade trees aims to minimize the exposition of the coffee plants to climatic risks as frosts, excesses of solar radiation, high temperatures and extreme winds, contributing for increasing of coffee plantation sustainability (Beer, 1987).

Some authors had observed the variation of meteorological elements in diverse types of coffee crop shaded in different producing regions of Brazil and in other countries (Barradas e Fanjul, 1986; Caramori et al., 1996). These works had evidenced the relation to microclimatic

differences in the wind speed, air temperature, vapor pressure deficit between the shaded and unshaded crops.

Coffee crop shaded tend to produce less than the unshaded crops because the greater stimulus to vegetative rather than flower buds (Cannell, 1976), and fewer nodes formed per branch and flower buds at existing (DaMatta, 2004). Unshaded plantations should be recommended in favorable climatic conditions for the coffee plantations and with the intensive use of agrochemical inputs, mechanization, irrigation and modern, high-yielding varieties are available (DaMatta, 2004).

On sub-optimal coffee producing areas, characteristic of area with low altitudes, with poor soils, water deficit, microclimatic stress and high wind-speed conditions, farms will receive the benefits of shaded systems (Camargo, 1990). In these sites, shade trees reduce excessive solar irradiance and large diurnal variations in air temperature and humidity.

This study presents results from a six-year study comparing, in conditions of São Paulo State, Brazil, the effects of shade species on microclimate and coffee (*Coffea arabica* L.) productivity.

MATERIAL AND METHODS

In this study was evaluated the coffee crop shaded by dwarf coconut palm (*Cocos nucifera* L.) at Garça (22º 14´ S, 49º 37´ W, 620 m.), the coffee crop shaded by banana *(Musa* sp.) 'Prata Anã', and the coffee crop shaded by grevílea trees (*Grevillea robusta*), both at Mococa (21º 28' S, 47º 01' W, altitude 665 m).

In coffee crop shaded by green dwarf coconut trees and unshaded coffee crop, at Garça, microclimatic measurements (air temperature, solar radiation and wind speed) were taken from November, 1999 to October, 2000.

In coffee crop shaded by banana plants, coffee crop shaded by grevílea and unshaded coffee crop, at Mococa, microclimatic measurements (solar radiation, air temperature, relative humidity and wind speed) and phenological and agronomic evaluations were taken from 2001 to 2006.

The microclimatic measurements were taken in one position of the unshaded coffee crop and in two different positions of the shaded coffee crop: one close to the nearest point to the shaded plants and another in the center of the shaded crop plot.

From July, 2001 to June, 2004 phenological data for the coffee crops were taken and the growth of the plants concerning height and diameter was also evaluated in coffee crop shaded by banana plants. The harvests of 2002, 2003, 2004, 2005 and 2006 were appraised by the production parameters, and their variability for different positions in shaded coffee crop at Mococa.

RESULTS

Microclimatic evaluations

Coffee crop shaded by dwarf coconut palm

Monthly maximum air temperature values obtained in shaded coffee crop were 1.8 ºC lower and 1.7 ºC higher than the values at the open sky condition according to the month evaluated and the site in the shaded crop. Concerning on minimum air temperature the shaded crop showed monthly mean values equal or higher (up to 1.0 ºC) than the unshaded crop. Also these differences reached up to 3.0 ºC during cold nights. The green dwarf coconut trees reduced the incoming solar radiation (Qg) of coffee crop from 38% during summer up to 45% during the spring, and an average to the year up to 42%. Also the coconut trees reduced the wind speed 60 to 99%.

Coffee crop shaded by banana

The microclimatic measurements were taken in one position of the unshaded coffee crop and in two different positions of the shaded coffee crop: one close to the nearest point to banana plants and another in the center of the shaded crop plot.

Banana plants reduced the incoming solar radiation of coffee crop by 21%, with monthly variation from 16 to 27%. Differences were also found concerning the reduction of the incoming solar radiation in different points of the shaded crop.

Concerning on air temperature and air moisture, differences were found differences only for the maximum temperature at the central point of the shaded crop, showing higher averages in relation to the unshaded crop during the summer and autumn, and also in relation to the nearest point to banana plants during the spring, summer and autumn. During cold nights, in spite of the shaded coffee crop showing smaller radiation loss, an increment of 0.3 ºC in the air temperature was noticed and for the leaves the values reached 0.5 ºC.

It was verified a windbreak action of fanlight of the banana trees in the shaded crop, promoting deviation in air temperature and humidity profiles, showing reduced gradients, in relation to the unshaded crop, above the coffee plants canopy. The mean values of the energy balance components for the different systems of cultivation did not show difference.

Coffee crop shaded by grevílea trees

The results showed that there was a reduction about 26% of solar global radiation (Qg) in the shaded systems with a 24 and 30% or variation. Due to the grevilea canopy spatial variation there was a difference in the transmition of Qg in the shaded system. There was a reduction about 35% in the wind velocity five days-average in the shaded system. The shaded system promoted a reduction of maximum air temperature and in the water deficit vapor during day-light period mainly in the amostral point near the grevilea tree.

Phenological and agronomic evaluations

The height and diameter vegetative growth showed higher increase during the spring-summer period in relation to the autumn-winter period. No significant differences in growth taxes, of phenological development and of the yield indexes were found. In the shaded crop, the nearest point to banana plants showed differences in relation to the other measurement points

regarding vegetative growth and phenological development for some seasons of the year. Regarding aspects related to the yield, the experiments had shown until 2006 harvesting, similar productions between shaded and unshaded coffee crop, with slightly superior yield in the coffee crop shaded by grevílea trees.

REFERENCES

Barradas V.L., Fanjul L. Microclimatic chacterization of shaded and apen-grow coffee (Coffea arabica L.) plantations in Mexico. Agricultural and Forest Meteorology, v. 38, p. 101-112, 1986.

Beer J. Advantages, disadvantages and desirable characteristics of shade trees for coffee, cacao and tea. Agroforestry Systems, v. 5, p. 3-13, 1987.

Camargo A.P. de. A arborização como meio de reduzir as adversidades climáticas e promover a sustentação da agricultura. In: Congresso Brasileiro De Pesquisas Cafeeiras, 16., Espirito Santo do Pinhal, 1990. Trabalhos apresentados. Rio de Janeiro: IBC, 1990. p.6-7.

Cannell M.G.R. Crop physiological aspects of coffee bean yield – a review. Kenya Coffee, v. 41, p. 245-253, 1976

Caramori P.H., Androcioli Filho A., Leal A.C. Coffee shade with Mimosa scabrella Benth. for frost protection in southern Brazil. Agroforestry Systems, v. 33, p. 205-214, 1996.

Damatta F.M. Ecophysiological constraints on the production of shaded and unshaded coffee: a review. Field Crops Research, v. 86, p. 99-114, 2004.

Evaluation of Productivity and Quality of Coffee Growing in *Cerrado* (Brazilian Savannah) Conditions and Irrigated Using the Drip System

A.L.T. FERNANDES[1], P.V. RABELO[1], L.C.D. DRUMOND[2], R. DE OLIVEIRA SILVA[1]

[1]University of Uberaba and FAZU, Uberaba, MG, Brazil
[2]Federal University of Viçosa, Rio Paranaíba, MG, Brazil

SUMMARY

Especially in regions considered marginal with respect to a lack of water, the use of irrigation has become more frequent for coffee growing. However, correct standards of measuring and handling are not always employed. (Drumond and Fernandes, 2001). It is necessary to bring together technical-economic subsidies that allow a more appropriate and effective orientation in each situation that arises, as a function of the size and characteristics of the fields, availability of water (quality and quantity), availability of energy and training of existing labor (Fernandes and Drumond, 2002). The generation and adaptation of technologies of coffee production under a total and supplementary irrigation system are essential, in order to ensure continues and ongoing high productivity without harm to the environment. Most experimental studies on coffee irrigation demonstrate increases of 20 to 30 beneficiated sacks per hectare, regardless of the systems used, and depending on the region under study (Matiello et al., 1995). In addition to the increase in productivity, it has been noted that irrigation induces several bloomings, leading to a single branch having fruits at a variety of stages of maturity. (Oliveira et al., 2002). The objective of this study was to evaluate the productivity and final quality of the coffee produced using drip irrigation, comparing it to non-irrigated plants. The research was conducted at the Research and Teaching Farm, at the University of Uberaba, with the plant *Catuaí Vermelho,* planted in spacing of 4.0 m x 0.5 m. To evaluate the final quality of the beverage obtained with the different irrigated treatments, samples of benefited coffee were collected, and after roasting and grinding, these were evaluated by the Ministry of Agriculture. Sensory analysis was performed with all beans harvested in each system, and the cherry beans, dry cherry beans and green coffee beans were not separated. To determine the amount of water to apply to the crop, climatic data furnished by an automatic meteorological station was used to estimate the evapotranspiration of the crop, using the Penman Monteith method, proposed by the FAO. All the nutritional and phytosanitary treatments were similar, with differences only relative to the moment of pest and disease control, always performed after the determination of the level of economic damage of the pests and diseases. After 7 harvests, it was concluded that in the soil and climate conditions of Uberaba, productivity of the dry cropping areas is hindered by the lack of water. The irrigated sections had productivity that was 95% higher than that of the control crops. Even using irrigation systems with uniformity of application inferior to drip and central pivot systems, irrigation is feasible in terms of productivity and income obtained from the crop. With regards to the final quality of the beverage, it was seen to be better than the control crop without irrigation, although with similar characteristics to that obtained from the drip irrigation system.

INTRODUCTION

Brazil already irrigates more than 10% of its land dedicated to coffee growing, an area that accounts for 21% of domestic coffee production (Santinato et al., 2008). Especially in regions considered marginal with regards to a lack of water, the use of irrigation has become more common for coffee growing; however, correct standards of quantity and handling are not always followed (Drumond and Fernandes, 2001). Therefore, it is necessary to study in detail, and in a comparative fashion, the different systems of coffee tree irrigation, in order to obtain information that can indicate practices to coffee growers, whether in the recovery of existing plantations, or in the enlargement of irrigated coffee growing in the *Triângulo* region of Minas Gerais state. It is necessary to gather technical and economic data that permit a more appropriate and effective orientation for producers in each situation that arises, as a result of the size and characteristics of the crop, availability of water (quality and quantity), availability of energy and training of workers present. (Fernandes and Drumond, 2002). The generation and adaptation of coffee production technologies under total and supplementary irrigation are essential, to enable continuous and economical high productivity, without leading to degradation of the environment. Most of the experimental work on coffee tree irrigation shows increases of some 20 to 30 beneficiated sacks per hectare, independently of the systems used, and dependent upon the region under study (Matiello et al., 1995). In addition to the increase in productivity, it has been noted that irrigation leads tress to blossoming several times, so that a single branch has beans in different stages of maturity. (Oliveira et al., 2002). Within this perspective, this study sought to evaluate the productivity and quality of coffee under drip irrigation, after 7 harvests, comparing it to a crop raised without irrigation.

MATERIAL AND METHODS

The experiment was conducted at the Experimental Field of the University of Uberaba - MG, in red-yellow latosol in a sandy phase, at an altitude of 820 meters with a Red Catuai coffee plantation H2077-2-5/144 with spacing of 4.0 meters between rows and 0.5 meters between plants (Figure 1). The treatments were: a) conventional drip irrigation (2 ha), with brand Netafim, model Tiram, with flow of 2.3 L h^{-1} and spacing of 0.75 m between emitters; b) Self-compensating drip irrigation (1.5 ha), brand Netafim, model RAM, with flow of 2.3 L h^{-1} and spacing of 0.75 meters between emitters; c) Control (0.5 ha) – non-irrigated area. In order to define the % of maturation of the samples, beginning at 30 months, 20 liters of coffee were harvested per plot, of which 100 beans were randomly selected for treatment into dry, dried cherry and undeveloped green beans. To evaluate the final quality of the beverage obtained with the irrigated and non-irrigated treatments, samples of beneficiated coffee were taken, which, after roasting and grinding, were evaluated by classifiers of the Ministry of Agriculture. The sensory analysis was performed with all the beans harvested in each system, and the cherry, dried cherry and green beans were not separated. The productivity data was submitted to statistical analysis with a significance level of 5%. To verify normality and homocedasticity, the Kolmogorov-Smirnov and Bartlett tests were used, respectively. After verification of the homocedasticity of the data, ANOVA was used. After verification of ANOVA significance, the Tukey test was used for multiple comparisons among treatment averages. To determine the quantity of water applied to the crop, climate data were used from an automatic meteorological station, brand/model Micrometos 300, installed at the test site, where the following meteorological elements were measured: temperature and relative humidity, precipitation, global solar radiation and wind speed, data that was used to estimate the evapotranspiration of the crop, by the Penman Monteith method, proposed by the FAO. All the nutritional and phytosanitary treatments were similar among the treatments, with the only differences relating to the time of pest and disease control in the different systems,

always performed after determination of the level of economic damage of the pests and diseases.

RESULTS AND DISCUSSION

Table 1 presents the results obtained in the 2000 to 2007 harvests, which show the significant superiority of the drip irrigation treatments, when compared to the control (without irrigation). Attributing a value of 100 to the non-irrigated control, it can be seen that the irrigation systems, after 7 harvests, were responsible for productivity increases ranging from 55 to 93%. The best productivities were obtained from the coffee plantations that were irrigated with self-compensating drip irrigation, which is superior to conventional drip irrigation and to the control. This can be explained by the fact that the emitters in this treatment were installed after the first harvest was obtained, which allowed the coffee plantation, until its first harvest, to have a more developed root system than that in which the drip irrigation system was installed since planting. (irrigation treatment with conventional drip). Expressive results in productivity gains with irrigation were obtained by Soares et al. (2005), in an experiment conducted in Patrocínio, MG. The authors evaluated the depth of irrigation in two harvests, comparing it with the non-irrigated control, and reached a preliminary conclusion that the irrigation depth referring to reposition of 100, 125 and 150% of evapotranspiration of the crop (ETc) led to gains of productivity of 153% compared to the non-irrigated control. In an experiment conducted in the *Triângulo* region in Minas Gerais state, evaluating different irrigation depths in coffee tree production parameters, Teodoro et al. (2005) obtained maximum production of 115 beneficiated sacks per hectare, with a depth of 164% of the evaporation of the class A tank, which represents an increase of 447% in relation to the non-irrigated treatment. With regards to the final quality of the coffee, superiority of the treatment without irrigation (control) was found, which obtained better results than the irrigated treatments, with the final classification soft and just soft (Table 2), except for the last two harvests. Rezende et al. (2006), in a study conducted in Lavras, MG, obtained similar results with the topaz coffee tree cut close to the ground and irrigated with drip irrigation. After two harvests, they concluded that the productivity and results tended to be higher; however, the irrigation delayed the maturation of the beans. It was noted that in all the harvests evaluated, the number of defects found in the control was lower than those found in the irrigated treatments.

Table 1. Results of 2001 to 2007 harvests for the irrigated and non-irrigated treatments, for Arabic coffee grown in *cerrado* conditions.
Farm School of the University of Uberaba, Uberaba - MG.

Treatments	**Productivity (beneficiated sacks / ha)**								**R%**
Systems	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	*Average*	
Self-compensating Drip	28,9 b	41,9 b	94,4 a	73,9 a	42,0 a	118,5 a	40,8 a	62,9	193
Non Self-compensating Drip	66,8 a	51,4 a	38,9 b	50,3 b	35,2 a	72,2 b	39,2 a	50,6	155
Control group	5,4 c	29,8 c	19,0 c	48,4 b	35,4 a	52,4 c	38,0 a	32,6	100
CV	26,76	32,09	23,31	34,50	43,36	25,216	27,983		

CONCLUSION

After 7 harvests, it can be concluded that under the climate and soil conditions of Uberaba, the productivity of non-irrigated plantations is low when compared to irrigated plantations; areas irrigated with drip irrigation, showed productivity that ranged from 55 to 93% higher than that of the control group

Table 2. Evaluation of the quality of the coffee beverage* produced from different irrigation systems, Farm School of the University of Uberaba, Uberaba - MG, from 2001 to 2007 (7 harvests).

Sample	Harvest	Sieve 16 or higher (%)	Beverage	Overall Quality**
Self-compensating Drip Irrigation	2001	73	Just soft	3,0
	2002	80	Hard	3,0
	2003	49	Slightly rushing	1,5
	2004	65	Hard	2,5
	2005	77	Rushing	1,0
	2006	75	Hard residue	1,0
	2007	67	Astringent hard	1,5
Conventional Drip Irrigation	2001	71	Soft	3,0
	2002	77	Just soft	2,0
	2003	59	Rushing	1,5
	2004	65	Just soft	3,0
	2005	68	Just soft	1,5
	2006	54	Hard residue	1,0
	2007	62	Astringent hard	2,5
Control (without irrigation)	2001	82	Mole	3,5
	2002	66	Just soft	2,5
	2003	67	Just soft	3,0
	2004	70	Just soft	3,0
	2005	75	Just soft	2,0
	2006	58	Hard residue	1,0
	2007	55	Irregular Hard	1,5

**Analysis conducted by Café Toledo Ltda. and by Qualicafex.*
***Overall condition of sample: 5 – excellent, 4 – very good; 3 – good; 2 – regular; 1 – poor.*

REFERENCES

Drumond L.C.D., Fernandes A.L.T. Coleção Cafeicultura Irrigada – Irrigação por Aspersão. Editora Universidade de Uberaba. 102p. 2001.

Fernandes A.L.T., Drumond L.C.D. Coleção Cafeicultura Irrigada – Gotejamento. Editora Universidade de Uberaba. 88p. 2002.

Matiello J.B., Miguel A.E., Vieira E. and Aranha E. Novas observações sobre os efeitos hídricos no pegamento da florada de cafeeiros. 21º Congresso Brasileiro de Pesquisa Cafeeira. Caxambu, (MG). p. 60.1995.

Oliveira L.A.M., Faria M.A., Alvarenga A.A., Silva M.L.O., Silva A.L., Garcia P.R., Costa H.S. Efeito da época da irrigação na emissão de flores e no estabelecimento de frutos of the coffee tree (Coffea arabica L.). In: Simpósio Brasileiro de Pesquisa em Cafeicultura Irrigada, 5., 2002, Araguari. Anais... Uberlândia: UFU, 2002. p.47-51.

Rezende F.C., Oliveira S.R., Faria M.A., Arantes K.R. Características produtivas do cafeeiro (*Coffea arabica* L. cv., Topázio MG-1190) recepado e irrigado por gotejamento. Coffee Science, Lavras, v.1, n.2, p.103-110, 2006.

Santinato R., Fernandes A.L.T., Fernandes D.R. Irrigação na Cultura do Café. 2. ed. Belo Horizonte: O Lutador, 583p., 2008.

Soares A.R., Mantovani E.C., Rena A.B., Coelho M.B., Soares A.A. Avaliação do efeito da aplicação de diferentes lâminas de irrigação na produtividade do cafeeiro para a região do cerrado de Minas Gerais. In: Simpósio Brasileiro de Pesquisa em Cafeicultura Irrigada, 7, 2005, Araguari. Anais..., p. 50-53.

Teodoro R.E.F., Melo B., Carvalho H.P., Guirelli J.E., Benedetti T.C., Bueno M.R. Influência de diferentes lâminas de irrigação nos parâmetros de produção do cafeeiro cultivado em região de cerrado.. In: Simpósio Brasileiro de Pesquisa em Cafeicultura Irrigada, 7, 2005, Araguari. Anais..., p. 161-165.

Effective Intercropping for Rehabilitating Old Unproductive *Coffea arabica* (Linneaus) on Mambilla Plateau, Nigeria

S.S. OMOLAJA* AND G.O. IREMIREN

Cocoa Research Institute of Nigeria (CRIN), P.M.B 5244, Ibadan, Nigeria.
*E-mail: dolaomolaja@yahoo.com

SUMMARY

In order to maximize labour use and rehabilitate old unproductive *Coffea arabica*, the effect of intercropping old unproductive coffee plants with some arable crops were studied on Mambilla Plateau, Taraba State, Nigeria. The systems evaluated were coffee/maize/cowpea, coffee/maize/melon, coffee/maize/groundnut and coffee/cocoyam/melon. The treatments were laid out in a randomized complete block design with four replications. Plot size was 17 m by 20 m. Sole crop plot measuring 17 x 20 m, was adjacent to the intercrop plots. Growth and development of coffee were evaluated by measuring the number of leaves, leaf colour, leaf area and yield (kg/ha). Data were subjected to analysis of variance (ANOVA) and the treatment means were compared using least significant difference at 5% level of probability. Result showed that growth performance in terms of leaf number, leaf colour and leaf area of coffee in the various systems was superior to that of coffee sole. The colour of leaf of coffee plants in the intercropped turned green from its previous yellowish green, thus facilitating efficient photosynthetic ability. The colour of leaves of coffee sole still remained yellowish green. There were significant differences in annual dry berry yield of coffee in the intercropping systems. The breakdown of dry coffee berry yield in different cropping systems was as follows: coffee/maize/cowpea mixture: 1,465.5 kg; coffee/maize/melon: 1,525.0 kg; coffee/maize/groundnut mixture: 1,706.0 kg; coffee/cocoyam/melon: 1,398.0 kg and coffee sole (control): 175.0 kg. This indicated that coffee/maize/groundnut intercrop performed best, while coffee sole was lowest. There was significant difference in economic return from sole coffee and the intercropped systems ($P < 0.05$). The coffee/maize/groundnut mixtures gave the highest economic return from the same land area. Weed incidence and frequency of weeding were three times more in coffee sole than in intercropped systems. This suggests that weed suppression was higher under intercropping than coffee sole. It is recommended that old coffee plantation could be rehabilitated by intercropping it with maize and groundnut. This brings higher economic returns per unit of land area and ensures a more efficient use of available soil nutrients and labour.

INTRODUCTION

Intercropping, which is the planting of two or more crops simultaneously on the same piece of land is the most practiced among the various forms of multiple cropping. Intercropping young coffee with plantain/banana is commonly practiced in Nigeria, primarily to provide shade for juvenile coffee. It however equally suppresses weeds and provides income to farmers before coffee starts to bear.

There had not been any serious attempt on the part of the Nigerian farmers to intercrop coffee with food crops either at the initial stage of establishment or when they have become senescent and unproductive. This was confirmed by Osajuyigbe and Obatolu (1984) in their survey of the coffee growing areas of Kwara State, in which they found out that decline in

coffee production was due to the initial high cost of production, which could not be defrayed by the farmers because most of them planted their coffee sole.

Most previous research on intercropping of coffee was with plantain/banana and maize (Okelana, 1982). Okelana (1982) reported the successful intercropping of coffee with maize using two different maize planting densities at 12,486 and 21,852 plants/ha at Kusuku, Mambilla Plateau, when coffee was still productive. Osajuyigbe and Obatolu (1986) suggested intercropping of coffee with yam, maize and cowpea at the initial stage of establishment before coffee comes into bearing and during rehabilitation to provide some foods and income to the farmers. However little is, known of the best cropping systems for rehabilitating old unproductive coffee plants. The objective of this study therefore is to evaluate various arable crop combinations that could rehabilitate and bring into profitable bearing, old and unproductive *Coffea arabica* plants.

MATERIALS AND METHODS

The study was carried out at Cocoa Research Institute of Nigeria (CRIN) Substation, Kusuku, Mambilla Plateau, Taraba State, Nigeria between 2006 and 2008. Mambilla Plateau is a cool high altitude (1550 metre above sea level) region in Nigeria where 90% of commercial *Coffea arabica* cultivation is concentrated. Average coffee yield on commercial plot on the Mambilla plateau ranges between 1.0 and 1.2 t/ha per year. The region extends from between Latitude 6.5° N to 8° N and Longitude 11° E to 12° E. The soil is an ultisol with a pH of 4.5 to 5.6. Average annual rainfall is 1860 mm, which spreads over nine months, from February to October.

The coffee plants used for this trial were 40 years old; with apparent signs of old age by its consistent low level of productivity for the past five years. Moreover, the leaves were yellowish green as a result of impoverished soil and irregular weeding. Weed could not be effectively carried out owing to inadequacy of labour to cope with timely weeding regime. Arable crop combinations were therefore introduced with a view of maximising available labour and examining the possibility of rehabilitating the plant for improved productivity.

Table 1. Crops used for intercropping.

Crop	Variety	Spacing (cm)	Planting density / ha
Maize (*Zea mays* L.)	Local	60 x 60	27,777
Cowpea (*Vigna unguiculata* L)	Local	25 x 25	160,000
Melon (*Colocynthus vulgaris* L)	Local	20 x 20	250,000
Groundnut (*Arachis hypogaea*)	Local	20 x 20	250,000
Cocoyam (*Xanthosoma sagittifolium*)	Local	120 x 120	13,888
Coffee (*Coffea arabica* L.)	Commercial	250 x 250	1,600

The four crop combinations studied were coffee/maize/cowpea, coffee/maize/melon, coffee/maize/groundnut and coffee/cocoyam/melon, with sole coffee as control. The treatments were laid out in a randomized complete block design with four replications. Plot size was 17 m by 20 m. Sole crop plot measuring 17 x 20 m, was adjacent to the intercrop plots. In April / May of each year, arable crops were planted. The old coffee plant inside which the arable crops were planted was established in 1967. Planting of arable crops was repeated twice annually. The varieties, spacing and planting densities used are shown in Table 1. Cocoyam was planted in mounds, while the other arable crops were planted on flat ground. The experimental plots were weeded by hand slashing when the weeds were 30 cm high.

Maize was thinned to two plants per stand three weeks after planting. Commercial NPK (15-15-15) fertilizer was applied at 200 kg / ha as blanket treatment to all crops.

Arable crops were harvested six (6) months after planting (MAP) for melon and cowpea, 6 MAP for maize and 10 MAP for cocoyam. Melon seeds were processed by the method of Ogunremi (1978) and the yield calculated at 6 % moisture content. Maize was shelled and dried to a moisture level of 14.5 % before the yield was estimated.

Growth and development of coffee were evaluated by measuring the number of leaves, leaf colour, leaf area and yield (kg/ha). Leaf area was estimated by multiplying the length by breadth and using a correction factor of 0.75. Coffee berries were harvested in November of every year and dried on concrete slabs to a moisture level of 6-8 %. Data were subjected to analysis of variance (ANOVA) and the treatment means were compared using least significant difference at 5% level of probability (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Leaf colour, leaf number and leaf area of coffee in the various intercropping systems are presented in Table 2. The number of leaf in intercropped coffee was higher than that of sole coffee. The numbers of leaf of coffee in coffee/maize/cowpea and coffee/maize melon mixtures were significantly higher than in coffee/cocoyam/melon and sole coffee. The colour of leaf of coffee plants in the intercropped turned green from its previous yellowish green, which is a sign of improved health, thus facilitating efficient photosynthetic ability. The colour of leaves of coffee sole still remained yellowish green. The leaf area of intercropped coffee was superior to that of coffee sole (control) in all cases. This was probably due to a more favourable microclimatic condition in the intercropping systems. According to Adeyemi (1989) adult coffee grew better when intercropped with cocoyam or maize, probably because the residues from the arable crops would have enriched the soil. Intercropping coffee with groundnut and melon showed similar results (Odegbaro, 1972). The earlier workers noted that the various crop combinations did not have any deleterious effect on coffee.

Table 2. Effects of intercropping on leaf characters of old *Coffea arabica* in the various cropping systems.

Cropping systems	Adult leaf colour	Leaf number	Leaf area (cm^2)
Coffee/maize/cowpea	Green	2,181	2.67
Coffee/maize/melon	Green	2,468	2.53
Coffee/maize/groundnut	Green	2,894	2.48
Coffee/cocoyam/melon	Green	1,982	2.57
Coffee (Control)	Yellowish green	1,061	1.72
LSD ($P \leq 0.05$)		56	0.23

There were significant differences in annual dry berry yield of coffee in the different planting conditions for the two years: 2006 and 2007 ($P < 0.05$) (Figure 1). On the average, dry berry yield in intercropped systems was better than coffee sole. The breakdown of dry coffee berry yield in different cropping systems was as follows: coffee/maize/cowpea mixture: 1,465.5 kg; coffee/maize/melon: 1,525.0 kg; coffee/maize/groundnut mixture: 1,706.0 kg; coffee/ cocoyam/melon: 1,398.0 kg and coffee sole (control): 175.0 kg. This indicated that coffee/maize/groundnut intercrop consistently performed best, while coffee sole was lowest (Table 3 and Figure 2). Yield of intercropped coffee was significantly higher than that of sole coffee because of improved soil condition and better weeding. Similar result was obtained in the coffee/maize trial (Okelana, 1982).

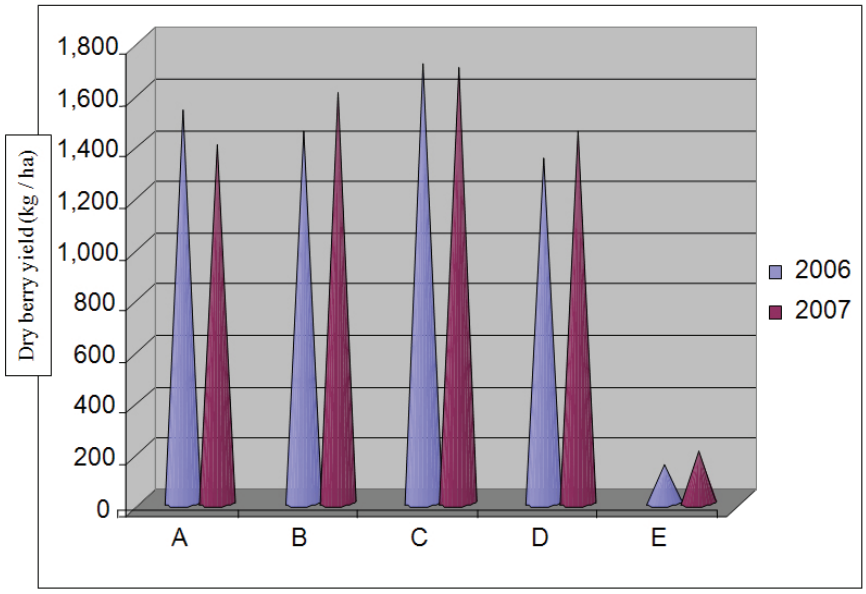


Figure 1. Dry berry yield (kg/ha) of *Coffea arabica* under different intercropping systems in 2006 and 2007. Cropping systems: A: coffee/maize/cowpea, B: coffee/maize/melon, C: coffee/maize/groundnut, D: coffee/cocoyam/melon and E: Coffee sole (control).

The income in US Dollar from the old coffee plants and arable crops in the intercropping system is presented in Table 4. There was significant difference in return from sole coffee and the intercropped systems ($P < 0.05$). The coffee/maize/groundnut mixtures gave the highest economic return from the same land area. This suggests that intercropping is an effective method of rehabilitating old and unproductive *C. arabica*. The best intercropping combination therefore was coffee/maize/groundnut in terms of income.

Table 3. Average annual yield (kg/ha) of arable crops and coffee under different intercropping systems.

Cropping systems	maize	cowpea	melon	groundnut	cocoyam	Coffee
A	542.0	347.0	-	-	-	1,465.5
B	674.0	-	12.8	-	-	1,525.0
C	552.0	-	-	301.0	-	1,706.0
D	-	-	11.5	-	1,120.0	1,398.0
E	-	-	-	-	-	175.0
LSD						68.87

Cropping systems: A: coffee/maize/cowpea, B: coffee/maize/melon, C: coffee/maize/ groundnut, D: coffee/cocoyam/melon and E: Coffee sole (control)

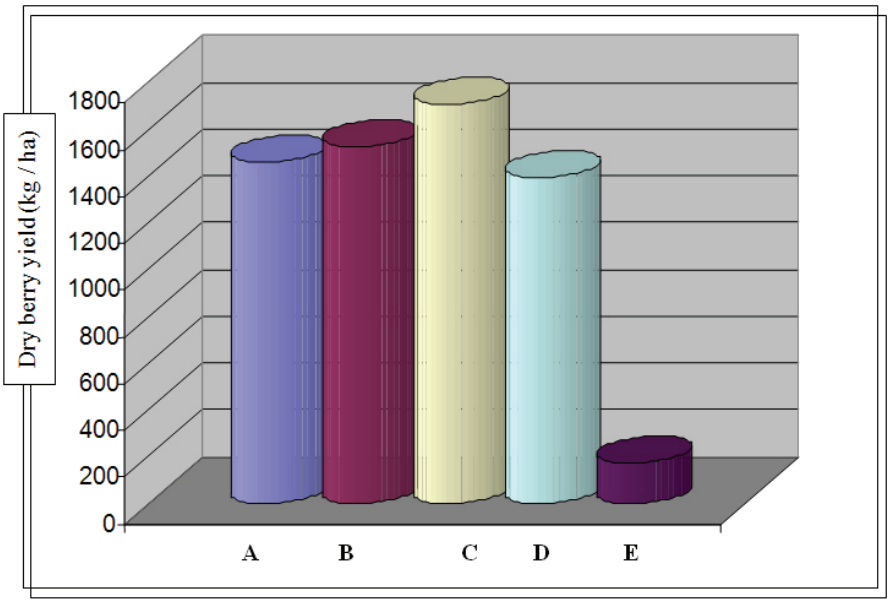


Figure 2. Average dry berry yield (kg/ha) of *Coffea arabica* under different intercropping systems for two years. Cropping systems: A: coffee/maize/cowpea, B: coffee/maize/melon, C: coffee/maize/ groundnut, D: coffee/cocoyam/melon and E: Coffee sole (control).

Weed suppression was higher under intercropping than for sole coffee, but there was no marked difference between the intercropping systems. Indeed weed incidence and frequency of weeding were three times more in coffee sole than in intercropped systems. This agrees with the result of Adeyemi (1989), who found out that weeds were more suppressed by intercropping coffee or cashew with arable crops.

Table 4. Income (US Dollar) from the output of various crops in different intercropping systems

Cropping systems	maize	cowpea	melon	groundnut	cocoyam	coffee	Total (US$)
A	109.0	116.6	-	-	-	615.75	841.72
B	135.93	-	15.10	-	-	640.75	808.59
C	111.32	-	-	126.47	-	716.80	954.60
D	-	-	13.5	-	188.23	587.39	789.13
E	-	-	-	-	-	73.52	73.52
LSD						23.45	30.41

Cropping systems: A: coffee/maize/cowpea, B: coffee/maize/melon, C: coffee/maize/ groundnut, D: coffee/cocoyam/melon and E: Coffee sole (control)

It is suggested that coffee production in Nigeria should be based on multiple cropping system so that farmers could benefit from the advantages derivable from the practice and, which according to Okigbo and Greenland (1975), included (i) higher yield returns per unit of land area, (ii) risk avoidance or crop insurance (iii) more efficient use of available nutrients and environmental factors (iv) control of pest and diseases (v) easier weed control and (vi) better use of labour.

CONCLUSION

Intercropping with various arable crops had no deleterious effect on the growth, development and yield of old coffee. Of the four systems tried, coffee/maize/groundnut was the best.

ACKNOWLEDGEMENT

The authors are grateful to all members of staff, CRIN Substation, Kusuku, Mambilla Plateau, for data collection and providing the enabling environment to write this paper, while the CTA is acknowledged for sponsorship to attend the conference.

REFERENCES

Gomez K.A. and Gomez A.A. (1984). Statistical Procedures for Agricultural Research (2nd Ed.). John Willey and Sons, Inc. New York. pp 315-322.

Effect of Training and Pruning Practices on Yield and Growth Parameters of Coffee (*Coffea arabica* L.) in Southwestern Ethiopia

E. TAYE, A. YILMA, A. NETSERE, T. KUFA, S. GEBREWOLD, T. SHIMBER, F. ASFAW

Jimma Agricultural Research Centre, P.O.Box 192, Jimma, Ethiopia.
E-mail: endaletw@yahoo.com

SUMMARY

An experiment was superimposed on rejuvenated coffee stands of the former hybrid coffee verification trial established at Metu Research Station that represents the southwestern coffee growing agro-ecology of Ethiopia. The objective was to evaluate yield and extension growth response of hybrid and pure line coffee varieties to training and pruning practices. Split plot design with two replicates was used where coffee varieties-three hybrids (Ababuna, Gawe and Melko-CH2 and two pure lines (745 and 75227) and five training treatments (capped single stem, uncapped single stem, capped multiple stem, uncapped multiple stem and free growth were assigned as main and sub plot treatments, respectively. The result revealed significant ($P < 0.05$) yield response due to varieties across the crop years. Similarly, in the over year's analysis, significant ($P < 0.01$) yield variations were noted among varieties. As a whole, the hybrid varieties were out yielded the pure lines where Ababuna produced the highest mean clean coffee yield across the crop seasons and over years. Similarly, coffee yield was significantly affected by training treatments in each crop year and over years. For the over year's analysis, free growth treatment resulted in the highest mean clean coffee yield of 1603.80 kg ha^{-1} pursued by uncapped multiple stem system with mean value of 1383.00 kg ha^{-1}. Although differences among varieties for growth parameters were not significant, training treatment had significantly ($P < 0.01$) influenced most of the parameters. Thus, at this stage of evaluation, free growth with two or more bearing heads had resulted in better coffee yield than the other treatments; however, to back up the present results, further study is essential by taking in to account the raw and cup quality parameters of coffee beans.

INTRODUCTION

Arabica coffee (*Coffea arabica* L.) which belongs to the Rubiaceae family has its primary center of origin and genetic diversity in its birth place, the highlands of southwest Ethiopia. Since the country is endowed with favorable agro-ecologies and environmental conditions, the crop grows on various soil types and climatic conditions. These environmental divergence might have greatly privileged the evolvement of immense genetic diversities within the Arabica coffee populations in the country, which could in turn provides a huge opportunity for genetic improvement (yield and other desired traits) of the crop.

Ethiopia is one of those countries that heavily depends on income generated from coffee export and exclusively produces arabica, which is the most important economic species in global coffee market, accounting for about 75% of the world coffee production. Being the dominant agricultural commodity in the country's economy, Arabica coffee was used to fetch over 65% of the foreign exchange income until recently. To date, it continues to be the

leading commodity generating about 40% of the foreign currency earnings (Negussie et al., 2007).

In spite of the aforesaid facts, the productivity of the crop is very low, about 660 kg ha^{-1} green coffee beans (CSA, 2006) as compared to the research results of the country which ranges between 1500 and 2000 kg ha^{-1} (Bayetta *et al*., 2000). Such low yield emanates from a number of production constraints *inter alias* heavy dependence on unimproved traditional coffee varieties and poor crop husbandry practices are the major factors. Among the inappropriate agronomic practices, lack of proper training and pruning practices remains the major bottleneck hampering yield and perhaps quality of the crop in the country.

Training and or pruning of coffee trees is one of the major coffee management practices because of its multiple advantages, *viz.* maintains suitable crop to leaf ratio, insures sustainable or uniform yield, pens tree to light and air for better flowering and fruiting, brings fresh vigor to the tree, assists disease and pest control, economizes chemical spraying, facilitates efficient picking cherries, and avoids overbearing die-back of primary branches and roots (Obiero, 1997). Regardless the aforementioned importance of pruning, little attempts have been made in our past coffee research endeavors and hence, research results in this area are very scanty. Nonetheless, about five years back, coffee research strategy of the country is geared towards this direction and thus, well designed activities on coffee training and/or pruning have been launched in different coffee growing agro-ecologies of the nation using their respective landrace or pipelines varieties. However, this paper solely presents results of the preliminary and pilot study undertaken on rejuvenated stands of coffee trees.

MATERIALS AND METHODS

A field trial was conducted at Metu Research Station that represents the southwestern coffee growing region of Ethiopia. The research station is located at 36° 0' E longitude and 7º 3' N latitude with an elevation of 1550 m above sea level (Paulos and Tesfaye, 2000) and receives an average annual rain fall of 1831 mm. The trial was superimposed on newly rejuvenated coffee stands of the former hybrid coffee verification trial which was planted in 1994. After completion of the verification trial, each coffee tree in the experimental plots was stumped back in February, 2002.

Two years later, training treatments were superimposed and evaluated for three consecutive crop years. Split plot design with two replications was used where five varieties *viz.* Ababuna, Gawe and Melko-CH2 (hybrids) and 745 and 75227 (pure lines) and training practices (capped single stem, uncapped single stem, capped multiple stem, uncapped multiple stem, and free growth were assigned as main and sub plot treatments, respectively. Each coffee tree in capped plots (capped multiple stem and capped single stem treatment) was topped at two meters height above the ground and its height restricted at this point. Moreover all multiple stem plots were trained on two bearing heads. However, each tree in free growth plot was maintained on two or more number of stems. Secondary branches were managed/pruned differently in each experimental unit; where seven to eight, five to six, four to five and three to four secondary branches were maintained per primary branch (a secondary branch alternately at each node) in capped single stem, uncapped single stem, capped multiple stem and uncapped multiple stem, in that order. However, no pruning operation was made at all on trees in free growth treatment.

In each crop year, red fresh cherry yield was selectively harvested from each experimental unit and weighed separately. The results were multiplied by the factor of 0.166 to convert the values into clean coffee yield and thus, reported in kilogram per hectare (kg ha^{-1}). Data on

extension growth parameters were recorded on four randomly selected trees per experimental unit. All other routine management practices were applied as of the recommendation of the area. Data recorded were statistically analyzed using Gene Stat computer program for each year and combined over years. LSD test at $P < 0.05$ probability level was employed to compare the difference between means whereas significant differences were obtained by analysis of variance (Mandefro, 2005).

RESULTS AND DISCUSSION

Coffee yield data of varieties and training treatments are presented in Table 1. Yield variations due to variety and training practices were significantly different across the crop years; however, their interaction effect was not significant in each crop year and for the over year's analysis. Generally, lowest mean clean coffee yield was recorded in the first crop year (2004/05) as compared to the other crop seasons. This could be attributed to the fact that the rejuvenated coffee trees did not reach to its potential bearing stage. In this season, hybrids, Ababuna and Gawe had significantly out yielded the remaining varieties with respective mean clean coffee yield of 1027.90 and 785.20 ka ha^{-1}; however, variation was not significant among the rest of the varieties. In subsequent crop year highest yield was recorded. In this case, among the varieties, the Ababuna-hybrid produced the highest mean clean coffee yield of 2454.50 kg ha^{-1} followed by Melko-CH2 (1949.00 kg $ha^{-1)}$ whereas 745 produced the lowest yield of 1397.20 kg ha^{-1} (Table 1). In the year 2006/07, yield tended to reduce as compared to the preceded crop year. This could be attributed to the mutual shading effect and hence competition for light and other resources, as canopies of the coffee trees were getting more and more close to one another (Table 2). In addition, the over year analysis had also revealed significant yield variation among verities where a hybrid variety-Ababuba gave highest mean clean coffee yield of 1738.70 kg ha^{-1} followed by Gawe and Melko-CH2 with respective mean clean coffee yield of 1345.40 and 1278.30 kg ha^{-1} (Table 1). In addition, as yield result the over years analysis revealed, the pure lines were found to be inferior to the hybrids.

In the first crop year (2004/05), coffee yield was significantly ($p < 0.05$) varied in response to training treatment. Accordingly, the highest and lowest mean clean coffee yield of 773.70 and 239.30 kg ha^{-1} was recorded from plots trained on free growth and capped single stem, respectively (Table 1). In the subsequent crop year (2005/06) significant ($P < 0.01$) coffee yield variations were also observed. Similar to the previous crop year, free growth treatment resulted in highest mean clean coffee yield of 2424.10 kg ha^{-1} pursued by uncapped multiple stem treatment with mean value of 2220.70 kg ha^{-1} (Table 1). Moreover, in 2006/07 crop season, significant ($P < 0.01$) yield differences were noted due to training or pruning treatment. Like the preceding crop year, trees of free growth treatment gave the highest yield; however, it was not significantly different from capped and uncapped multiple stem treatments. Furthermore, in the over-year analysis, results revealed significant yield variations due to training or pruning treatment. In this regard, the free growth treatment produced the highest yield followed by uncapped multiple stem system with mean value of 1603.80 and 1383.00 kg ha^{-1} clean coffee, respectively (Table 1).

Data of growth parameters collected in the third crop year are depicted in Table 2. Though, results revealed non-significant differences among varieties for each parameter, the hybrids were found to be superior over the pure lines in number of main stem node, plant height, and total number of primary branches (Table 2). On the other hand, the height up to first primary branch was shorter for hybrid than pure line varieties; indicating the more potential bearing surface of the former. Length of primary branches ranged between 120.35 and 130.89 cm for

Table 1. Mean clean coffee yield as influenced by coffee varieties and training practices at Metu Research Station.

Treatment	Yield (kg ha^{-1})			
	2004/05	2005/06	2006/07	Mean
Variety	*	*	*	**
Ababuna	1027.90 a	2454.50 a	1733.80 a	1738.70 a
Gawe	785.20 a	1655.40 c	1595.70 ab	1345.40 b
Melko-CH2	236.40 b	1949.00 b	1649.60 a	1278.30 b
745	147.50 b	1397.20 c	1108.00 ab	884.20 c
75227	212.00 b	1804.40 bc	576.50 c	864.30 c
SE (±)	106.86	105.43	261.65	97.17
CV (%)	48.64	27.11	26.30	30.90
Training practices	*	**	**	**
Capped single stem	239.30 c	1384.00 b	1036.80 c	886.70 d
Uncapped single stem	539.50 b	1585.80 b	1181.40 bc	1102.20 c
Capped multiple stems	435.80 bc	1645.90 b	1324.30 abc	1135.30c
Uncapped multiple stems	420.70 bc	2220.70 a	1507.60 ab	1383.00 b
Free growth	773.70 a	2424.10 a	1613.50 a	1603.80 a
SE (±)	74.04	158.80	110.84	69.11
CV (%)	48.64	27.11	26.30	30.90

**, ** =significant at 0.05 and 0.01 probability level, respectively. Means within a column followed by same letter(s) are not significantly different at 0.05 probability level.*

Table 2. The influence of variety and training on extension growth of stumped coffee stands at Metu Research Station.

Treatment	NMSN	PH (cm)	TNPB	HFPB (cm)	LPB (cm)	CD (cm)
Variety	NS	NS	NS	NS	NS	NS
Ababuna	59	278.60	110	46.27	121.28	255.70
Gawe	59	282.93	104	47.72	120.35	265.73
Melko-CH2	61	278.60	111	41.14	127.33	275.67
745	56	255.80	103	50.45	130.89	278.98
75227	51	261.97	89	50.36	125.40	260.56
SE (±)	2.35	7.73	3.82	3.34	2.03	5.55
CV (%)	8.89	6.88	11.68	16.88	7.15	6.31
Training	**	**	**	**	NS	**
Capped single stem	22 c	200.00 c	43 c	52.38 ab	129.11	258.15 bc
Uncapped single stem	47 b	323.90 ab	86 b	55.05 a	126.18	252.37 c
Capped multiple stem	44 b	200.00 c	83 b	46.80 b	127.84	285.69 a
Uncapped multiple stem	85 a	307.90 b	148 a	45.21 b	121.99	270.18 ab
Free growth	90 a	326.10 a	157 a	36.50 c	120.12	270.25 ab
SE (±)	1.61	5.95	5.08	2.52	1.76	5.33
CV (%)	8.89	6.88	11.68	16.88	7.15	6.31

*NS, ** = non-significant and significant at 0.01 probability level, respectively. Means within a column followed by same letter(s) are not significantly different at 0.05 probability level. NMSN= Number of main stem node, PH=Plant height, TNPB=Total number of primary branch, HFPB= Height up to first primary branch, LPB=Length of primary branch and CD= Canopy diameter.*

Gawe and pure line (745) varieties, respectively. Furthermore, tree canopy diameter ranged between 255.70 and 278.98 cm for Ababuna and 745, respectively (Table 2). The relatively short primary branch length and narrow canopy diameter of the Ababuna hybrid noted in the present study, agrees with the report of Bayetta *et al. (*1998).

On the other hand, training practices had significantly ($P < 0.01$) affected most of the growth parameters evaluated except length of primary branch. Uncapped multiple stem and free growth treatments resulted in significantly highest mean value of number of main stem nodes and total number of primary branches; where as trees trained on single stem gave the lowest value. Moreover, plant height was also significantly affected by training or pruning treatments where free growth and uncapped single system training or pruning system increased plant height than the rest of the treatments. Although not statistically different, length of primary branches of capped trees were greater than that of uncapped and free growth plots. This is apparently due to the removal of apical dominance and thus, initiation of the lateral extension growth of capped trees.

CONCLUSION

Varieties had a substantial influence on coffee yield. In most of the cases, hybrids were superior over the pure lines with Ababuna out yielding the rest of the varieties. Similarly, training or pruning treatment affected coffee yield across crop years and for the over year's analysis where the free growth treatment gave better yield followed by uncapped multiple stem treatment. Extension growth parameters were not considerably affected due to varietal differences; however, training and pruning treatment had altered almost all parameters studied. Consequently, at this stage of evaluation, free growth treatment with two or more bearing heads seems to be better in enhancing coffee yield; however, to back up the present result, further study is essential by taking in to account the raw and cup quality parameters of coffee beans.

ACKNOWLEDGMENT

The authors would like to express their gratitude to the Coffee Breeding and Genetics Research Division of the Jimma Agricultural Research Center for provision of the research plot and other relevant information of the coffee varieties. Our appreciation also goes to the European Union-CIP IV project for the financial support rendered to the first author to participate in and present the results of this work on the 22nd International Coffee Science Conference held in Campinas, Brazil.

REFERENCES

Bayetta Bellachew, Behailu Atero and Fekadu Tefera. 2000. Breeding for resistance to coffee berry disease in Arabica coffee: Progress since 1973. P. 85-98. In*:* Proceedings of the Workshop on Control of Coffee Berry Disease (CBD) in Ethiopia. 13-15 Aug. 1999. Addis Ababa, Ethiopia.

Bayetta Bellachew, Behailu Atero and Gibramu Temesgen. 1998. Description and production recommendation for new cultivars of Arabica coffee. Research report No: 34. Institute of Agricultural Research. Addis Ababa, Ethiopia.

Central Statistical Agency (CSA). 2006. Agricultural sample survey 2005/ 06 (September 2005-February 2006). Volume I. Report on area and production of crops (private peasant holdings, Meher season). Statistical bulletin 361, Addis Ababa, Ethiopia.

Obiero S.O. 1997. Better Coffee Farming: Pruning. In: Kenya Coffee. P. 2553-2555. Vol. 63, No 734.

Nigussie M. (2005). Statistical Procedures for Designed Experiments. EARO, Addis Ababa, Ethiopia.

Nigussie Efa, Derese Teshome and Endale Taye (2007). Innovative technology transfer for perennial trees. The case of coffee stumping. Research report 73, Addis Ababa, Ethiopia.

Paulos Dubale and Tesfaye Shimber (2000). Some ecological parameters occurring in the major coffee growing southwestern and south Ethiopia. Pp. 107-124. In: Proceedings of the Workshop on Control of Coffee Berry Disease (CBD) in Ethiopia. 13-15 Aug. 1999. Addis Ababa, Ethiopia.

Effect of Different Indigenous Shade Trees on the Quality of Wild Arabica Coffee in the Afromontane Rainforests of Ethiopia

A. YADESSA[1*], J. BURKHARDT[2], M. DENICH[1], T. WOLDEMARIAM[3], E. BEKELE[3], H. GOLDBACH[2]

[1]University of Bonn, ZEF Bonn, Walter-Flex-Str. 3, D-53113, Bonn, Germany.
Permanent address: Ethiopian Institute of Agricultural Research, Forestry Research Center, P.O. Box 30708, Addis Ababa, Ethiopia. [*]E-mail: abebeyadessa@yahoo.com
[2]University of Bonn, INRES-PE, Karlrobert-Kreiten-Str. 13, 53115 Bonn, Germany
[3]Addis Ababa University, P.O. Box 28513, Addis Ababa, Ethiopia

SUMMARY

A study on four commonly found indigenous coffee shade tree species (*Acacia abyssinica, Albizia gummifera, Albizia schimperiana,* and *Cordia africana*) was conducted in the Afromontane rainforests of Ethiopia to assess their effects on the physical and sensory quality of wild arabica coffee. Results showed that sensorial differences in wild arabica coffees were detected due to tree species. Tree species significantly affected beverage acidity ($p = 0.005$), flavour ($p = 0.044$), aftertaste ($p = 0.002$) and overall cup quality ($p = 0\ .015$). This means coffee samples collected under *A. abyssinica* and *C. africana* were more acidic, with better flavour and overall cup quality as compared to those collected under both *Albizia* species. Although not statistically significant, coffees under *Acacia* were more aromatic than those under other shade tree species. But no apparent difference in body of the brew could be detected due to tree species. Moreover, the proportion of marketable beans (screen 14 plus in Ethiopian case) was higher under *Acacia* (92.73%) and *Cordia* (91.79%) than under *A. schimperiana* (89.48%) and *A. gummifera* (88.42%). To the contrary, the proportion of very small beans (which are rejects in most cases) was significantly higher under *A. gummifera* and A. *schimperiana* than under *Acacia* or *Cordia*. Coffee aroma was inversely related with the proportion of very small beans; the higher the proportion of very small beans the poorer the quality of the coffee, especially its aroma. Generally, coffee beverages prepared from samples under *Acacia* and *Cordia* were more appreciated by the tasters than those under both *Albizia* species. This finding coincides well with the local farmers' perception for *Acacia*, but not for *Cordia*. In Ethiopia, *C. africana* is a valuable timber tree but endangered species, and hence its positive effect on coffee quality as obtained by the present finding is an added advantage. In light of the present findings, the practical significance the study in terms of shade coffee production and biodiversity conservation are discussed.

INTRODUCTION

The shade trees in coffee production systems provide several economic and ecological benefits. Studies elsewhere have demonstrated the multiple uses of coffee shade trees; and their role in soil fertility management (Yadessa et al., 2001; Kimemia, 2007; Muleta et al., 2008), biodiversity conservation (Sonto-Pinto et al., 2000; Perfecto et al., 2005), carbon sequestration (Jong et al., 1997; Harmand and Hergoualc'h, 2007), micro-climate regulation and prevention of coffee plants from damages by frost or other extreme conditions (Barradas and Fanjul, 1984; Caramori et al., 1996; Lin, 2007) and cash income generation from the sale of timber and non-timber products (Beer, 1987; Beer, 1988; Vaast et al., 2006) have been well documented. And there is no exception to the shade tree-coffee association in the

Afromontane rainforests of Ethiopia. In addition to providing wild coffee, the Afromontane rainforests in Ethiopia are reservoir of several products and services.

Traditionally coffee plants grow under the shade of trees. In these tree-coffee associations, studies have showed that coffee shade trees have positive impacts on coffee quality (Muschler, 2001; Vaast et al., 2006; Avelino et al., 2007), by lengthening of the maturation period of coffee berries and hence a better bean filling and also through the modification of microclimate for coffee growing underneath shade trees (Lin, 2007). Moreover, shade improves the quality of coffee by allowing the beans to accumulate greater amounts of sucrose as compared to sun grown beans (Steiman, 2003). According to Guyot et al. *(*1996: cited in Steiman, 2003), bean size increased slightly with shade as did the chemical constituents: chlorogenic acids by 10%, total acidity by 16%, and caffeine by 4% and sucrose by 3%. But trigonelline content decreased by 10%, and bitterness among the organoleptic properties decreased by 18%. Muschler (1998) also observed the effects of various shade regimes on two coffee varieties (Caturra and Catimor). Both varieties exhibited a substantial bean size increase as well as improvement in visual appearance. The total amount of defects was less under shade for both varieties. Acidity and body improved with shade but aroma was slightly negatively affected in Catimor (Steinman, 2003). These studies suggest that shade positively affects coffee quality. However, due to the interaction of shade with the environment and genetic composition, the effects might be site specific.

Indigenous shade trees are very common features of the coffee production systems in Ethiopia, although currently the existing valuable shade tree species are being depleted from the Afromontane rainforest ecosystem by selective cutting for timber (eg. *Cordia*). Jotie (2005) have listed the major important indigenous shade tree species in the coffee production systems of SW Ethiopia, and this was supplemented by field observation and reconnaissance survey during the study. The effects of some of these shade trees on yield and agronomic traits of coffee have been documented in Ethiopia (Taye, 2007; Tesfaye et al., 2002). But their effects on coffee quality, notably under natural forest coffee ecosystem is scarce. The present study, therefore, focused on the effect of the four most common indigenous coffee shade tree species (*Acacia abyssinica, Albizia gummifera, Albizia schimperiana,* and *Cordia africana*) on the physical and sensory quality of wild arabica coffee in the Afromontane rainforests of SW Ethiopia.

MATERIALS AND METHODS

The study was carried out in Yayu area, Illubabor Zone of Oromia Regional State in south western Ethiopia. The shade trees were selected based on farmers ranking of the major shade trees for suitability, field observations and reconnaissance survey and available literature. Accordingly, the four major species were *Acacia abyssinica (*Sondii*)*, *Albizia gummifera(*Hambabeessa*)*, *Albizia schemperiana(*Alalee*)*, and *Cordia africana* (Waddeessa) in order of farmers' preference ranking for suitability as shade for coffee. Coffee cherries were harvested both under the canopies of different shade trees and also outside the canopies of respective trees (tree species and sub-habitat) in November 2007 when they were at full maturity. This means two factors were involved, namely: tree species and sub-habitats (under tree canopy vs. outside canopy). Red cherries were selectively hand picked and dry processed with careful handling at Jimma Agricultural Research Center with close supervision of the Coffee Quality and Processing Division staffs. The dried cherries were manually depulped and the beans were made ready for cup tasting. The coffee samples were medium roasted and medium ground. Sensory evaluation was then assessed using the major cup quality criteria: fragrance, aroma, flavour, acidity, body, aftertaste and overall quality; and scoring was based on a scale of 1-10, corresponding to the total absence or presence of the criterion in the coffee beverage,

respectively. The beverage was prepared by brewing 9 g of roasted coffee in 180 millilitres of hot water. The coffee brews were evaluated by a panel of four experienced tasters at Robera Coffee Cup Tasting Laboratory http://www.roberacoffee.com/qualitycon.html) in Addis Ababa, Ethiopia. For the statistical tests, the means of the values attributed by all the tasters were used.

RESULTS AND DISCUSSION

Results showed that there were significant differences in organoleptic properties of wild Arabica coffees collected under different indigenous shade tree species. Tree species significantly affected beverage acidity ($p = 0.005$), flavour ($p = 0.044$), aftertaste ($p = 0.002$) and overall cup quality ($p = 0.015$). But the tasters could not detect significant differences in some of the organoleptic properties the coffee brews, especially for the body (Figure 2). According to the present findings, coffee samples collected under *A. abyssinica* and *C. africana* were more acidic, with better flavour and good overall cup quality as compared to those collected under both *Albizia* species (Figures 1 and 2). Although not statistically significant, coffees under *Acacia* were more aromatic than those under other shade tree species. But no apparent difference in the body of the coffee beverage could be detected due to tree species (Figure 2), which may be related to other factors such as soil properties (Yadessa et al., ASIC this volume). Generally, coffees under *A. abyssinica* tree were best followed by those under *C. africana*. Coffees under both *Albizia* species were less preferred by a panel of tasters (Figure 2). This is especially interesting for *Cordia* as it is not leguminous species. This might be due to the fact that nitrogen might be less important for cup quality as compared to other soil properties like available P and K (Yadessa et al., ASIC this volume).

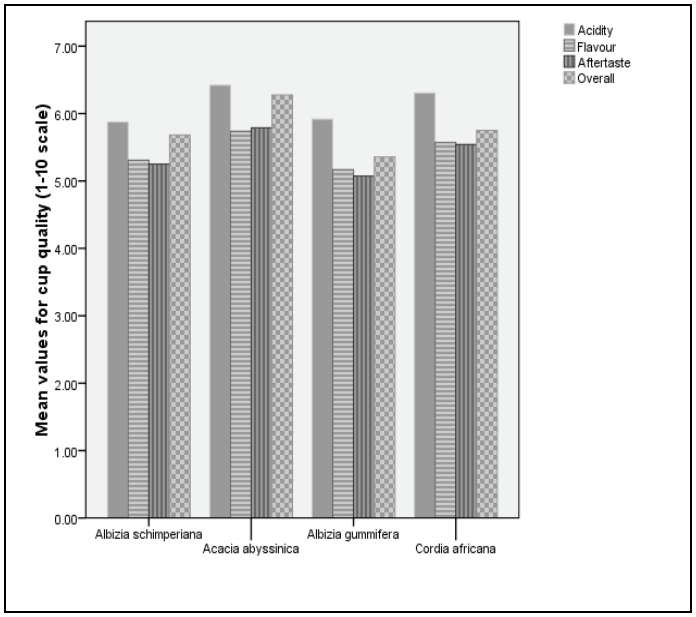


Figure 1. Organoleptic properties of wild arabica coffee as influenced by indigenous shade tree species.

As indicated in Figure 3, the proportion of marketable beans (screen 14 plus in Ethiopian case) was higher under *Acacia* (92.73%) and *Cordia* (91.79%) than under *A. schimperiana* (89.48%) and *A. gummifera* (88.42%). The proportion of very small beans (which are rejects in most cases) was significantly higher under *A. gummifera* and *A. schimperiana* than under *Acacia* or *Cordia*. Coffee aroma was inversely related with the proportion of very small

beans; the higher the proportion of very small beans the poorer the quality of the coffee, especially its aroma (Figure 4).

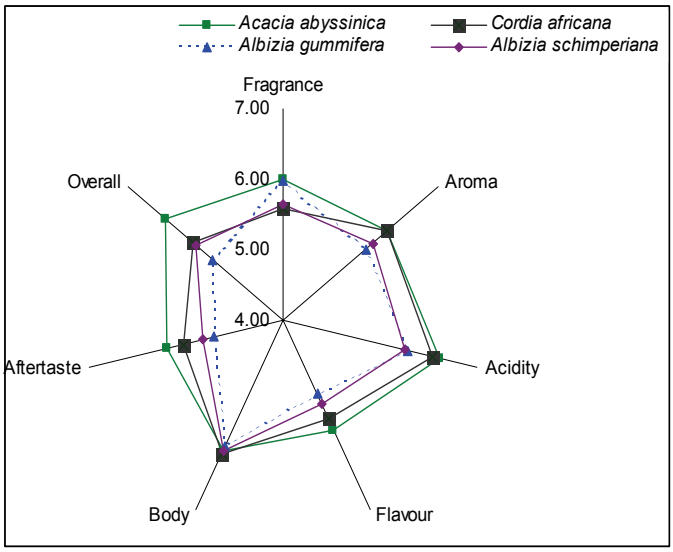


Figure 2. Organoleptic properties of coffees collected under different shade trees.

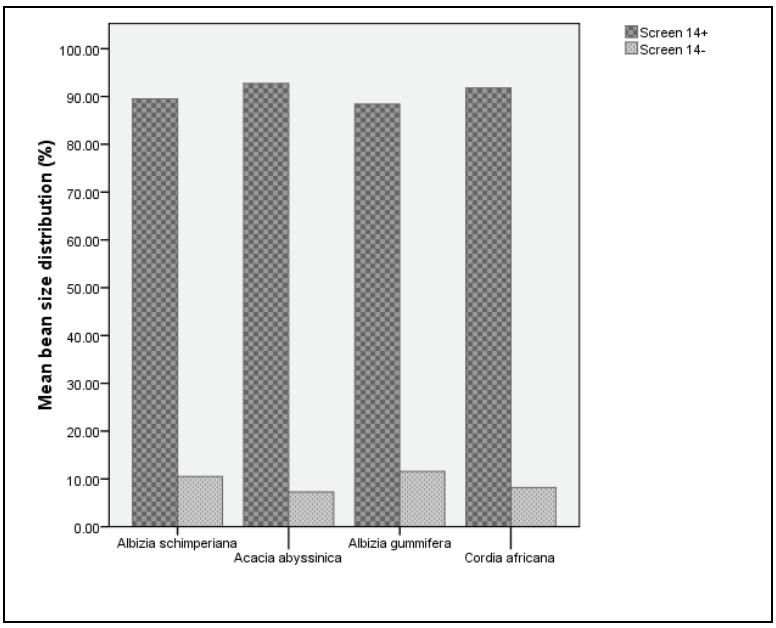


Figure 3. Bean size distribution of wild arabica coffee as influenced by indigenous shade tree species.

But farmers' preference ranking of shade trees for improving coffee quality was in the order of *A. abyssinica* > *A. gummifera* > *A. schemperiana* > *C. africana*.

The results of the present study thus coincide well with the local farmers' perception for *Acacia*, but not for *Cordia.* In Ethiopia, *C. africana* is a valuable timber tree species, but it is an endangered species. And cutting of *Cordia* is banned by proclamation in the country (TGE, 1994.), although selective cutting of the tree from the forest ecosystem and elsewhere is unabated. Hence, the positive impact of *Cordia* on coffee quality as obtained by the present finding could be an added advantage. And this may help to boost the conservation efforts of the species as coffee shade. Studies also show that shaded coffee plantations have been proposed as refugees for biodiversity conservation since they can potentially preserve high

diversity of organisms (Perfecto et al., 2005). Promotion of indigenous shade trees in coffee production systems should be considered as a potential adaptive strategy for *in situ* conservation of biodiversity and valuable species in the area. Study by Derero et al., (2007) revealed that the scattered *Cordia* trees on farmlands also harbor substantial genetic diversity comparable to the continuous populations, and hence shade trees can be used as sources of genetic materials as well. *Cordia* is also a good soil ameliorator; higher concentrations of soil nutrients such as available P were reported under the canopies of *Cordia* trees as compared to its nearby open area (Yadessa et al., 2001).

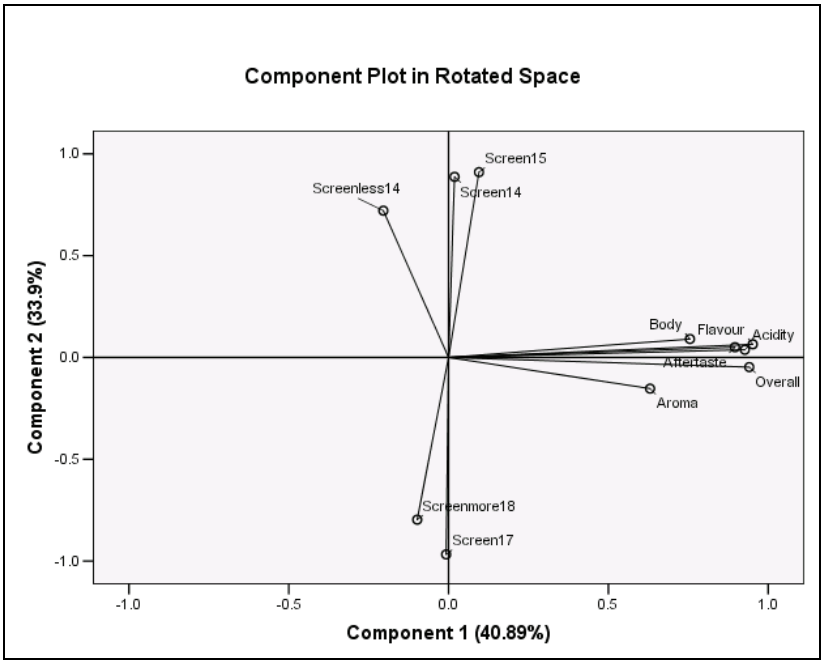


Figure 4. Principal components taking into account coffee bean size distribution and cup quality traits in the Afromontane rainforests of SW Ethiopia.

Coffee aroma was inversely related with the proportion of beans less than screen 14 minus; stated differently, coffee aroma was positively related with the proportion of beans greater than screen 14. This was the trend observed in SW Ethiopia, but the reverse trend was observed in SE Ethiopia (data not shown). This shows the site-specific nature of coffee quality.

However, there was no significant difference in coffee quality between samples under the canopy of shade trees and 'outside' the canopy of shade trees although the difference was significant for the tree species. This might be due to the fact that in forest or semi-forest coffee systems the presence of purely open coffee is less likely because of the density of shade trees. There is no proper 'open area' or 'full sun' in forest or semi-forest coffee production systems of the Afromontane rainforests in Ethiopia. The density of upper canopy trees in the Afromontane rainforests of Ethiopia was reported in detail by Senbeta (2006). This could be the probable reason for lack of significant difference in the beverage quality of coffees collected from different sub-habitats although significant differences were reported in literature in coffee plantations (Muschler, 2001; Vaast et al., 2006; Avelino et al., 2007).

CONCLUSION

The present results revealed that indigenous shade trees considerably influenced the organoleptic properties of wild Arabica coffee in the Afromontane rainforests of SW

Ethiopia, especially beverage acidity, flavor, aftertaste, and overall cup quality. The taste of the coffee brew was relatively best under *Acacia*, followed by *Cordia*, but lower under both *Albizia* species. Coffee samples collected under *A. abyssinica* and *C. africana* were more acidic and better flavour than those collected under both *Albizia* species. Generally, coffees under *Acacia* and *Cordia* were more appreciated by the tasters than those under *Albizia* species. This finding coincides well with the local farmers' perception for *Acacia*, but not for *Cordia*. This could be an added economic advantage for *Cordia* as it also provides other economically useful products (e.g. timber) for the people and ecologically important services (e.g. soil amelioration) for the ecosystem. These economic and ecological benefits of shade trees in the Afromontane rainforests of Ethiopia thus presents a good opportunity to develop programs for sustainable natural resources management by combining conservation and economic goals if shade trees of these species are promoted for coffee production in the buffer zone areas or other parts of the country. Similar opportunities were also reported in Central America in coffee plantations (Perfecto et al., 2005). And thus in coffee trading, shade coffee certification has recently emerged as a conservation-oriented marketing strategy. Globally there is an increasing demand for shade coffee as they are environmentally friendy (Steinman, 2003). It is therefore advantageous and economically sound to promote shade trees in coffee production systems, and the shade coffee certification programs that take into consideration the relationship between biodiversity and coffee quality should be considered in the future.

REFERENCES

Avelino J., Barbora B., Davrieux F. and Guyot. 2007. Shade effects on sensory and chemical characteristics of coffee from very high altitude plantation in Costa Rica. *In*: Second International Symposium on Multi-Strata agroforestry systems with perennial crops: Making ecosystem services count for farmers, consumers and the environement, September 17-21, 2007 Turrialba, Costa Rica.

Barradas V.L. and Fanjul L., 1986. Microclimatic characterization of shaded and open grown coffee (*Coffea arabica* L.) plantations in Mexico. Agricultural and Forest Meteorology, 38: 101-112.

Beer J., 1987. Advantages, disadvantages and desirable characteristics of shade trees for coffee, cacao and tea. Agroforestry Systems, 5, 3-13.

Beer J., Muschler R., Kass D., Somarriba E., 1998. Shade management in coffee and cacao plantations. Agroforestry Systems, 38, 139-164.

Caramori P.H., Filho A.A. and Leal A.C. 1996. Coffee shade with Mimosa scabrella Benth. for frost protection in southern Brazil. Agroforestry Systems, 33: 205-214.

Derero A., Gailing O., Finkeldey R., 2007. Comparative Genetic Variation in *Cordia africana* between Trees in Traditional Agroforestry Systems and Continuous Populations in Ethiopia. Tropentag 2007.

Harmand J.M. and Hergoualc'h K. 2007. Carbon Sequestration in aerial biomass and derived products from coffee agroforestry systems in Central America. *In*: Second International Symposium on Multi-Strata agroforestry systems with perennial crops: Making ecosystem services count for farmers, consumers and the environement, September 17-21, 2007 Turrialba, Costa Rica. web.catie.ac.cr/cd_multiestrata/Poster/session2/Carbon _sequestration.pdf

Jong B.H., Soto-Pinto M.L., Montoya-Gómez G., Nelson K., Taylor J., Tipper R., 1997. Forestry and agroforestry land-use systems for carbon mitigation: an analysis in Chiapas, Mexico. *In*: Adger, W.N., Pettenella, D., Whitby, M. (Eds.), Climate-Change Mitigation and European Land-Use Policies. CAB International, pp. 269-246.

Jotte J. 2005. The influence of indigenous institutions in the conservation and use of *Coffea arabica.* The case of Yayo Forest in Ilu Abba Bora Zone, Southwest Ethiopia. Addis Ababa University, School of graduate studies, Department of Sociology and Social Anthropology, Addis Ababa, Ethiopia.

Kimemia J.K., 2007. Influence of an *Albizzia* shade tree on soil chemical properties, coffee plant growth and yields in Kenya, pp.1199-1202. In: 21th ASIC Proceedings, Montpelier, France.

Lin B.B., 2007. Agroforestry management as an adaptive strategy against potential microclimate extremes in coffee agriculture. Agricultural and Forest Meteorology, 144: 85-94

Muleta D., Assefa F., Nemomissa S. and Granhall U., 2008. Distribution of arbuscular mycorrhizal fungi spores in soils of smallholder agroforestry and monocultural coffee systems in southwestern Ethiopia. Biology and Fertility of Soils, 44: 653-659.

Muschler R. 2001. Shade improves coffee quality in a sub-optimal coffee zone of Costa Rica. Agroforestry Systems, 51: 131-139.

Muschler R. 1998. Tree Crop Compatibility in Agroforestry: Production and Quality of Coffee Grown under managed tree shade in Costa Rica. Ph.D. Dissertation, University of Florida. 219 pp.

Perfecto I., Vandermeer J., Mas A. and Soto Pinto L.S. 2005. Biodiversity, yield, and shade coffee certification. Ecological Economics, 54: 435-446.

Senbeta F., 2006. Biodiversity and ecology of Afromontane rainforests with wild *Coffea arabica* L. populations in Ethiopia. Ecology and Development Series No. 38, 2006.

Soto-Pinto L., Perfecto I., Castillo-Hernandez J. and Caballero-Nieto J., 2006. Shade effect on coffee production at the northern Tzeltal zone of the state of Chiapas, Mexico. Agriculture, Ecosystems and Environment, 80: 61-69

Steiman S., 2003. Shade versus sun coffee: a review. http://www.grayskies.net/honeybear/shade.htm

Taye Kufa, Alemseged Yilma, Tesfaye Shimber, Anteneh Nestere and Endale Taye. 2007. Yield performance of *Coffea arabica* cultivars under different shade trees at Jimma Research Center, southwest Ethiopia. *In*: Second International Symposium on Multi-Strata agroforestry systems with perennial crops: Making ecosystem services count for farmers, consumers and the environement, September 17-21, 2007 Turrialba, Costa Rica.

Tesfaye Shimber, Taye Kufa and Alemseged Yilma. 2002. The effect of established shade trees on the growth and yield of arabica coffee in two planting patterns. Proceedings of the International Conference on Coffee Science (ASIC), May 14th-18th, 2001, Trieste, Italy.

TGE (Transitional Government of Ethiopia), House of Representatives. 1994. NEGARIT GAZETA of The Transitional Government of Ethiopia, 53rd Year - No. 80, Proclamation No. 94/1994. Forestry Conservation, Development and Utilization Proclamation, p.372.

Vaast P., Van Kanten R., Siles P., Dzib B., Franck N. and Harmand J.M. 2006. Shade: A Key Factor for Coffee Sustainability and Quality, 887-896. ASIC 2006, Montpellier, France.

Yadess, A., Itanna I. and Olsson M., 2001. Contribution of indigenous trees to soil properties: the case of scattered trees of Cordia africana Lam. in croplands of western Oromia. Ethiopian Journal of Natural Resources, 3: 245-270.

Use of Shade Trees in Coffee as a Green House Gas Mitigation Strategy

J.K. KIMEMIA* AND D.A. ODENY

Coffee Research Foundation, P O Box 4 00232 Ruiru, Kenya. *E-mail: jkimemia@crf.co.ke

SUMMARY

Coffee, one of the most important crops in Kenya, has been mainly grown as a mono crop. While this management system has enabled important gains in productivity, the intensive coffee monoculture often results in decreased plantation longevity. Other negative long-term environmental impacts include water pollution/contamination, loss of biodiversity and emission of green house gases (ghg) such as N_2O. One of the ways to reduce the emission of green house gases would be the use of intercrops or shade trees. Several studies have shown that shade provides substantial benefits over coffee grown in full sun. This paper reviews the work carried out on use of shade trees in coffee and their potential for carbon sequestration. It also looks at the possibility of enabling farmers benefit from carbon credits of the World Bank Bio-Carbon fund.

INTRODUCTION

Over the last decade, phenomenal changes in global weather patterns have been observed.

According to Intergovernmental Panel on Climate Change (IPPC), the projected global average surface temperature would increase by between 1.4-5.8 °C over the period 1990 – 2100 (Figure 1). Temperatures have already increased by an average of 0.6 °C (IPCC, 2001) although higher increases have been observed. Benisten (2007) indicated that the mean temperatures in Basel, Switzerland are 6 °C higher than the 1961-1990 average, and hotter than any other time since 1901. These increases are bound to have a significant effect on coffee production.

Arabica coffee *Coffea arabica* originated from the Ethiopian highlands where it grows naturally as an under storey plant. It therefore developed under shade conditions with low temperature range. This determines to a greater extent the adaptability, productivity and quality of the crop under commercial conditions. Any change in temperature may have a profound effect on coffee production.

Coffee is a crucial crop to over 26 countries in Africa and Central America. An increase in temperatures would have detrimental effect on the coffee production in these countries. In Uganda a warming of only 2 °C would massively cut the land suitable for coffee production. In Brazil, if mean temperatures were to increase by 5.8 °C the suitable areas for farming coffee will decrease and even an increase in precipitation may not counter the damage of the increased temperature (IPCC, 2001).

Effect of temperature change on coffee

Arabica coffee prefers a temperature range 15-24 °C. Temperatures over 25 °C reduce photosynthesis and above 30 °C leaf damage occurs (Wilson, 1999). Besides this temperature range, arabica coffee does not tolerate a diurnal range of more than 19 °C (Mwangi, 1983).

Shading reduces the diurnal range of ambient air temperatures. This is important particularly in the high altitude areas where 'hot and cold' disorder characterized by crinkling of the leaves is a problem. Shading is known to considerably reduce this disorder by reducing the magnitude of the diurnal temperature range (Tapley, 1961). More important is that shading lowers coffee leaf temperatures. It has been shown that high leaf temperatures reduce the rate of photosynthesis. Kumar and Tieszen (1980) observed that photosynthetic rates decreased above 25 ^{o}C which was apparently due to a decline in the mesophyl conductance, as standard conductance remained more or less unchanged between 25 ^{o}C and 35 ^{o}C in Kenya. The extent to which shading can reduce leaf temperatures may be considerable. Hardy (1962) reported on experiments from Costa Rica where unshaded coffee leaves reached 47 ^{o}C whilst shaded leaves were only 28 ^{o}C.

Shading also reduces soil temperatures and this may be critical particularly during the seedling and early establishment stage of coffee growth. High soil temperatures may also increase the rate of evaporation from the surface soil layer and the rate of organic matter breakdown. This may lead to poor soil structure and increased susceptibility to erosion (Wiley, 1975).

The relative humidity may be increased by shading and this in turn reduces transpiration hence the overall water requirement of the crop decreases and consequently diminishes the likelihood of water stress.

An increase in temperature will also affect the rainfall, both amount and variability. For coffee to flower, it requires a 4-6 weeks moisture stress followed by rainfall. An increase in temperature may mean: prolonged stress that affects the coffee trees adversely, variable rainfall that will not coincide with the peak moisture demand for coffee resulting in light 'hungry' beans of lower quality, faster breakdown of organic matter and vaporization of the nitrogenous compounds of decomposition resulting in reduced organic matter in the soil.

Coffee is attacked by a number of pests. There are over 1000 insect pests which attack different parts of the coffee tree. The behaviour and life cycle of insects is greatly affected by temperatures. A change in temperature would therefore affect the dynamics of these pests with some increasing in number and voracity, some minor insect pests becoming major while some may be wiped out.

Arabica coffee is attacked by numerous diseases but those of economic importance are Coffee Berry Disease (CBD) caused by *Colletotrichum kahawae* and coffee Leaf Rust caused by *Hemileia Vastatrix*. CBD is favoured by high precipitation and is more common in the upper coffee zones. It causes losses of up to 100%. On the other hand, Coffee Leaf Rust is favoured by high temperatures and is more common in the lower altitude coffee areas. A rise in temperature will therefore increase significantly the leaf rust incidences. Already leaf rust has been observed in the higher altitudes of Kenya where leaf rust was not a problem in the past Kairu (2007) This could be attributed to global warming and and the costs of protecting coffee from these two diseases would be very high and may render coffee production uneconomical.

To realize the high quality potential of Arabica coffee, the coffee undergoes wet processing. The coffee is then dried through a strict and controlled process lasting over one month. The coffee should never get into contact with water during this drying period. A rise in temperature would drastically affect the drying process and reduce the coffee quality.

As already indicated, high temperatures will also result in sporadic and unexpected rains. Rewetting of coffee encourages mould growth rendering the coffee unsuitable for human consumption.

SHADE TREES AS MITIGATION STRATEGIES

The impact of climate change will affect the developing countries more than the developing ones (IPCC 2003). Coffee is mainly grown by these developing countries in Africa, Asia, South and Central America. There is increasing acceptance that even the very ambitious climate change mitigation measures would not be sufficiently effective to halt the green gas concentrations in the medium term and that other adaptation measures are needed. Carbon sequestration through land use change and forestry is a possible mitigation strategy under the Clean Development Mechanisms. Since land for expanding forestry is limited due to competition with growing food crops, then Agroforestry offers a unique opportunity to increase carbon sequestration. The trees are able to store large amounts of carbon in the living biomass, in the soil and in the durable wood products.

One disadvantage of shade trees is that they do compete with the coffee crop for water. The degree of competition would depend on the particular cropping pattern and the nature of the shade tree root system relative to that of the crop (root distribution and mass) (Willey, 1975). The moisture status will be affected by whether or not the shade trees shed their leaves during the dry season. In situations where shading is likely to increase the moisture stress during the dry season, deciduous trees would seem desirable, but of course, this would have to be equated with other aspects of shading, such as the need to lower temperatures. Shade trees do shed leaf litter which may act as mulch and as such reduce soil temperature, protect against erosion, increase organic matter and nutrients in the upper soil layers (Willey, 1975). Inclusion of leguminous trees will lead to production of non CO_2 emissions gases but the amounts are less compared to the amount of carbon captured and stored

The amount of organic matter added would depend on the rate of decomposition, and it has been shown that shade tree leaves, a major component of the litter do decompose much slower than coffee leaves, and these slower than coffee flowers and fruits (Guenca et al., 1983). For the nutrient issue to be positive, the shade trees must be able to tap some nutrient resources which are not normally available to the crop either through fixation of nitrogen by leguminous tree species, or tap lower soil horizons than the crop and recycle the nutrients. This recycling of nutrients would depend on the nutrient requirements of the trees. Some of the nutrients tapped will be used in growth, so the net loss or gain of nutrients to the crop will depend on such factors as the rate of shade tree growth, whether pruning is carried out whether prunings are returned to the soil.

To get full advantage of shade trees as a measure against global warming, the following factors need to be considered while choosing suitable shade trees:

- To have the same life span as coffee
- The wood should not be brittle
- The growth should be fairly rapid

- It must respond to training and be able to produce a clear straight trunk for at least 5-6 m before branching with a spreading habit.
- The leaves should be feathery
- The tree should not be leafless during the dry season,
- It should be deep rooted,
- It should have no adverse effect on the coffee immediately near it, not should it be an alternative host of any of the pests or diseases in coffee.

As it is not likely to get an ideal tree, there are some trees which are possible candidates but will require more thorough investigation before they are recommended. Some of them are: *Albizia maraguensis, Grevillea robusta, Albizzia sinesis, Cordia holstii, Leucaena leucocephala, Sesbania aegyptiaca, Cassia didymobotria, Colliandra calothyrus* among others.

CONCLUSION

The issue of global warming is already here and will have serious implications on coffee production and consequently consumption. A decline in coffee fortunes will also adversely affect the economies of many coffee producing countries. Some of the mitigating strategies include use of shade trees in the medium term. More is required particularly the measurements of the amounts of green house gases released to the atmosphere and the amounts of carbon the indigenous trees can capture. Besides the carbon capture gains coffee grown under shade could also be sold as specialty coffee fetching more income to the producers. The long term strategy would be to breed varieties that will withstand the increased temperatures and reduction of Green House Gases (GHC). If these efforts become fruitful, it will then be possible to reverse the global warming and coffee will continue as a major beverage in the world.

REFERENCES

Beniston M., 2007: Sustainability of the landscape of a UNESCO World Heritage Site in the Lake Geneva region (Switzerland) in a greenhouse climate. International Journal of Climatology. In press

Gallagher R.S., E.C.M. Fernandes and E.C. McCallie (1999). Weed management through short term improved fallow in tropical agroecosystems. Agroforestry Systems 47:197-221.

Guenca G., Aranguren J. and Herrera R. 1983. Root growth and litter decomposition in a coffee plantation under shade trees. Plant and Soil 71: 477-486

Hardy F. 1962. Cacao soils III. The problem of shade for cacao. Gordian 62: 685-690

Impala (2001). First Annual Report of the IMPALA Project October 2000-December 2001

Intergovernmental Panel on Climate Change, 2001. Climate Change 2001: The scientific basis United Nations Environmental Programme Nairobi Kenya.

Kairu G.M. (2007) Possible effects of global warming on coffee diseases. Proceedings XVI International Plant Protection Congress, Glasgow, 2007.

Kumar D. and Tieszen L.L. 1980. Photosynthesis in *Coffea arabica*. 1. Effects of light and temperature. Experimental agriculture 16: 13-19.

Mwangi C.N. 1983. Coffee Growers Handbook. Coffee Research Foundation Ruiru Kenya

Njoroge J. M. and J. K. Kimemia (1989). A comparison of different weed control methods in Kenya coffee. *I* 55:863-870.

Willey R.W., 1975. The use of shade in coffee, cocoa and tea. Hort. Abstracts 45: 791-797.

Test of an Agrometeorological Model for Estimating the Beginning of the Flowering Period for Coffee Crop

A.O. ZACHARIAS, M.B.P. CAMARGO

Instituto Agronômico de Campinas, Centro de Pesquisa e Desenvolvimento de Ecofisiologia e Biofísica, Campinas, São Paulo, Brazil

SUMMARY

According to Camargo and Camargo (2001), the flowering buds complete the maturation and reach to the dormancy of the buds, being ready for the main flowering when the accumulated value of potential evapotranspiration (ETp), starting from April, reaches about 350 mm. The objective of this work was the parametrization (calibration) and validation of the agrometeorological-phenological model for estimating the beginning of the full flowering period for arabic coffee, for the tropical conditions of Brazil. The calculation of the sequential water balance, for estimating of the availability soil water, for the regions of Campinas and Mococa, State of São Paulo, was based on the method of Thorhwaite and Mather (1955), in ten-days time scale. By means of the water balance ten-days time values of the atmospheric demand had been generated, represented for the ETp, estimating for the method of Thorthwaite. Phenological observations of adult coffee crops, variety Mundo Novo and Catuaí, were obtained from archieves of the "Instituto Agronomico de Campinas" (IAC) during the years of 1993 to 2005. Results had indicated that they are necessary accumulated values of ETp of 335 mm or 1,579 GDD, for the flower buds to reach the maturation, and a minimum value of 7 mm of rainfall, to break the dormancy of mature buds, presented better capacity to indicate the period of the full flowering for arabic coffee, with less errors of estimating comparing with the original model. Thus, the parametrized agrometeorological-phenological model will serve of important tool for models for monitoring and estimating the coffee productivity, resulting in more consistent estimates, exactly in years with many adversities climatic.

INTRODUCTION

More consistent models are necessary to subsidize the works of anticipated forecast of the harvest of coffee in Brazil, to allow for a more secure and accurate. Agrometeorological models that relate environmental conditions, such as temperature and water availability in the soil, with phenology, bienalidade and productivity of coffee are being developed for coffee crops regions of Brazil. These models consider that each factor carries some climate control in the productivity of culture by influence in certain critical phenological periods, as in the flower induction, the bloom, the formation and maturing fruits of cofee (Camargo et al., 2003; Carvalho et al., 2003).

The agrometeorological model proposed by Camargo and Camargo (2001) considered the value of 350 mm relative to the ten days accumulated values ETp, starting from April, and the minimum amount of rainfall of 10 mm in the ten day period, necessary so that the mature buds are induced to antesis, presented relative capacity to indicate the beginning of the period of the full flowering of the arabic coffee, presenting errors in estimation of more than two periods of ten days. However, to be incorporated into models of monitoring and estimation of

loss of productivity, they need this important information phenological, we need more studies to determine more precisely the limits temperature and water for the maturation of flower buds and the break of dormancy for the antesis (Santos and Camargo, 2006).

The objectives of this study had been to parametrize and to validate an agrometeorological-phenological model for estimate the beginning of the full flowering (main) of the arabic coffee tree for the tropical conditions of Brazil.

MATERIAL E METHODOS

Phenological data and of productivity of the *Coffea arabica* (L.) to variety Mundo Novo and Catuaí, in adult phase, were obtained from archieves of the "Instituto Agronomico de Campinas" (IAC) of experiments and comments carried through during thirteen years (1993 the 2005), for the regions of Campinas and Mococa, State of São Paulo. For both regions, believed to date the beginning of the full flowering, or when the flower buds mature turned into flowers. The fertilizer, pesticide treatments and cultural treatment were usually recommended for commercial cultivation of coffee, without application of irrigation. Daily data centre of rainfall (mm) and maximum and minimum temperatures of air (°C) were obtained from the archives of the Center of Ecophisiologe and Biophysics of the IAC, from the meteorological stations of Mococa and Campinas.

The calculation of the sequential water balance, for estimating of the availability soil water was based on the method of Thorhwaite and Mather (1955), in ten-days time scale. By means of the water balance ten-days time values of the atmospheric demand had been generated, represented for the ETp, estimating for the method of Thorthwaite (1948). The ETp is a important climatological element considered by Thorthwaite to indicate the availability of solar energy in the region, being constituted an index of thermal efficiency of the region, seemed to the Growing Degree Day (GDD), however being express in millimeters (mm) of evaporation equivalent (Camargo and Camargo, 2000).

As indicating of the related thermal factor with the phenology of the cofee tree, it was considered initially the suggested value of 350 mm relative to the ten days accumulated ETp values starting from April, and the minimum amount of rainfall of 10 mm in the ten day period, necessary so that the mature buds are induced to antesis, such as the original model. Different accumulated values of ETp and Growing Degree Day (GDD) and different values of minimum rainfall (1 to 10 mm) also were considered.

For the evaluation of the estimates of the full flowering, analyses of linear regression had been used.

RESULTS E DISCUSSION

Examining all combinations of values of ETp and accumulated rainfall, both for the region of Campinas and for Mococa, selected are those that have the best statistical indices. It appears that, in Campinas, the major indexes considered statistical values accumulated ETp between 330 and 335 mm, and rainfall from 1 to 10 mm. The values of R^2 ranged from 0.66 to 0.69, indicating a small dispersal of data obtained from the average. For Mococa, there were better results for the values of ETp accumulated between 330 to 340 mm, and rainfall of 6 to 10 mm. The R^2 were also higher, 0.93 and 0.75 respectively, indicating good precision and accuracy of the performance of the model to estimate the early florada full. Considering the average statistical results of Campinas and Mococa, got up the accumulated values of 335 mm of ETp, from April, and rain of at least 7 mm as the best interaction of phenological-

agrometeorlogical model to estimate the time of occurrence of full flowering of coffee, tropical conditions for the State of Sao Paulo. These results are in line with Damatta and Rena (2002) which suggested that 5 to 10 mm of rain are sufficient to break the dormancy of flower buds, similar to the value of 7 mm here observed. The application of the parametrizated model is shown in Figure 1. It has been rainfall distributions and the accumulated value of ETp to Campinas, in the period from 2007, showing the estimated time of maturation of buds and flowering and the real flowering. After the accumulation of ETp of 335 mm, the flower buds are ready to be stimulated to antesis. The full flowering, however, occurs about one week after the occurrence of a water shock in the bud by rain of at least 7 mm in ten-days time. According to the model, the flower buds are mature in last ten-days period of the August (21-31), but not quantity rains theoretically enough to break the dormancy of flower buds and lead to antesis. Thus, the flowering occur only in the third ten-days time of the October, after 8.1 mm of rain accumulated in the second ten-days time of October, inducing the full flowering of coffee crop, coinciding with the actual flowering (Figure 1).

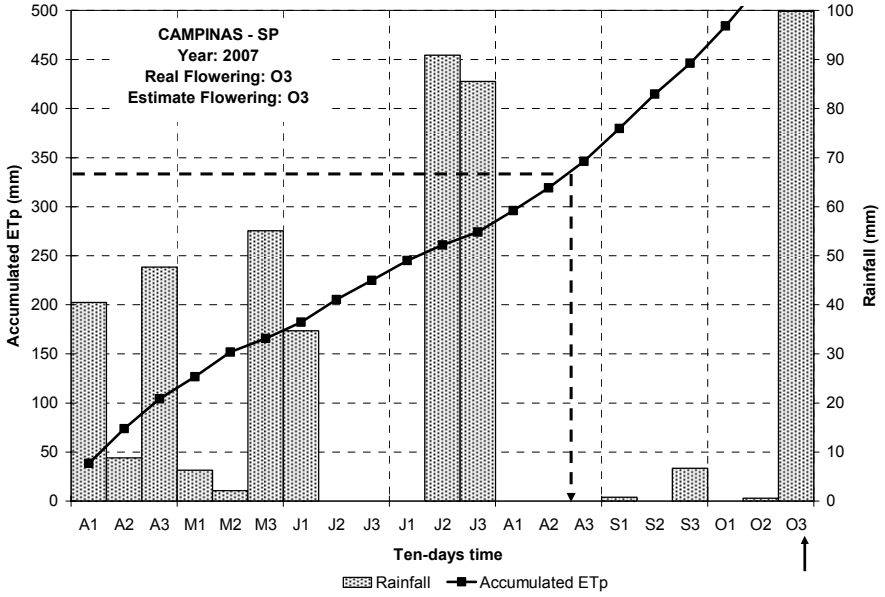


Figure 1. Distribution pluviometric and accumulated value of ETp from April, in ten-days time levels, with indications of the values of 335mm of ETp and estimated times of full flowering of coffee crop to Campinas, State of Sao Paulo, in 2007 period.

CONCLUSIONS

The calibration agrometeorological model, which considers the value of ETp accumulated from April equal to 335 mm for the flower buds to reach maturity, and a minimum of 7 mm of rain to break the dormancy of buds mature, showed good ability to indicate the time of full flowering of Arabic coffee, with errors of less than the original model estimate of Camargo and Camargo (2001) and may be incorporated into the agrometeorological models for estimate of loss of productivity, they need this important information phenological.

REFERENCES

Camargo A.P., Camargo M.B.P. Uma revisão analítica da evapotranspiração potencial. Artigo de Revisão. Bragantia, Campinas, v.59, n.2, p.125-137, 2000.

Camargo A.P., Camargo M.B.P. Definição e esquematização das fases fenológicas do cafeeiro arábica nas condições tropicais do Brasil. Bragantia, Campinas, v.60, n.1, p.65-68, 2001.

Camargo M.B.P., Santos M.A., Pedro Junior M.J., Fahl J.I., Brunini O., Meireles E.J.L., Bardin L. Modelo agrometeorológico de monitoramento e de estimativa de quebra de produtividade como subsidio à previsão de safra de café (*Coffea arabica L.*): resultados preliminares. In: Simpósio de Pesquisa dos Cafés do Brasil, 3., 2003, Porto Seguro. Anais. Porto Seguro: Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café, 2003. p.75-76.

Carvalho L.G. de, Sediyama G.C., Ccecon P.R., Ramos Alves H.M. Avaliação de um modelo agrometeorológico para previsão de produtividade de café em três localidades da região sul do Estado de Minas Gerais. Revista Brasileira de Agrometeorologia, Santa Maria, v.11, n.2, p.343-352, 2003.

Damatta F.M., Rena A.B. Relações hídricas no cafeeiro. In: Simpósio De Pesquisa Dos Cafés Do Brasil, 1., 2000, Poços de Caldas. Anais**.** Brasília, DF: Embrapa Café, 2002. p.9-44.

Santos M.A., Camargo M.B.P. Parametrização de modelo agrometeorológico de estimativa de produtividade do cafeeiro nas condições do Estado de São Paulo. Bragantia, Campinas, v.65, n.1, p.173-183, 2006.

Thornthwaite C.W. An approach toward a rational classification of climate. Geography Review, v 38, p. 55-94, 1948.

Thornthwaite C.W., Mather J.R. The water balance. Centerton, NJ: Drexel Institute of Technology - Laboratory of Climatology, 1955. 104p. (Publications in Climatology, v. 8, n.1).

Climate Change and Coffee Sustainability in Tanzania: Use of Historical Data Trends for Impact Assessment

G.P. MARO* AND J.M. TERI

Tanzania Coffee Research Institute, P.O.Box 3004, Moshi, Tanzania.
Tel. +255 027 2756868, Fax +255 027 2756773.
*E-mail: marogp2002@yahoo.com, tacriced@yahoo.com − Website: http://www.tacri.org

SUMMARY

A study of climatic and yield data trends was conducted in the slopes of Mt. Kilimanjaro, for the purpose of assessing the climate change impact on coffee. Variables studied included rainfall, maximum and minimum temperatures. The time span was 10 years replicated 3 times 1946-1955, 1966-1975 and 1996-2005 (denoted as Periods 1, 2 and 3 respectively). Data were extracted from Lyamungu Meteorological Station. Total rainfall did not differ significantly over the chosen periods. Its distribution, however, seems to have been slightly changed, with long rain peaks getting steeper. Mean maximum temperature differed significantly over the years and periods. It oscillated evenly around a mean of 25 °C, which is still normal for coffee. As for mean minimum temperature, periods showed significant difference, though years within periods did not. Period 3 showed to deviate most from the mean of 15 °C, experiencing a rise of almost 1 °C. This seems to be the closest parameter among those studied, to be used as an evidence of global warming. Yield trends were not significantly affected by variation in climate. These observations suggest that coffee in Tanzania is not yet a casualty of climate change. TaCRI's adaptive research strategies to possible future climate change effects are discussed.

INTRODUCTION

Climate change is a distortion of weather trends over time, due to natural variability or human activity. Coffee *(Coffea* sp.) is a vulnerable crop with specific growth requirements. Arabica coffee, according to Wrigley (1988) and Kimemia (2007), thrives well between the altitudes of 1400 and 2200 m above sea level. The temperature range is 15-25 °C. Above 25 °C, photosynthesis is reduced, whereas above 30 °C, leaves may be damaged. Rainfall should be 1000mm or more per annum with a 2-month dry spell.

The global concern on climate change, and the special reference to Mt. Kilimanjaro, whose slopes constitute the core of Arabica coffee in Tanzania (Agrawala, 2003), prompted the Tanzania Coffee Research Institute (TaCRI) to undertake an assessment of weather trends and coffee yields over its southern catchment. The objective was to determine the extent to which historical weather data, where available, can be used to assess impacts of climate change; and whether coffee in Tanzania is already a casualty of climate change.

MATERIALS AND METHODS

An assessment of weather trends (rainfall, maximum and minimum temperatures) was done in the slopes of Mt. Kilimanjaro. Three time series of 10 years each were used; 1946-1955, 1966-1975 and 1996-2005 (denoted as Periods 1, 2 and 3 respectively). Data were extracted from Lyamungu Meteorological Station. Coffee yield data at the Lyamungu Research Centre

were collected and evaluated as well. Data were mainly analysed in Excel, with regression done by using COSTAT package.

RESULTS AND DISCUSSION

Rainfall data were available continuously from 1935 to 2005, and were all plotted as shown in Figures 2(a) and (b) below, before defining the study periods. The temperature data were not continuous, so three fully represented periods of 10 years were picked for further analysis. Total rainfall did not differ significantly over the chosen periods, except the onset of the long rains shifting to March, and peaks getting steeper.

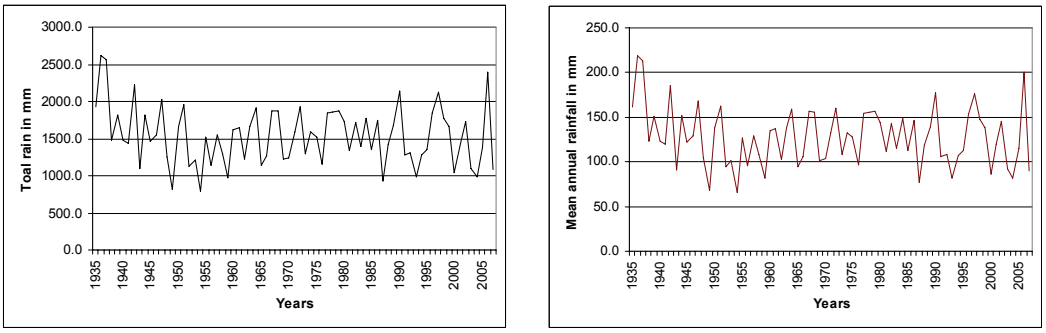


Figure 2. Variation in total rainfall (a) and mean rainfall (b) over the past 7 decades.

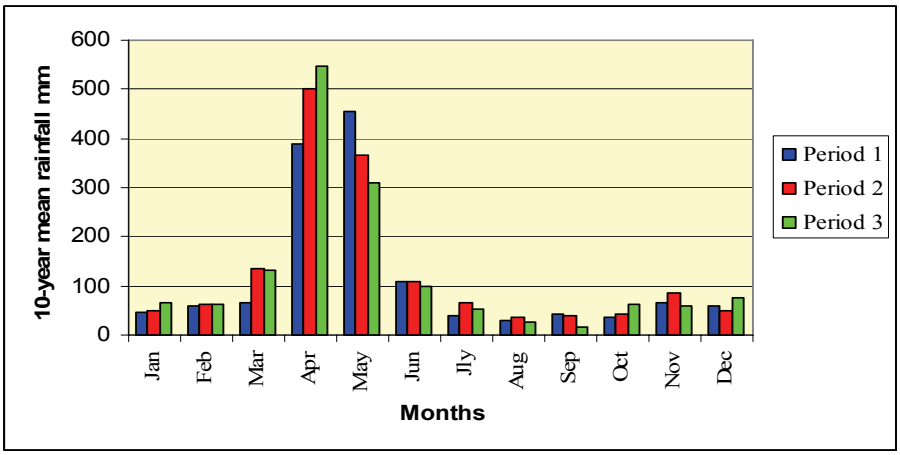


Figure 3. Monthly rainfall variability for the three periods at Lyamungu.

Mean maximum temperature (Figure 4a) differed significantly over the years and periods. It oscillated around a mean of 25 °C, which is still normal for coffee. As for mean minimum temperature (Figure 4b), periods varied significantly, though years within periods did not. Period 3 showed to deviate most from the mean of 15 °C, experiencing a rise of almost 1 °C. This seems to be the closest parameter among those studied, to be an appropriate indicator of climate change around the study area. The averages of 25 °C and 15 °C for maximum and minimum temperatures found in this work are still suitable for coffee and therefore, no serious threat is posed as yet.

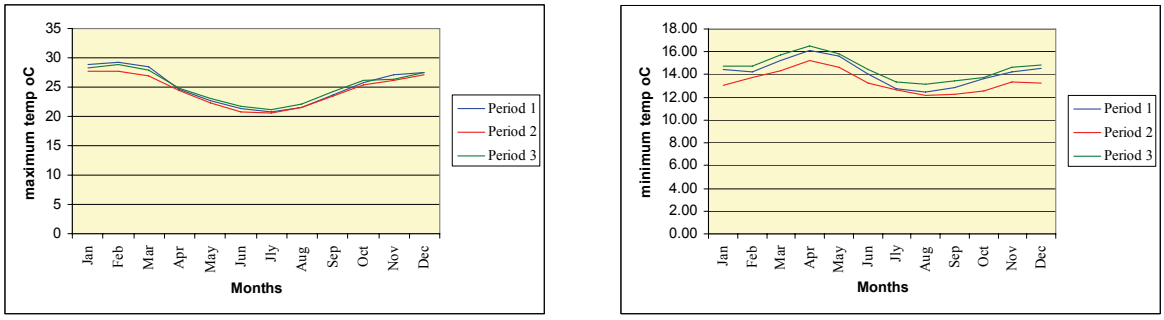


Figure 4. Variation in mean maximum (a) and minimum (b) temperatures at Lyamungu.

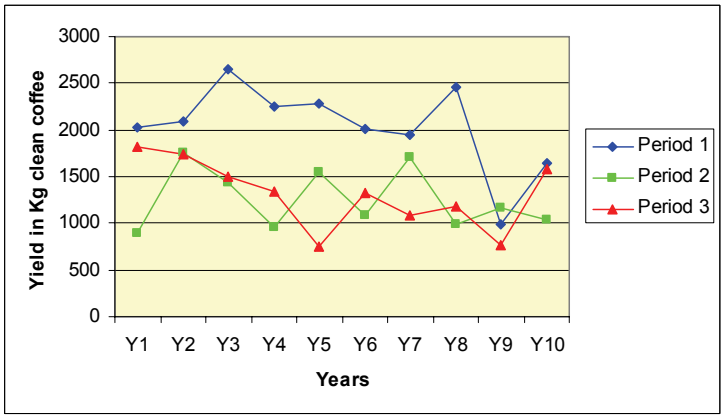


Figure 5. Variation in coffee yield over the study periods, Lyamungu CRS/TaCRI.

Figure 5 shows the variations in parchment yield over the study periods. Best yields were reported in Period 1. Periods 2 and 3 showed more or less the same yield trend. This means that yield has not significantly changed from what it was in the 1960's and 70's, which puts into question the observation by Orindi et al (2006) that one of the expected impacts of climate change is an increase in coffee production where the challenges of pests and diseases can be overcome. Analysis of variance at 0.05 level did not show significant variation for periods or years within periods.

Table 1. Regression model between coffee yields and climatic factors.

Model	**B**	**t**	**Sig.**
Rainfall	0.56	1.72	0.098
Tmax	-18.096	-0.082	0.935
Tmin	34.85	0.275	0.785
Constant	668.13	0.12	0.906
R^2 = 0.166		F = 1.719	

A regression model (Table 1) relating coffee yields to the three climatic variables studied found no significant relationship at 0.05 levels. Only rainfall would be significant if the level was extended to 0.1. Negative relation in Tmax and positive one in Tmin were both expected. This implies that yield is not a function of climate alone – other factors cited by Van Ranst (1999) as soil physical and chemical variables and crop management levels play an important role.

TACRI'S STRATEGIES TO COPE WITH FUTURE THREATS OF CLIMATE CHANGE

The new Strategic Action Plan, 2008-13 has taken climate change seriously. To keep track of weather trends, we are planning to maintain the available weather station at Lyamungu, and establish new ones in each of the 5 substations. Research priorities will be on breeding for stress tolerance (including heat, drought, pest and disease pressure, etc.) and research on grafting, developing and disseminating appropriate technologies for cost-effective productivity, soil and water conservation (mulching, trickle irrigation), rainwater harvesting and use of ecological pulpers. We have also included issues of afforestation for carbon sequestration, agroforestry, strategies of income diversification such as bee-keeping, and biodiversity.

CONCLUSION

This study has compared the analysis of long-term climate trends as indicators of climate change and their relationship with coffee yields. It was observed that trends in rainfall, maximum and minimum temperatures were very irregular and have no significant impact on yield. As such, coffee in Tanzania is not yet a casualty of climate change.

ACKNOWLEDGEMENT

This work was financed from FMO 94-99 STABEX CONTRACT No. DLO/COF99/01/14 Project No. TA STA COF99 01. We are grateful to the EU and Tanzania Government for this support.

REFERENCE

Agrawala S., Moehner A., Hemp A., van Aalst M., Hitz S., Smith J., Meena H., Mwakifwamba S.M., Hyera T. and Mwaipopo O.U. 2003. Development and climate change in Tanzania: focus on Mount Kilimanjaro. OECD Environmental Directorate, 2 rue André Pascal, 75775 Paris, Cedex 16, France. 136pp.

Kimemia J.K. 2007. Effects of global warming on Arabica coffee production. Proc. IACO Conference, Yaounde, Cameroon, 14-22 November, 2007.

Orindi V.A., Kibona E. and Moindi D.M. 2006. Addressing climate change in the development process in Tanzania. African Centre for Technology Studies (ACTS) Ecopolicy Series no. 18, Acts Press, P.O. Box 45917, Nairobi, Kenya. 34pp.

Van Ranst E., Verdoodt A. and Louwagie G. 2002. Land evaluation: Practical exercises manual. Lab. Of Soil Sci, Ghent University, Belgium. 117pp.

Wrigley G. 1988. Coffee: Tropical Agriculture Series. Longman Scientific and Technical, John Wiley and Sons Inc., NY 10158

Air Temperature in *Coffea arabica* Microclimate Arborized with Dwarf Coconut Palm and Rubber Tree in Mococa, SP, Brazil*

M.B.P. CAMARGO[1], G.S. ROLIM[1], P.S. SOUZA[2], P.B. GALLO[2]

[1]Instituto Agronômico, Campinas, SP, Brazil. E-mail: mcamargo@iac.sp.gov.br
[2]Pólo APTA Nordeste Paulista, Mococa, SP, Brazil
*Supported by FAPESP

SUMMARY

The arborization, shading management system, is a crop production system that has found increasing use in Brazil. Moreover, it has been considered appropriate for areas that present risks of high temperatures, as well as frosts, providing crop sustentability, higher yields and beverage quality improvement. The purpose of this work is to verify the effect of 8 year old arborized systems on daily air maximum (TMAX) and minimum (TMIN) temperature for *Coffea arabica* in Mococa (21º28' S, 47º01' W, 665 m), a region of subtropical climate and representative of the crop production zones of São Paulo state, Brazil. Data of air temperature were collected every 15 minutes and at 1.5 m inside the canopy for three different systems during 1.5 years: unshaded systems (without arborization) (UNS), arborized with dwarf coconut palm (*Cocos nucifera* L.) (PAL) with spacing of 8m x 8m, and arborized with rubber tree (*Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg.) (RUB) with spacing of 16m x16m. For all the systems data of net radiation and wind velocity were collected at each 15 minutes at 0.5m above the canopy, beside the data of the final yield treatments. It was observed that the TMAX were more affected than the TMIN. Comparing the arborized and the unshaded systems, the former presents an average decrease in TMAX up to 1.5 °C for PAL and up to 2.0 °C for RUB, mainly when air temperature is near to 38°C. The two systems did not show significant differences in relation to the minimum temperatures.

INTRODUCTION

The arborization, a shading management system, is a crop production system that has found increasing use in Brazil. Moreover, it has been considered appropriate for areas that present risks of high temperatures, as well as frosts, providing crop sustentability, higher yields and beverege quality improvement.

In the arborizated systems, due to the heterogenous nature of the canopies, the physical environment interacts in a more complex way with the coffe plants as compared with the conventional system (Pezzopane et al., 2003).

The effect of arborization in relation to the microclimate has been studied by many authors (Baggio et al., 1997; Miguel et al., 1995; Beer et al., 1998; Peeters et al., 2002). These papers describe the arborization in a qualitative manner about the type of the tree utilized and crop density. However several papers are found in literature that characterize the microclimate quantitatively such as in a coffee and dwarf coconut system in Mococa, Brazil (Pezzopane et al., 2003), in an arborizated systems in Colômbia (Farfan-Valencia et al., 2003), in an agroflorestal system in Mexico (Barradas and Fanjul, 1986) and in an arborizated system with 'bracatinga' in Londrina, Brazil (Caramori et al., 1996). These works showed that the microclimate is strongly influenced by wind, soil type, water deficit vapor, temperature and

solar radiation and these elements are dependent on climate, species utilized for arborization and crop density.

The objective of this work is to verify the effect of 8 year old arborized systems on daily air maximum (TMAX) and minimum (TMIN) temperature for *Coffea arabica* in Mococa (21º28' S, 47º01' W, 665 m).

MATERIAL AND METHODS

Data on air temperature were collected every 15 minutes at 1.5 m inside the canopy for three different systems during 1.5 years: unshaded systems (without arborization) (UNS), arborized with dwarf coconut palm (*Cocos nucifera* L.) (PAL) with spacing of 8 m x 8 m, and arborized with rubber tree (*Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg.) (RUB) with spacing of 16m x16m in Mococa (21º28' S, 47º01' W, 665 m), a region of subtropical climate and representative of the crop production zones of São Paulo state, Brazil. For all the systems, data of net radiation and wind velocity were collected every 15 minutes at 0.5m above the canopy, beside the data of the final yield treatments.

RESULTS AND DISCUSSION

It was observed that TMAX (Figure 1.A and B) was more affected than TMIN (Figure 1.C and D). Comparing the arborized and the unshaded systems, the former presents an average decrease in TMAX up to 1.5 °C for PAL and up to 2.0 °C for RUB, mainly when air temperature is near to 38 °C. The two systems did not show significant differences in relation to the minimum temperatures.

Those results were due to an average reduction in net radiation of 31% for PAL and 86% for RUB, beside wind velocity that was 50% lower for RUB in comparison to PAL. The plot yield in 2006 was approximately 26, 29, 37 sacks.ha^{-1} for UNS, PAL and RUB, respectively. These results show the effect of reduction in maximum temperatures as a result of the arborization system, as it was already demonstrated by Caramori et al. (1996), making the environment more suitable for *Coffea arabica*, once it is adapted to conditions of higher altitude tropical climate, demanding colder temperatures. Therefore, the arborization has become a technological option to yield increase in subtropical climates.

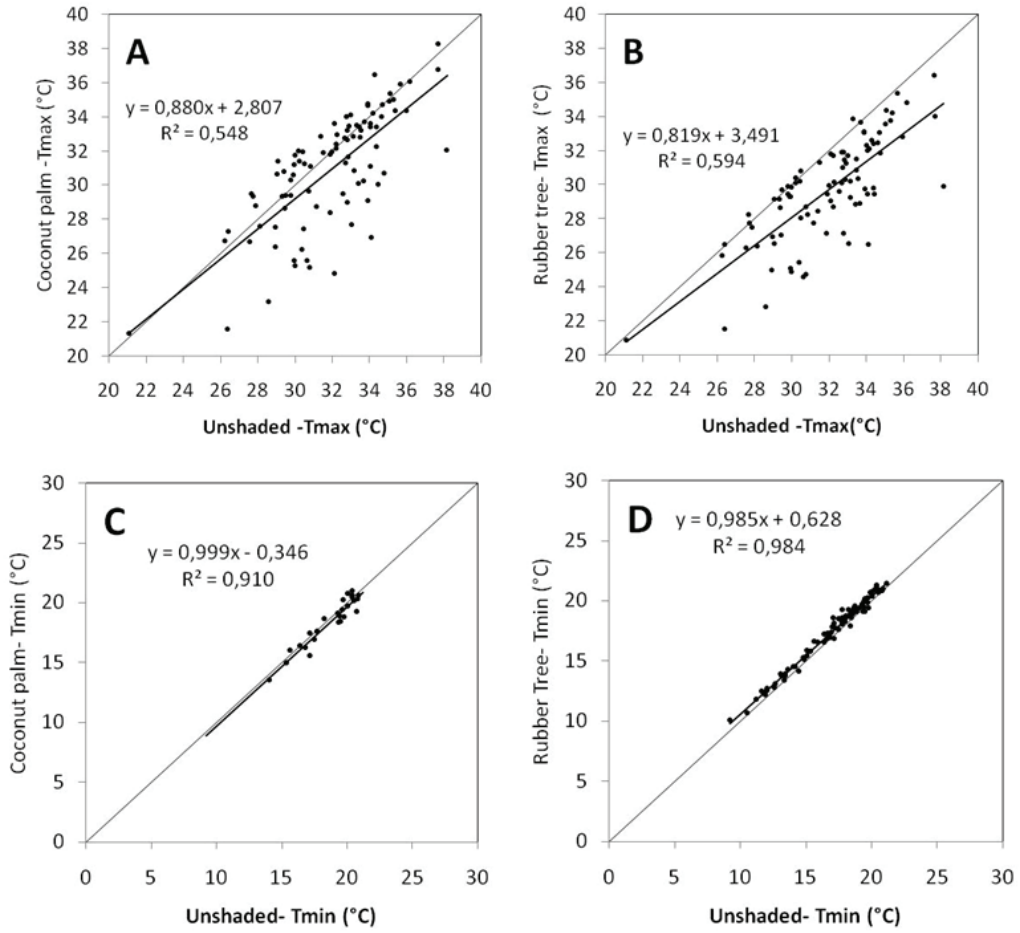


Figure 1. Relation between five days average values of absolute maximum temperatures (Tmax) for unshaded coffee and dwarf coconut palm (A) and rubber tree (B) and of absolute minimum temperatures (Tmin) for unshaded coffee and dwarf coconut palm (C) and rubber tree (D), during the period of 08/31/2006 to 12/31/2007.

CONCLUSION

The arborizated systems decreased the maximum temperature up to 1.5 °C and 2.0 °C in dwarf coconut palm and rubber tree systems, respectively, and promoted the increase of yield.

REFERENCES

Baggio A.J., Caramori P.H., Androcioli A., Montoya L. Productivity of southern Brazilian coffee plantations shaded by different stockings of Grevillea robusta. Agroforestry Systems, Amsterdam, v. 37, n. 2, p.111-120, 1997.

Barradas V.L., Fanjul L. Microclimatic characterization of shaded and open-grow coffee (*Coffea arabica* L.) plantations in Mexico. Agricultural and Forest Meteorology, Amsterdam, v. 38, p. 101-112, 1986.

Beer J., Muschler R., Kass D., Somarriba E. Shade management in coffee and cacao plantations. Agroforestry Systems, Amsterdam, v. 38, p. 139-164, 1998.

Caramori P.H., Androcioli Filho A., Leal A.C. Coffee shade with *Mimosa scabrella* Benth. for frost protection in southern Brazil. Ag*roforestry Systems*, Amsterdam, v. 33, p. 205-214, 1996.

Farfan-Valencia F., Arias-Hernandez J.J., Rianoherrera N.M. Deasarrollo de una metodologia para medir sombrio en sistemas agroflorestales com café. Cenicafé, Chinchina. 54, n.1, p. 24-34, 2003.

Miguel A.E., Matiello J.B., Camargo A.P., Almeida S.R., Guimarães, S.R. Efeitos da arborização do cafezal com *Grevillea robusta* nas temperaturas do ar e umidade do solo, Parte II. In: Congresso Brasileiro De Pesquisas Cafeeiras, 21., Rio de Janeiro, 1995. Trabalhos apresentados... Rio de Janeiro: PROCAFE, 1995. p. 55-60.

Peeters L.Y.K., Soto-Pinto L., Perales H., Montoya G., Ishiki M. Coffee production, timber, and firewood in traditional and Inga-shaded plantations in Southern Mexico.Agriculture, Ecossystems and Environment, Amsterdam, v.82, p. 1-13, 2002.

Pezzopane J.R.M., Gallo P.B., Pedro Júnior M.J., Ortolani A.A. Caracterização microclimática em cultivo consorciado café/coqueiro-anão verde. Revista Brasileira de Agrometeorologia, Santa Maria, v. 11, n. 2, p. 293-302, 2003.

Maximum Global Irradiance and Photosynthetically Active Radiation Intercepted by *Coffea arabica* Canopy Under Different Types of Pruning in Mococa SP, Brazil

G.S. ROLIM[1*], P.S. SOUZA[2], M.J. PEDRO-JÚNIOR[1], P.B. GALLO[2], J.R.M. PEZZOPANE[3]

[1]Instituto Agronômico, Campinas, SP, Brazil. [*]E-mail: rolim@iac.sp.gov.br
[2]Pólo APTA Nordeste Paulista, Mococa, SP, Brazil
[3]Universidade Federal do Espírito Santo, São Mateus, ES, Brazil

SUMMARY

Pruning in coffee has different purposes, such as the decrease of pathogens population, increase of crop robustness, flowering uniformity and increase in final yield. The objective of the paper is to analyze the influence of different pruning types on microclimate in coffee canopy. The cultivar growing in the area used for this work was Ouro Verde, with 9 years old and 3.5 m x 0.75 m of spacing. Three different types of pruning were tested: traditional (or 'decote', the most common in Brazil, consists basically of removing the apical part of the orthotropic branch at 1.6 m height), skeleton cut (equal to traditional and plus the plagiotropic branches) and trunking (the most drastic, because it is removed all above-soil part of the plant). The experiment was performed in Mococa (21º28' S, 47º01' W, 665 m), a region of subtropical climate and a representative area of the most important coffee production zone in São Paulo State. Data of air temperature, global radiation (Qg), photossynthetically active radiation (PAR) and relative humidity were collected in three heights inside coffee canopy at each 15 minutes: 50cm above canopy, 1.5 m inside the canopy and 50cm inside the canopy above soil. These measurements started 3 days before pruning and ended 3 days after it, during 2007/August with two repetitions in the experimental plot of about 10.5 m x 7.5 m. As a result it was observed that among all meteorological variables measured, the Qg and PAR were the ones that suffered more influence of pruning. After pruning there was always an increase of Qg and PAR available to coffee, without significant changing of average daily temperature and relative humidity. The PAR above the canopy in all conditions studied was approximately of half the Qg.

INTRODUCTION

Pruning in coffee has different purposes, such as the decrease of pathogens population, increase of crop robustness, flowering uniformity and increase in final yield and drink quality. These modifications are consequences of physiology modification in the plans mainly due to the source/drain relationship (Kramer and Boyer, 1995; Alves and Livramento, 2003; Laviola et al., 2007).

There are many types of pruning in coffee, among them, the most used (traditional) in Brazil consists basically of removing the apical part of the orthotropic branch at 1.6m height of the lateral shoots. The skeleton cut is equal to traditional pruning but the plagiotropic branches are removed as well. Finally, the trunking is the most drastic because it is removed all above-soil part of the plant, use only in special occasions, normaly when the coffee plants are in bad conditions (Serra, 2008).

When pruning is used there are modifications in the canpoy microclimate, nevertheles few studies are found quantifying these changes. This quantification could be used for a better pruning planing in the area avoiding extremes weather.

The objective of the paper is to analyze the influence of different pruning types on microclimate in coffee canopy regarding maximum global irradiance (Qg) and photossynthetically active radiation (PAR).

MATERIAL AND METHODS

The cultivar growing in the area used for this work was Ouro Verde, with 9 years old and 3.5 m x 0.75 m of spacing. Three different types of pruning were tested: traditional (or 'decote', the most common in Brazil,), skeleton cut and trunking (the most drastic,). The experiment was performed in Mococa (21º 28' S, 47º 01' W, 665 m), a region of subtropical climate and a representative area of the most important coffee production zone in São Paulo State. Data of air temperature, global radiation (Qg), photosynthetically active radiation (PAR) and relative humidity were collected in three heights inside coffee canopy at each 15 minutes: 50 cm above canopy, 1.5 m inside the canopy and 50cm inside the canopy above soil. These measurements started 3 days before pruning and ended 3 days after it, during 2007/August with two repetitions in the experimental plot of about 10.5 m x 7.5 m.

RESULTS AND DISCUSSION

As a result it was observed that among all meteorological variables measured, the Qg and PAR were the ones that suffered more influence of pruning. After pruning there was always an increase of Qg and PAR available to coffee, without significant changing of average daily temperature and relative humidity. The PAR above the canopy in all conditions studied was approximately half of the Qg.

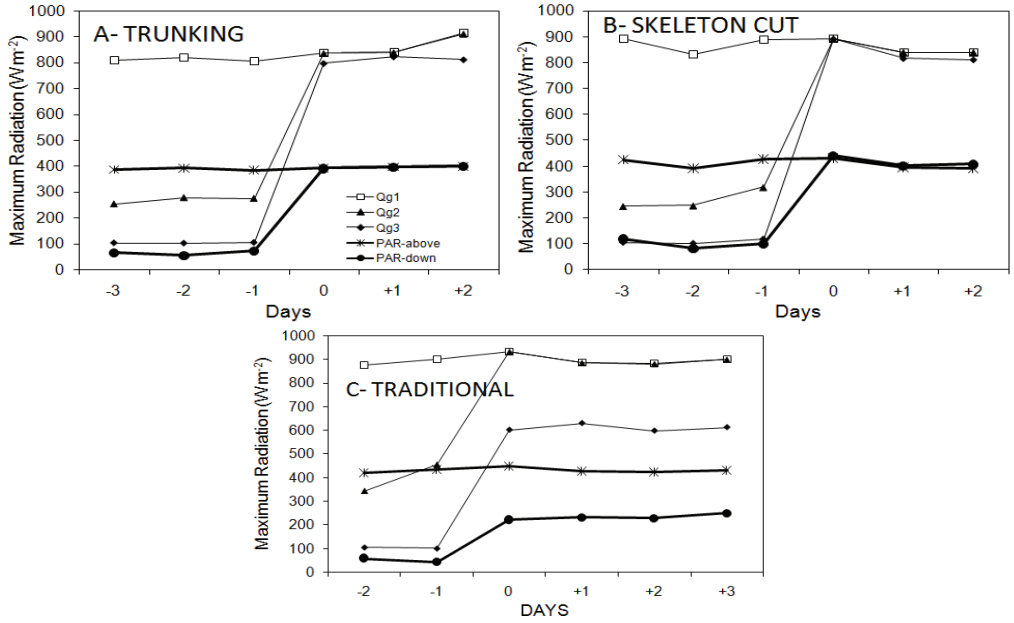


Figure 1. Maximum daily global radiation and photossynthetically active radiation observed in 3 days before and 3 days after pruning of trunking (A), skeleton cut (B) and traditional (C) in 2007/August, Mococa, SP, Brazil. Each point is an average of two repetitions inside the experimental plot, 50 cm above the canopy (1), 1.5 m inside the canopy (2) and 50 cm inside the canopy, above soil (3).

The trunking and skeleton cut pruning provided the same conditions after the pruning date (day 0, Figure 1), because maximum values of Qg and PAR of 1.5 m and 50 cm inside the canopy became equal of those at 50 cm above the canopy. Only the traditional pruning made the Qg at 50 cm inside the canopy did not reach the same values at the top of the canopy, occurring an attenuation of approximately 30%. The same condition was observed for PAR that after the pruning it remained almost 50% observed at the top of the crop.

CONCLUSION

The type of pruning in coffee affects directly the amount of solar irradiation and photossynthetically active radiation in the area. The traditional pruning elevated 50% of Qg and PAR inside the canopy, near the ground, nevertheless the trunking and the skeleton cut turned the conditions inside the canopy equal at the top.

REFERENCES

Alves J.D., Livramento D.E. Morfologia e fisiologia do cafeeiro. Lavras: UFLA/FAEPE. 46p. 2003. 46p. (Especialização em Cafeicultura Empresarial: Produtividade e Qualidade)

Laviola B.G., Martinez H.E.P., Salomao L.C.C. et al. Alocação de fotoassimilados em folhas e frutos de cafeeiro cultivado em duas altitudes. Pesq. agropec. bras., Nov. 2007, vol.42, no.11, p.1521-1530. ISSN 0100-204X.

Serra J.R.M. Poda de café: decote, recepa e esqueletamento, o que fazer.Informativo Garcafé, dezembro de 1998. http://www.coffeebreak.com.br/ocafezal.asp?SE=8&ID=60. Acessed in August,25, 2008.

Air Temperature of Coffee Canopy Before and After Different Types of Pruning in Mococa SP, Brazil

G.S. ROLIM[1*], P.S. SOUZA[2], M.J. PEDRO-JÚNIOR[1], P.B. GALLO[2], J.R.M. PEZZOPANE[3]

[1]Instituto Agronômico, Campinas, SP, Brazil. [*]E-mail: rolim@iac.sp.gov.br
[2]Pólo APTA Nordeste Paulista, Mococa, SP, Brazil
[3]Universidade Federal do Espírito Santo, São Mateus, ES, Brazil

SUMMARY

One of the desirable effects of pruning is to make the environment more suitable for growing and development of coffee plantations. The air temperature has a direct effect on all phonological stages of *Coffea arabica* and according to CAMARGO (1977), the range of optimal temperatures for its development stays between 18 °C and 22 °C. The objective of the paper is to analyze the influence of different pruning types on microclimate in coffee canopy. The cultivar growing in the area used for this work was Ouro Verde, with 9 years old and 3.5 m x 0.75 m of spacing. Three different types of pruning were tested: traditional (or 'decote', the most common in Brazil, consists basically of removing the apical part of the orthotropic branch at 1.6m height), skeleton cut (equal to traditional and plus the plagiotropic branches) and trunking (the most drastic, because it is removed all above-soil part of the plant). The experiment was performed in Mococa (21° 28' S, 47° 01' W, 665m), a region of subtropical climate and a representative area of the most important coffee production zone in São Paulo State. Data of air temperature were collected in three different heights inside coffee canopy at each 15 minutes: 50 cm above canopy (T1), 1.5m inside the canopy (T2) and 50cm inside the canopy above soil (T3). These measurements started 3 days before pruning and ended 3 days after, during 2007/August with two repetitions in the experimental plot of about 10.5 m x 7.5 m. As a result it was observed that the temperatures of all heights measured before the pruning was similar, making evident the rule of thermal moderator of the coffee canopy. After pruning it was verified that the minimum daily temperatures near the soil (T3) had a tendency to be lower than those observed above the canopy (T1), otherwise the maximum daily temperatures near the soil would have a tendency to be higher than those observed above the canopy. Therefore one can concludes that after the pruning there is a tendency to thermal amplitude increase inside the canopy for all pruning kinds.

INTRODUCTION

One of the desirable effects of pruning is to make the environment more suitable for growing and development of coffee plantations. Pruning in coffee has different purposes, such as the decrease of pathogens population, increase of crop robustness, flowering uniformity and increase in final yield and drink quality. These modifications are consequences of phisiology modification in the plans mainly due to the source/drain relationship (Kramer and Boyer, 1995; Alves and Livramento, 2003; Laviola et al., 2007).

The air temperature has a direct effect on all phenological stages of *Coffea arabica* and according to Camargo (1977), the range of optimal temperatures for its development stays between 18 °C and 22 °C.

There are many types of pruning in coffee, among them, the most used (traditional) in Brazil consists basically of removing the apical part of the orthotropic branch at 1.6m height of the lateral shoots. The skeleton cut is equal to traditional pruning but the plagiotropic branches is removed as well. Finally, the trunking is the most drastic because it is removed all above-soil part of the plant, use only in special occasions, normaly when the coffee plants are in bad conditions (Serra, 2008).

When pruning is used there are modifications in the canopy microclimate, nevertheles few studies are found quantifying these changes. This quantification could be used for a better pruning planing in the area avoiding extremes weather.

The objective of the paper is to analyze the influence of different pruning types on maximum daily temperature in different levels of coffee canopy.

MATERIAL AND METHODS

The cultivar growing in the area used for this work was Ouro Verde, with 9 years old and 3.5 m x 0.75 m of spacing. Three different types of pruning were tested: traditional, skeleton cut and trunking. The experiment was performed in Mococa (21° 28' S, 47° 01' W, 665 m), a region of subtropical climate and a representative area of the most important coffee production zone in São Paulo State. Data of air temperature were collected in three different heights inside coffee canopy at each 15 minutes: 50 cm above canopy (T1), 1.5m inside the canopy (T2) and 50cm inside the canopy above soil (T3). These measurements started 3 days before pruning and ended 3 days after, during 2007/August with two repetitions in the experimental plot of about 10.5 m x 7.5 m.

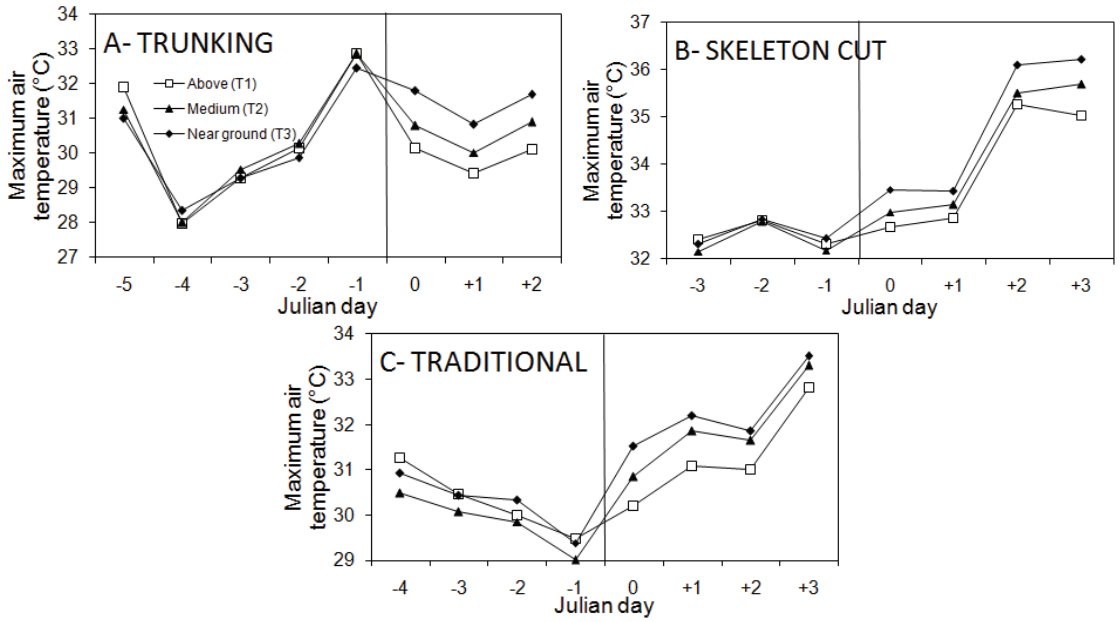


Figure 1. Maximum daily temperature above the canopy (T1), 1.5 m inside the canopy (T2) and 50 cm above the soil (T3), before and after pruning of trunking (A), skeleton cut (B) and traditional (C) in August, Mococa, SP, Brazil. Each point is an average of two repetitions inside the experimental plot.

RESULTS AND DISCUSSION

As a result the temperatures of all heights measured before the pruning was similar, making evident the rule of thermal moderator of the coffee canopy. After pruning, the maximum daily

temperatures near the soil (T3) had a tendency to be greater than those observed above the canopy (T1) (Figure 1) indicating the effect of soil temperature in warming the coffee canopy.

CONCLUSION

The coffee canopy act as a termal regulator, when it is pruned, there is an increase of temperature up to 1 °C in the traditional and skeleton cut areas in comparison to the temperature measured above the canopy.

REFERENCES

Alves J.D., Livramento D.E. Morfologia e fisiologia do cafeeiro. Lavras: UFLA/FAEPE. 46p. 2003. 46p. (Especialização em Cafeicultura Empresarial: Produtividade e Qualidade).

Laviola B.G., Martinez H.E.P., Salomao L.C.C. et al. Alocação de fotoassimilados em folhas e frutos de cafeeiro cultivado em duas altitudes. *Pesq. agropec. bras.*, Nov. 2007, vol.42, no.11, p.1521-1530. ISSN 0100-204X.

Serra J.R.M. Poda de café: decote, recepa e esqueletamento, o que fazer.Informativo Garcafé, dezembro de 1998. http://www.coffeebreak.com.br/ocafezal.asp?SE=8&ID=60. Acessed in August,25, 2008.

Relative Humidity in *Coffea arabica* Microclimate Arborized with Dwarf Coconut Palm and Rubber Tree at Mococa, SP, Brazil

G.S. ROLIM[1*], M.B.P. CAMARGO[1], P.S. SOUZA[2], P.B.GALLO[2]

[1]Instituto Agronômico, Campinas, SP, Brazil. [*]E-mail: rolim@iac.sp.gov.br
[2]Pólo APTA Nordeste Paulista, Mococa, SP, Brazil
Supported by FAPESP

SUMMARY

Relative humidity (RH) is an important meteorological element directly related to evapotranspiration rate, occurrence of pests and diseases, beverege quality and crop final yield. The purpose of this work is to verify the effect of 8 years old arborized systems on daily air relative humidity (RH) for *Coffea arabica* in Mococa (21º 28' S, 47º 01' W, 665 m), a region of subtropical climate and representative of the crop production zones of São Paulo state, Brazil. Data of air temperature were collected at each 15 minutes and at 1.5 m inside the canopy for three different systems during 1.5 years: unshaded systems (without arborization) (UNS), arborized with dwarf coconut palm (*Cocos nucifera* L.) (PAL) with spacing of 8m x 8m, and arborized with rubber tree (*Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg.) (RUB) with spacing of 16m x16m. For all the systems data of net radiation and wind velocity were collected at each 15 minutes at 0.5 m above the canopy, besides the data of the final yield treatments. As a general result, it was observed that the arborized systems make possible the maintenance of high RH during the day, as it was already demonstrated by other authors (Caramori et al., 1996; Alfaro-Villatoro et al., 2004). The RUB system kept the relative humidity higher than the PAL system. It was also observed that the RH hourly amplitude of the arborized systems was significantly lower than that of the UNS systems and also that the PAL system amplitude was lower than that of RUB. The most extreme conditions were observed during the days without wind, such as on day 285 that the RH in UNS was of 18% at 14h00 and in the arborized systems it was of 65% and 81% for PAL and RUB, respectively

INTRODUCTION

Relative humidity (RH) is an important meteorological element directly related to evapotranspiration rate, occurrence of pests and diseases, beverege quality and crop final yield.

The arborizated systems, due to heterogeneous nature of the canopies, the physical environment interacts in a more complex way to the coffe plants compared with the conventional system (Pezzopane et al., 2003).

The effect of arborization in relation to the microclimate has been studied for many authors (Baggio et al., 1997; Miguel et al., 1995; Beer et al., 1998; Peeters et al., 2002). These papers describe the arborization in a qualitative manner about the type of the tree utilized, crop density. However few papers are found in literature that caracterize the microclimate quantitatively such as in a coffee and dwarf coconut sistem in Mococa, Brazil (Pezzopane et al., 2003), in an arborizated systems in Colômbia (Farfan-Valencia et al., 2003), in an agroflorestal system in Mexico (Barradas and Fanjul, 1986) and in an arborizated system with

'bracatinga' in Londrina, Brazil (Caramori et al., 1996). These works showed that the microclimate is strongly influenced by wind, soil type, water deficit vapor, temperature and solar radiation and those elements are dependent to the climate, the species utilized for arborization and the crop density.

The purpose of this work is to verify the effect of 8 years old arborized with dwarf coconut palm and rubber tree on daily air relative humidity at Mococa, SP, Brazil

MATERIAL AND METHODS

This paper aims is to verify the effect of 8 years old arborized systems on daily air relative humidity (RH) for *Coffea arabica* in Mococa (21º 28' S, 47º 01' W, 665 m), a region of subtropical climate and representative of the crop production zones of São Paulo state, Brazil. Data of air temperature were collected at each 15 minutes and at 1.5 m inside the canopy for three different systems during 1.5 years: unshaded systems (without arborization) (UNS), arborized with dwarf coconut palm (*Cocos nucifera* L.) (PAL) with spacing of 8 m x 8 m, and arborized with rubber tree (*Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg.) (RUB) with spacing of 16 m x16 m. For all the systems data of net radiation and wind velocity were collected at each 15 minutes at 0.5 m above the canopy, besides the data of the final yield treatments.

RESULTS AND DISCUSSION

As a result, it was observed that the arborized systems make possible the maintenance of high RH during the day (Figure 1), as it was already demonstrated by other authors (Caramori et al., 1996; Alfaro-Villatoro et al., 2004). The RUB system kept the relative humidity higher than the PAL system.

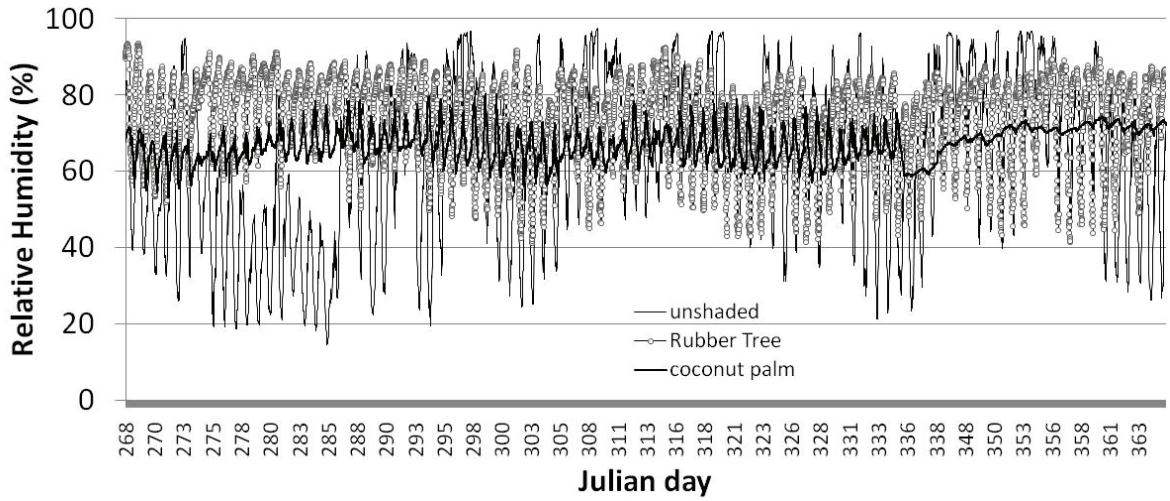


Figure 1. Relative Humidity in unshaded systems, arborized with rubber tree and with dwarf coconut palm between the days 09/24/2007 (Julian day 268) to 12/31/2007 (Julian day 365) in Mococa, SP, Brazil.

It was also observed that the RH hourly amplitude of the arborized systems was significantly lower than that of the UNS systems and also that the PAL system amplitude was lower than that of RUB. The most extreme conditions were observed during the days without wind, such as on day 285 that the RH in UNS was of 18% at 14h00 and in the arborized systems it was of 65% and 81% for PAL and RUB, respectively.

CONCLUSION

The arborizated systems maintained high air relative humidity compared to the unshaded area.

REFERENCES

Alfaro-Villatoro M.A. et al. Produção de Café em Sistema Agroflorestal. Seropédica: EMBRAPA-Agrobiologia, 2004. 36p. (EMBRAPA-Agrobiologia. Documentos, 187).

Baggio A.J., Caramori P.H., Androcioli A., Montoya L. Productivity of southern Brazilian coffee plantations shaded by different stockings of Grevillea robusta. Agroforestry Systems, Amsterdam, v. 37, n. 2, p.111-120, 1997.

Barradas V.L., Fanjul L. Microclimatic characterization of shaded and open-grow coffee (*Coffea arabica* L.) plantations in Mexico. Agricultural and Forest Meteorology, Amsterdam, v. 38, p. 101-112, 1986.

Beer J., Muschler R., Kass D., Somarriba E. Shade management in coffee and cacao plantations. Agroforestry Systems, Amsterdam, v. 38, p. 139-164, 1998.

Caramori P.H., Androcioli Filho A., Leal A.C. Coffee shade with *Mimosa scabrella* Benth. for frost protection in southern Brazil. Ag*roforestry Systems*, Amsterdam, v. 33, p. 205-214, 1996.

Farfan-Valencia F., Arias-Hernandez J.J., Rianoherrera N.M. Deasarrollo de una metodologia para medir sombrio en sistemas agroflorestales com café. Cenicafé, Chinchina. 54, n.1, p. 24-34, 2003.

Miguel A.E., Matiello J.B., Camargo A.P., Almeida S.R., Guimarães S.R. Efeitos da arborização do cafezal com *Grevillea robusta* nas temperaturas do ar e umidade do solo, Parte II. In: Congresso Brasileiro De Pesquisas Cafeeiras, 21., Rio de Janeiro, 1995. Trabalhos apresentados... Rio de Janeiro: PROCAFE, 1995. p. 55-60.

Peeters L.Y.K., Soto-Pinto, L., Perales H., Montoya G., Ishiki M. Coffee production, timber, and firewood in traditional and Inga-shaded plantations in Southern Mexico. Agriculture, Ecossystems and Environment, Amsterdam, v.82, p. 1-13, 2002.

Pezzopane J.R.M., Gallo P.B., Pedro Júnior M.J., Ortolani A.A. Caracterização microclimática em cultivo consorciado café/coqueiro-anão verde. Revista Brasileira de Agrometeorologia, Santa Maria, v. 11, n. 2, p. 293-302, 2003.

Global Solar Radiation in *Coffea arabica* Microclimate Arborized with Dwarf Coconut Palm and Rubber Tree in Mococa, SP, Brazil*

G.S. ROLIM[1*], P.S. SOUZA[2], M.B.P. CAMARGO[1], P.B. GALLO[1], T.J.G. DE SALVA[1]

[1]Instituto Agronômico, Campinas, SP, Brazil. [*]E-mail: rolim@iac.sp.gov.br
[2]Pólo APTA Nordeste Paulista, Mococa, SP, Brazil
Supported by FAPESP

SUMMARY

Global solar radiation, that reaches the earth surface after passing through the atmosphere, is greatly affected in arborized (shading) systems. The purpose of this work is to verify the effect of 8 years old arborized systems on daily global radiation (Qg) for *Coffea arabica* in Mococa (21º 28' S, 47º 01'W, 665 m), a region of subtropical climate and representative of the crop production zones of São Paulo state, Brazil. Data of air temperature were collected at each 15 minutes and at 1.5 m inside the canopy for three different systems during 1.5 years: unshaded systems (without arborization) (UNS), arborized with dwarf coconut palm (*Cocos nucifera* L.) (PAL) with spacing of 8m x 8m, and arborized with rubber tree (*Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg.) (RUB) with spacing of 16 m x16 m. For all the systems data of net radiation and wind velocity were collected at each 15 minutes at 0.5m above the canopy, besides the data of the final yield treatments. As a general result it was observed that Qg was lower in the arborized systems in comparison with the UNS systems in September, October, November and December (Figure 1), mainly between 10 h 00 and 17 h 00. It was observed that in those months the UNS systems presented the maximum values of global radiation, which were higher than 700 W m^{-2} between 12 h 00 and 14 h 00, while for PAL and RUB those values stayed between 300 Wm^{-2} and 100 W m^{-2}, respectively. The photossynthetic rate is directly affected by this decrease in availible radiation. In spite of that, the RUB system yield reached approximately 40 sacks ha^{-1}, while in PAL and UNS it was of 28 sacks ha^{-1} and 25 sacks ha^{-1}, respectively. Under those conditions there were no significant changes in terms of beverege quality comparing the different systems in relation to contents of caffeine, trigoneline, total chlorogenic acids, sacarose and total titulable acidity and sensorial analysis.

INTRODUCTION

Global solar radiation, that reaches the earth surface after passing through the atmosphere, is greatly affected in arborized (shading) systems. The arborizated systems, due to heterogeneous nature of the canopies, the physical environment interacts in a more complex way to the coffe plants compared with the conventional system (Pezzopane et al., 2003).

The effect of arborization in relation to the microclimate has been studied for many authors (Baggio et al., 1997; Miguel et al., 1995; Beer et al., 1998; Peeters et al., 2002). These papers describe the arborization in a qualitative manner about the type of the tree utilized, crop density. However few papers are found in literature that caracterize the microclimate quantitatively such as in a coffee and dwarf coconut sistem in Mococa, Brazil (Pezzopane et al., 2003), in an arborizated systems in Colômbia (Farfan-Valencia et al., 2003), in an agroflorestal system in Mexico (Barradas and Fanjul, 1986) and in an arborizated system with

'bracatinga' in Londrina, Brazil (Caramori et al., 1996). These works showed that the microclimate is strongly influenced by wind, soil type, water deficit vapor, temperature and solar radiation and those elements are dependent to the climate, the species utilized for arborization and the crop density.

The purpose of this work is to verify the effect of 8 years old arborized systems on daily global radiation (Qg) for *Coffea arabica* in Mococa (21º 28' S, 47º 01' W, 665 m), a region of subtropical climate and representative of the crop production zones of São Paulo state, Brazil.

MATERIAL AND METHODS

Data of global radiation were collected at each 15 minutes and at 1.5 m inside the canopy for three different systems during 1.5 years: unshaded systems (without arborization) (UNS), arborized with dwarf coconut palm (*Cocos nucifera* L.) (PAL) with spacing of 8m x 8m, and arborized with rubber tree (*Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg.) (RUB) with spacing of 16 m x16 m. For all the systems data of net radiation and wind velocity were collected at each 15 minutes at 0.5 m above the canopy, besides the data of the final yield treatments.

RESULTS AND DISCUSSION

As a general result it was observed that Qg was lower in the arborized systems in comparison with the UNS systems in September, October, November and December (Figure 1), mainly between 10 h 00 and 17 h 00.

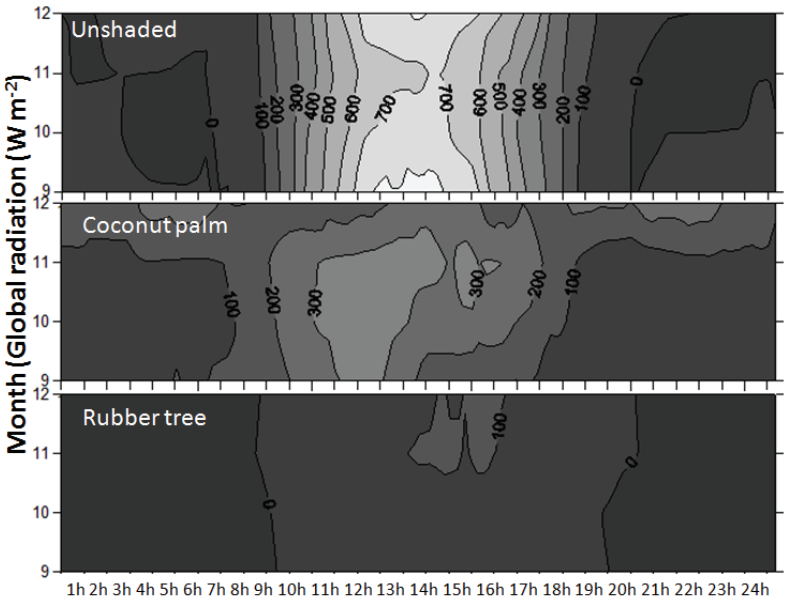


Figure 1. Global solar radiation in unshaded systems, arborized with rubber tree and with dwarf coconut palm between 09/24/2007 and 12/31/2007 in Mococa, SP, Brazil.

It was observed that in those months the UNS systems presented the maximum values of global radiation, which were higher than 700 W m^{-2} between 12 h 00 and 14 h 00, while for PAL and RUB those values stayed between 300 Wm^{-2} and 100 W m^{-2}, respectively. The photossynthetic rate is directly affected by this decrease in available radiation. In spite of that, the RUB system yield reached approximately 40 sacks ha^{-1}, while in PAL and UNS it was of

28 sacks ha^{-1} and 25 sacks ha^{-1}, respectively. Under those conditions there were no significant changes in terms of beverege quality comparing the different systems in relation to contents of caffeine, trigoneline, total chlorogenic acids, sacarose and total titulable acidity and sensorial analysis.

CONCLUSION

The arborizated systems decreased the maximum global radiation in all conditions increasing the yield and not affecting the beverage quality.

REFERENCES

Baggio A.J., Caramori P.H. Androcioli A., Montoya L. Productivity of southern Brazilian coffee plantations shaded by different stockings of Grevillea robusta. Agroforestry Systems, Amsterdam, v. 37, n. 2, p.111-120, 1997.

Barradas V.L., Fanjul L. Microclimatic characterization of shaded and open-grow coffee (*Coffea arabica* L.) plantations in Mexico. Agricultural and Forest Meteorology, Amsterdam, v. 38, p. 101-112, 1986.

Beer J., Muschler R., Kass D., Somarriba E. Shade management in coffee and cacao plantations. Agroforestry Systems, Amsterdam, v. 38, p. 139-164, 1998.

Caramori P.H., Androcioli Filho A., Leal A.C. Coffee shade with *Mimosa scabrella* Benth. for frost protection in southern Brazil. Ag*roforestry Systems*, Amsterdam, v. 33, p. 205-214, 1996.

Miguel A.E., Matiello J.B., Camargo A.P., Almeida S.R., Guimarães S.R. Efeitos da arborização do cafezal com *Grevillea robusta* nas temperaturas do ar e umidade do solo, Parte II. In: Congresso Brasileiro de Pesquisas Cafeeiras, 21., Rio de Janeiro, 1995. Trabalhos apresentados... Rio de Janeiro: PROCAFE, 1995. p. 55-60.

Peeters L.Y.K., Soto-Pinto L., Perales H., Montoya G., Ishiki M. Coffee production, timber, and firewood in traditional and Inga-shaded plantations in Southern Mexico.Agriculture, Ecossystems and Environment, Amsterdam, v.82, p. 1-13, 2002.

Pezzopane J.R.M., Gallo P.B., Pedro Júnior M.J., Ortolani A.A. Caracterização microclimática em cultivo consorciado café/coqueiro-anão verde. Revista Brasileira de Agrometeorologia, Santa Maria, v. 11, n. 2, p. 293-302, 2003.

Evapotranspiration Components and Dual Crop Coefficients of Coffee Trees during Crop Production

D.L. FLUMIGNAN[1], R.T. FARIA[2]

[1]Graduate Student, M.S., Universidade Estadual de Londrina/Londrina – Brazil
[2]Researcher, PhD, Instituto Agronômico do Paraná/Londrina – Brazil.
E-mail: rtfaria@iapar.br

SUMMARY

Quantifying crop consumptive use is essential for many applications in agriculture, such as crop zoning, yield forecast and irrigation management. The objective of this study was to determine evaporation (E), transpiration (T) and dual crop coefficients (Ke and Kcb) of coffee trees during crop production (3rd and 4th year of cultivation) conducted under sprinkler and drip irrigation and no irrigation, in Londrina, PR, Brazil. Crop evapotranspiration (ET) was measured by weighing lysimeters cultivated with plants of cultivar IAPAR 59, E was measured by microlysimeters installed on the lysimeters and T was obtained by the difference between ET and E. Crop coefficient (Kc) was determined for the irrigated treatments by the ratio of coffee crop ET and reference evapotranspiration (ETo). In addition, evaporation coefficient (Ke) and basal crop coefficient (Kcb) were determined by the ratio of E and T, respectively, to the value of ETo, which was estimated by the ASCE Penman-Monteith method on hourly basis. The measurements of E and Ke varied due to atmospheric demand and irrigation method. Those factors, in addition to crop phenology and growth of leaf, influenced T and Kcb. The measured values of E corresponded to 21 to 57%, 21 to 54% and 21 to 66% of ET, while T was 43 to 79%, 46 to 79% and 34 to 79% of ET measured on sprinkler, drip and no irrigation treatments, respectively. The Ke values varied from 0.19 to 0.79 and 0.15 to 0.39, while Kcb varied from 0.35 to 1.09 and 0.33 to 0.79 for sprinkler and drip irrigation, respectively.

INTRODUCTION

Crop consumptive use can be expressed as crop evapotranspiration (ET), which includes soil evaporation (E) and plant transpiration (T). The magnitude of these processes is governed by the atmospheric demand, also named as reference evapotranspiration (ETo). Quantifying crop ET is essential for many applications in agriculture, such as crop zoning, yield forecast and irrigation management.

A practical method for calculation of ET is the FAO method (Allen et al., 1998), in which crop ET is determined by the product of ETo, estimated from climatic variables from weather stations located in the region of interest, and a crop coefficient (Kc), determined experimentally. According to that method, Kc can be split into two components, a basal crop coefficient (Kcb), expressing the ration T/ETo, and a evaporation coefficient (Ke), expressing the ration E/ETo.

Accurate ET can be determined by weighting lysimeters in intervals less than one day (Howell et al., 1985; Faria et al., 2006). A useful approach for partition of ET measured in a cropped lysimeter is determine E from microlysimeters (Flumignan and Faria, 2007) installed on the lysimeter and find T by subtracting E from ET.

This study had as objectives to determine, for a coffee crop, during productive stage, grown in Londrina, PR, Brazil: a) E and T for treatments under irrigation (sprinkler and drip irrigation) and no irrigation, and b) Ke and Kcb coefficients for the irrigated treatments.

METHODOLOGY

The experiment was conducted at the Instituto Agronômico do Paraná (IAPAR), in Londrina, PR, Brazil (latitude 23° 18' S, longitude 51° 09' W and altitude of 585 m), during September 1, 2004 to August 31, 2006. The soil is a Red Latosol classified as Nitossol (Embrapa, 1999) and climate is classified as subtropical humid (Cfa), according to Köppen classification, characterized by mean annual temperature of 21.5 °C and annual precipitation varying from 1,400 to 1,600 mm (Iapar, 1994).

Evapotranspiration was measured in five weighting lysimeters (Figure 1A) consisting of a double tank (1.4 m width, 1.9 m length and 1.3 m depth) supported by a load cell balance with electric signal recorded by an datalogger (Faria et al., 2006). Coffee plants of the cultivar IAPAR 59 were at the 3rd and 4th year after transplanting, in spacing of 2 x 1.6 m, with two plants. In a experimental area of 115 m^2 (8 x 14.4 m). Two lysimeters were irrigated by sprinkler and one by drip irrigation, maintaining other two lysimeters without irrigation. Water was applied once to twice a week to maintain soil close to field capacity. Variation of mass was taken every 3 sec to obtain 10 min average, which was stored and then used to calculate hourly and daily ET by water balance from the system input and output.

Daily soil E was measured using microlysimeters (Figure 1B) adapted from Boast and Robertson (1982) by Flumignan and Faria (2007). They were built by double PVC tubes, in which the internal tube (internal diameter of 100 mm and length of 150 mm) was craved into the lysimeter to be filled with undisturbed soil at the same moisture as the surrounding soil. After extraction, the hole was filled with the external tube; the internal tube with soil was closed at one end by a cap and then returned into the hole. Six microlysimeters were installed in each lysimeter and weighting was taken daily at the same time to evaluate mass variation, which corresponded to soil E, since there was no rain during the measurements. T was calculated daily by the difference between ET measured by the lysimeter and soil E determined by microlysimeters.

Hourly values of ETo were determined by the ASCE Penman-Monteith method (Walter et al., 2000) using the program REF-ET (Allen, 2000) with hourly meteorological data from nearby SIMEPAR weather station as input. Kc was determined for the irrigated treatments by the ratio between coffee crop ET and ETo. Ke and Kcb coefficients were determined by the ratio of E to ETo and T to ETo, respectively.

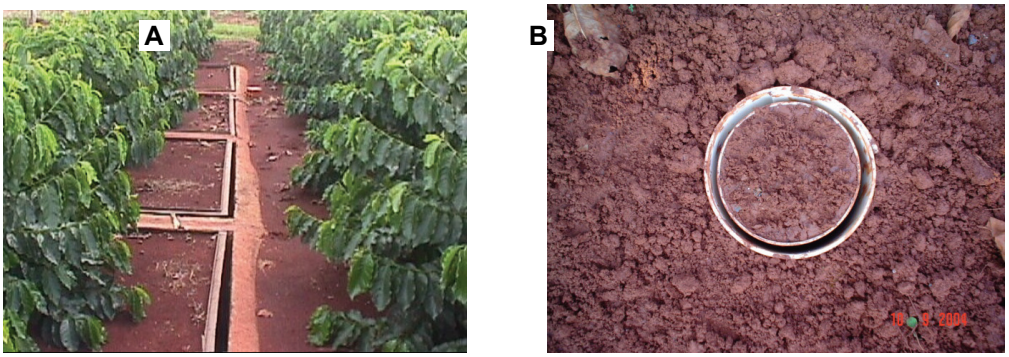


Figure 1. Lysimeter (A) and microlysimeter (B).

RESULTS AND DISCUSSION

During September, 2004 to August, 2006 coffee tress increased height from 1.05 to 1.75 m and leaf area index (LAI) varied from about 4 during Spring and Winter of the 3rd year to about 15 to 20 during fall and winter of the 4th year. Soil moisture was maintained close to field capacity in the irrigated treatments, while some periods of water deficits (October, 2004, March to May, 2005 and May to August, 2006) occurred in the no irrigated treatment.

A summary of the results are presented in Table 1. Since LAI was high (at least 4), soil E behaved as a function of atmospheric demand and water application method, varying from 0.52 to 3.19 mm day^{-1}, 0.41 to 1.41 mm day^{-1} and 0.32 to 1.92 mm day^{-1} for the sprinkler, drip and no irrigated treatments, respectively. In general, the rates of E were 21 to 66% of ET. Higher E rates were measured for the treatment with sprinkler irrigation, during spring and summer (1st, 2nd, 4th and 5th series), coinciding to high ETo (> 3 mm day^{-1}). For the same conditions, the treatment under drip irrigation had E close to the rates of no irrigated treatment, due to application of water in a partial area, under the canopy. Similarly to E, Ke varied as function of atmospheric demand and irrigation method, from 0.19 to 0.79 and 0.15 to 0.39 for treatments under sprinkler and drip irrigation, respectively.

Table 1. Mean reference evapotranspiration (ETo), evapotranspiration (ET), soil evaporation (E), plant transpiration (T), single crop coefficient (Kc), evaporation coefficient (Ke) and basal crop coefficient (Kcb) for coffee trees irrigated by sprinkler, drip and no irrigation, during six periods (series) in Londrina, PR, Brazil.

Period	ETo		Sprinkler	Drip	No Irrigated	Coefficients	Sprinkler	Drip
	(mm day^{-1})							
Sept 20-23, 2004	4.33	ET	4.23	3.43	3.55	Kc	0.98	0.80
		E	2.08	-	1.82	Ke	0.48	-
		T	2.16	-	1.73	Kcb	0.50	-
Feb 01-03, 2005	4.04	ET	-	-	5.76	Kc	-	-
		E	3.19	-	1.92	Ke	0.79	-
		T	-	-	3.84	Kcb	-	-
Jun 07-10, 2005	2.06	ET	2.80	2.10	1.46	Kc	1.38	1.02
		E	0.59	0.46	0.62	Ke	0.29	0.22
		T	2.21	1.64	0.84	Kcb	1.09	0.79
Sept 20-23, 2005	3.05	ET	2.29	2.05	1.98	Kc	0.74	0.68
		E	1.54	1.27	1.55	Ke	0.45	0.39
		T	1.14	1.06	0.81	Kcb	0.35	0.33
Jan 31- Feb 03, 2006	5.44	ET	5.60	4.69	4.82	Kc	1.04	0.87
		E	2.15	1.41	1.22	Ke	0.40	0.27
		T	3.45	3.28	3.59	Kcb	0.64	0.61
May 16-19, 2006	2.74	ET	2.48	1.97	1.53	Kc	0.91	0.72
		E	0.52	0.41	0.32	Ke	0.19	0.15
		T	1.96	1.56	1.21	Kcb	0.72	0.57

Coffee plants T behaved as a function of atmospheric demand, water application method, phenological stage and leaf area evolution, varying from 1.14 to 3.45 mm day^{-1}, 1.06 to 3.28 mm day^{-1} and 0.81 to 3.84 mm day^{-1} for the sprinkler, drip and no irrigated treatments, respectively. In general, the rates of T were 34 to 79% of ET. Higher rates of T were measured on the irrigated treatments due to more frequent water application. The no irrigated

treatment had lower T only during periods of water deficit (1st, 3rd and 6th series) when soil moisture limited plant withdraw. Independently of treatment, higher T rates were measured during the summer (2nd and 5th series). During these periods, high ETo, associated to high LAI, good water supply and intense plant physiological activity were determinant for achieving high T rates. The lower T rates occurred during September, 2005 (4th series). During this period, mean ETo was 3.05 mm day^{-1}, while T was 1.14, 1.06 and 0.81 mm day^{-1} for sprinkler, drip irrigation and no irrigation treatment. The low T rate related to ETo can be attributed to small plant leaf area and also to low physiological activity, since trees were at them senescence period. The values of Kcb were also highly variable, varying from 0.35 to 1.09 and 0.33 to 0.79, for the sprinkler and drip irrigation, respectively.

CONCLUSION

The measured values of E represented 21 to 57%, 21 to 54% and 21 to 66% of ET, while T was 43 to 79%, 46 to 79% and 34 to 79% of ET measured on sprinkler, drip and no irrigation treatments, respectively. The Ke values varied from 0.19 to 0.79 and 0.15 to 0.39, while Kcb varied from 0.35 to 1.09 and 0.33 to 0.79 for sprinkler and drip irrigation, respectively.

REFERENCES

Allen R.G. Ref-Et: Reference evapotranspiration calculation software. Version 2.0. Moscow: University of Idaho, 2000. 82p.

Allen R.G., Pereira L.S., Raes D., Smith M. Crop evapotranspiration: Guidelines for computing crop water requirements. Rome: FAO, 1998. 300p. (FAO Irrigation and Drainage Paper 56)

Boast C.W., Robertson T.M. A “micro-lysimeter” method for determining evaporation from bare soil: Description and laboratory evaluation. Soil Science Society of America Journal, Madison, v.46, n.4, p.689-696, 1982.

EMBRAPA. Sistema brasileiro de classificação de solos. Rio de Janeiro: EMBRAPA-CNPS, 1999. 412p.

Faria R.T., Campeche L.F.S.M., Chibana E.Y. Construção e calibração de lisímetros de alta precisão. Revista Brasileira de Engenharia Agrícola e Ambiental, Campina Grande, v.10, n.1, p.237-242, 2006.

Flumignan D.L., Faria R.T. Teste de um microlisímetro para medição da evaporação do solo. In: Congresso Brasileiro De Agrometeorologia, 15., 2007, Aracaju. Anais... Aracaju: Sociedade Brasileira de Agrometeorologia, 2007. CDROM.

Howell T.A., Mccormick R.L., Phene C.J. Design and installation of large weighing lysimeters. Transactions of the American Society of Agricultural Engineers, Saint Joseph, v.28, n.1, p.106-117, 1985.

IAPAR. Cartas climáticas do Estado do Paraná. Londrina, IAPAR, 1994. 49p. (Documento 18)

Walter I.A., Allen R.G., Elliott R., Jensen M.E., Itenfisu D., Mecham B., Howell T.A., Snyder R., Brown P., Echings S., Spofford T., Hattendorf M., Cuenca R.H., Wright J.L., Martin D. Asce’s standardized reference evapotranspiration equation. In: National Irrigation Symposium, 4., 2000, Phoenix. Proceedings... Phoenix: American Society of Agricultural Engineers, 2000. p.?-?.

Estimation of Duration of the Flowering-Maturation Stage for Different Arabic Coffee Cultivars

F.L. NUNES[1], M.B.P. DE CAMARGO[2], L.C. FAZUOLI[2], J.R.M. PEZZOPANE[3]

[1]Curso de PG em Agricultura Tropical e Subtropical, IAC, Campinas, SP, Brazil
[2]Instituto Agronômico, Campinas, SP, Brazil
[3]Universidade Federal do Espírito Santo, São Mateus, ES, Brazil

SUMMARY

The full maturation of the arabic coffee fruits happens when at least 50% of the grains reach the cherry stage, that according to the literature happens when the sum of the potential evapotranspiration values after the flowering phase complete about 700 mm. However, to be incorporate in estimation of coffee yield models are necessary determinations with larger precision of the required thermal accumulations for the complement of the maturation of the fruits. The objective of this work was to identify the best agrometeorological model for quantification of thermal sum in the estimate of the duration of the growth stage of flowering-maturation of the fruits for four coffee cultivars (Mundo Novo, Catuai, Obatã and Tupi). Phenological data were collected from scientific research files of the "Instituto Agronomico" (IAC) for Campinas region and Mococa, Sao Paulo State, Brazil, for the growing seasons from 2001 to 2007. Different thermal sums were used based on evapotranspirations: potencial (ETp), actual (ETa) and a combination between ETa and ETp; and based on growing degree-day: classical (GD) and GDcorr corrected by water availability factor (GDcorr). The thermal sums using ETr and GDcorr presented the best estimates compared to the others thermal sums. The thermal accumulations requirements for the complement of the flowering-maturation phase were 751.2 mm (ETa) and 2770.5 GD (GDcorr) for cv. Mundo Novo, 766.3mm (ETa) and 2838.5 (GDcorr) for cv. Catuai, for cv. Obatã 704.5 mm (ETa) and 2904.3 GD (GDcorr) and for cv Icatu 682.9 mm (ETa) and 2814.2 GD (GDcorr).

INTRODUCTION

The reproductive period of the coffee plant corresponds to the flowering phase up to the maturation phase. Usually the flowering happens in September with the start of the rainy season. High temperatures associated to water deficit during bloomed can cause abortion of the flowers. After the fecundation the pin heads and fruit expansion phases come that usually goes until December. Severe drought in this phase can harm the growth of the fruits. The fruit expansion happens when the internal liquids solidify, giving formation to the coffee beans. Usually happens in the middle of summer, January to March. Severe droughts in that phase can result in badly formation of the endosperm of the beans. The maturation of the fruits usually happens between April and June, and the full maturation happens, when at least 50% of the grains reach the cherry phase.

Agricultural production forecasts are the great importance for the establishment of the coffee politics in Brazil. Agrometeorological models that relate environmental conditions, such as air temperature and soil water availability, with coffee phenology and yield are being developed for the Brazilian coffee areas. Those models consider that each meteorological variable

exercises control on the crop productivity for influencing in certain critical phenological periods such as flowering, fruit expansion and fruit maturation.

Observations (Camargo et al., 2001) made in adult coffee plants in different tropical conditions indicated that the floral gems complete the maturation and they enter in dormant numbness, being ready for the full anthesis when the sum of ETp starting from the beginning of April reaches about 350mm. The authors also indicated that after the flowering, the fruit maturation is reached when the sum of ETp reaches about 700 mm. These values are indicators of the amount of accumulated energy in each phonological sub period of the coffee crop. According to Camargo et al. (2005), that phonological model has reasonable capacity to indicate the beginning of the flowering and the maturation phases.

The value of 700mm of the sum of ETp according to Pezzopane et al. (2005) is equivalent to the accumulation of about 2800 degree-day (GD). However, to be incorporate in agrometeorological models are necessary, more studies to determine with larger precision the thermal and water requirement limits for the maturation of the fruits (Pezzopane et al., 2006).

The objective of this project was the identification and parametrization of the best agrometeorological model considering different accumulations values (ET and GD) in the estimate of the duration of the flowering-fruit maturation for the coffee cultivars Mundo Novo, Catuaí, Obatã, and Icatu.

MATERIAL AND METHODS

The coffee phenological data related to Mundo Novo, Catuai, Obatã and Tupi cultivars were obtained at the Agronomic Institute (IAC/APTA) files from breeding and agrometeorological experiments accomplished in Campinas (Lat.: 22° 54' S; Long.: 47° 05' W and altitude of 669 m) and Mococa (Lat.: 21° 28' S; Long.: 47° 01' W and altitude of 665 m). Daily meteorological data such as maximum and minimum air temperatures and rainfall were obtained from weather stations closed to the experimental areas in Campinas and Mococa.
Phenological data related to Mundo Novo, Catuai, Obatã and Tupi cultivars were collected from scientific research files of the “Instituto Agronomico” (IAC) for Campinas (Lat.: 22° 54' S; Long.: 47° 05' W and altitude of 669 m) and Mococa, (Lat.: 21° 28' S; Long.: 47° 01' W and altitude of 665 m) regions, Sao Paulo State, Brazil, for the years from 2001 to 2007. Several “flowering-maturation” cycles (2001/02 to 2006/07 growing seasons) were analyzed for the different cultivars.

Different thermal sums models were used to estimate the duration of the growth stage of flowering-maturation of the coffee fruit cultivars based on:

a) Evapotranspirations: potencial (ETp), actual (ETa) and a combination between ETa and ETp (ETa and ETp);
b) Growing degree-day: classical (GD) and another one corrected by water availability factor (GDcorr).

The ETp and ETa values were estimated by the Thornthwaite and Mather (1955), method. ETp is a fundamental climatic element to indicate the available solar energy in the area, it constitutes an index of thermal efficiency, similar to the degree-day, however being expressed in millimeters (mm) of equivalent evaporation (Camargo and Camargo, 2000), that it is a quantitative physical unit (Camargo, 1962). Besides the method of ET, the classic method of growing degree day (GD) was used according to PEREIRA et al. (2002).

"ETa", "ETa and ETp" and "GDcorr" (Tb = 10.5 °C) thermal sums were used as proposed by PEZZOPANE et al. (2005) considering them since the first eight ten-day periods after the flowering. After that, ETp sums or classic GD sums (Tb = 10.2 °C) were considered starting at the ninth ten-day period up to the maturation phase. This procedure was adopted, to consider the influence of periods with water deficits in the initial development phase of the coffee fruits. That critical phase corresponds to the development of the pin heads and expansion of the fruits up to eighth ten-day periods after the flowering.

RESULTS AND DISCUSSION

The mean values of thermal sum for ETp were 812.0 mm, 819.8 mm, 862.9 mm, and 845.1 mm respectively for the cultivars Mundo Novo, Catuai, Obatã and Icatu. These values were superior to the value (700mm) originally suggested by Camargo and Camargo (2001).

The thermal sums using ETa and GDcorr presented the best estimates compared to the others thermal sums. The thermal accumulations requirements for the complement of the flowering-maturation phase were 751.2 mm (ETr) and 2770.5 GD (GDcorr) for cv. Mundo Novo, 766.3 mm (ETr) and 2838.5 (GDcorr) for cv. Catuai, for cv. Obatã 704 mm (ETr) and 2904.3 GD (GDcorr) and for cv Icatu 682.9 mm (ETr) and 2814.2 GD (GDcorr).

These results indicate the need to consider the factor water for the quantification of the thermal sums, for ET or GD, necessary for estimating the flowering-maturation phase.

REFERENCES

Camargo A.P. Contribuição para a determinação da evapotranspiração potencial no Estado de São Paulo. Bragantia, Campinas, v. 21, n. 12, p. 163-203, 1962.

Camargo A.P. Florescimento e frutificação de café arábica nas diferentes regiões cafeeiras do Brasil. Pesquisa Agropecuária Brasileira, Brasília, v.20, n.7, p. 831-839, 1985.

Camargo A.P., Pereira A.R. Agrometeorology of the coffe crop. Gebeve. World Meteorological Organization, 1994. 96p. (Agricultural Meteorology CaM report, 58). 1994.

Camargo A.P., Camargo M.B.P. Uma revisão analítica da evapotranspiração potencial. Artigo de Revisão. Bragantia, Campinas, v.59, n.2, p.125-137, 2000.

Camargo A.P., Camargo M.B.P. Definição e esquematização das fases fenológicas do cafeeiro arábica nas condições tropicais do Brasil. Bragantia, Campinas, v. 60, n. 1, p. 65-68, 2001.

Camargo M.B.P., Santos M.A., Brunini O., Fahl J.I., Meireles E.J.L., Lorena B. Teste de modelo agrometeorológico de monitoramento e de estimativa de produtividade do cafeeiro no Estado de São Paulo. In: Simpósio de Pesquisa dos Cafés do Brasil, 4., 2005. Londrina, Anais, Londrina: Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café, 2005. p. 25-29.

Pereira A.R., Angelocci L.R., Sentelhas P.C. Agrometeorologia Fundamentos e Aplicações Práticas. Guaíba, SP, 478p. 2002.

Pezzopane J.R.M., Pedro Jr M.J., Camargo M.B.P., Fazuoli L.C. Temperatura-Base e Graus-dia com correção pela disponibilidade Hídrica para o cafeeiro 'Mundo Novo' no período florescimento-colheita. Congresso Brasileiro De Agrometeorologia, Campinas, SP, 2005.

Pezzopane J.R.M., Pedro Jr M.J., Camargo M.B.P., Fazuoli L.C. Índices agrometeorológicos para estimativa da duração do período florescimento-colheita do cafeeiro Mundo Novo. In: IV Congresso Brasileiro de Biometeorologia, 2006, Ribeirão Preto, SP. Mudanças climáticas: impacto sobre homens, plantas e animais. Ribeirão Preto, SP: Sociedade Brasileira de Biometeorologia, 2006. v. 1, p. 2-2.

Thornthwaite C.W., Mather J.R. The water balance. Centerton, N. J. 1955, 104p. (Publ. in Climatology, v. 8, n. 1), 1955.

Vegetative Development and Leaf Water Potential of Irrigated Coffee Tree, cv Catuaí, Cultivated under Different Densities of Plantation in Mococa, SP

E. SAKAI[1], V.A.QUEIROZ[1], E.S. KOBAYASHI[1], P.S. SOUZA[2], J.M.C. SILVEIRA[2], R.C.M. PIRES[1], F.B. ARRUDA[1]

[1]Instituto Agronômico/APTA/Campinas-SP – Brazil. E-mail: emilio@iac.sp.gov.br
[2]Pólo Nordeste Paulista/APTA /Mococa-SP – Brazil

SUMMARY

Canopy development and predawn leaf water potential (Ψ_{am}) of irrigated and non-irrigated coffee trees, cv Catuaí, were evaluated in a field experiment in Mococa, SP, Brazil. The experimental design was a 6 x 2 factorial scheme (plantation densities and irrigation) in randomized blocks. The Ψ_{am} varied according to the amount of water in the soil, which oscillated due to the irrigations and seasonal precipitations. Irrigation provided greater stability for the coffee trees: smaller variation of Ψam and crop coefficient (Kc) as well as better vegetative development.

INTRODUCTION

Coffee plantation is one of the most important agricultural activities in Brazil, with relevant influence in the country's socioeconomic aspect. The crop was first introduced to regions where it could easily adapt due to the favorable water availability. Later, it also spread into regions with water deficit. Nowadays, more than 10% of total Brazilian coffee area is under irrigation (Santinato et al., 2008). Such fact has lead to a constant modernization of irrigation systems and to the search of trustworthy values of relevant parameters that can assist in the management of the water in agriculture. An important indication of soil water deficit is the leaf water potential. This parameter, measured before the sunrise, is indicative of the level of soil water storage, since there is a trend to equilibrium between the water conditions of the plant and the soil when the water deficit is not intensified (Silva et al., 2003). The present work evaluates the predawn leaf water potential (Ψ_{am}) and the vegetative development of irrigated coffee trees, cv Catuaí, in order to assist in the management and proper use of water resources in the drip irrigation systems.

MATERIAL AND METHODS

A field work with a two-year-old *Coffea arabica* L. plants, cv Catuaí, was carried out in Mococa, SP, Brazil, in 2007. The experiment was implemented as a 6 x 2 factorial scheme (densities of plantation and irrigation), in random blocks with four replications. The treatments consisted of six spacing arrangements: E1 (1.60 x 0.50 m), E2 (1.60 x 0.75 m), E3 (1.60 x 1.00 m), E4 (3.20 x 0.50 m), E5 (3.20 x 0.75 m) and E6 (3.20 x 1.00 m), with irrigated (I) and non-irrigated (NI) factors. Irrigation started in September. During the experimental period, data concerning average air temperature, precipitation, relative humidity of air, wind speed and solar radiation were recorded on a daily basis from the Automatic Meteorological Station (AMS) closely located to the experimental area. Reference evapotranspiration (ET_0) was calculated using Penman-Monteith method (Allen et al., 1998). After July, soil moisture

was monitored every 10 cm up to 1 m depth using a Sentek probe (model Diviner 2000). With these values, crop evapotranspiration (ET_C) was estimated from a field water balance. The crop coefficient (Kc) was estimated using the data regarding ET_0 and ET_C. For the assessment of vegetative development of the coffee trees, measurements of the height (h) and the canopy diameter (cd) in four plants randomly selected in each experimental plot were accomplished on December 17, 2007. The Ψ_{am} of the coffee tree was assessed every other week between 4 and 5 pm using a Scholander pressure chamber (PMS Instrument, model 1000, Corvallis)

RESULTS AND DISCUSSION

Table 1 shows the monthly averages of meteorological variables, ET_0 and the precipitations observed during the experimental period. ET_0 values ranged from 3.49 to 5.43 mm day^{-1}, with mean value of 4.37 mm day^{-1}. Kobayashi (2007), in an experiment also performed in Mococa, found ET_0 mean values of 3.90 mm day^{-1}. Figure 1 illustrates the results regarding to the soil water storage for irrigated (E1-I; E3-I and E4-I) and non-irrigated (E1-NI) coffee trees. After the beginning of September, the variations of soil water storage occurred due to both the irrigations and the precipitations. This fact contributed to the increase of the monthly average Kc (Table 2), which were 0.61, 0.69 and 0.48 for the treatments E1-I, E3-I and E4-I, respectively. These values are in agreement with those found by Villa Nova et al. (2002). Using Duncan's test ($p < 0.05$), statistically significant differences between irrigated and non-irrigated treatments were observed (Table 3), but not for h and cd variables among the irrigated factors.

Table 1. Monthly averages of meteorological variables at the region of Mococa, SP, from January to November 2007.

Month/ Year	WS ± SE ($m\ s^{-1}$)	RH ± SE (%)	AT ± SE (ºC)	LR ± SE ($MJ\ m^{-2}\ day^{-1}$)	ET_0 ± SE ($mm\ day^{-1}$)	Precipitation (mm)
Jan/07	1.80 ± 0.08	84.05 ± 0.96	23.34 ± 0.23	9.22 ± 0.63	2.73 ± 0.19	606.23
Feb/07	1.78 ± 0.06	72.76 ± 1.42	24.36 ± 0.23	13.73 ± 0.66	4.25 ± 0.21	170.17
Mar/07	1.53 ± 0.07	70.19 ± 1.65	24.53 ± 0.23	12.84 ± 0.58	4.09 ± 0.20	174.23
Apr/07	1.63 ± 0.08	72.92 ± 1.46	24.76 ± 0.61	10.97 ± 0.43	3.54 ± 0.14	28.20
May/07	2.86 ± 0.15	70.72 ± 1.95	18.74 ± 0.62	8.33 ± 0.54	3.02 ± 0.17	86.60
Jun/07	2.74 ± 0.12	64.01 ± 1.45	19.47 ± 0.35	8.86 ± 0.38	3.42 ± 0.14	16.15
Jul/07	2.97 ± 0.15	63.35 ± 2.39	18.88 ± 0.52	8.50 ± 0.44	3.49 ± 0.23	59.40
Aug/07	2.43 ± 0.09	52.89 ± 1.57	20.99 ± 0.25	11.24 ± 0.14	4.42 ± 0.12	0.00
Sep/07	2.64 ± 0.08	47.06 ± 1.55	24.07 ± 0.36	12.59 ± 0.22	5.43 ± 0.12	19.00
Oct/07	2.41 ± 0.08	54.19 ± 2.96	25.05 ± 0.46	11.68 ± 0.71	4.94 ± 0.32	60.80
Nov/07	2.43 ± 0.09	71.97 ± 1.68	23.25 ± 0.28	10.07 ± 0.76	3.56 ± 0.25	144.52

WS: wind speed; RH: relative humidity of air; AT: average air temperature; LR: calculated liquid radiation; ET_0: reference evapotranspiration; SE: standard error of the mean.

Table 2. Monthly average values of crop coefficient (Kc) in Mococa, SP, from July to November 2007.

Date	E1 (I)	E1 (NI)	E3 (I)	E4 (I)
Jul/07	0.42	0.44	0.50	0.46
Aug/07	0.28	0.31	0.37	0.42
Sep/07	0.61	0.12	0.62	0.37
Oct/07	0.89	0.18	0.91	0.59
Nov/07	0.87	0.21	1.04	0.54
Average	0.61	0.25	0.69	0.48

Table 3. Statistical results of the spacing and irrigation parameters of plant height and foliage diameter.

Parameters	Treatments	Height, cm	Foliage Diameter, cm
Spacing regimes	E1	89.8 a	82.0 a
	E2	86.0 a	80.5 a
	E3	87.9 a	85.9 a
	E4	87.1 a	81.5 a
	E5	84.8 a	80.9 a
	E6	81.6 a	80.5 a
Irrigation	(I)	91.1 a	86.3 a
	(NI)	81.3 b	77.8 b
CV (%)		10.7	13.4

Means followed by the same letter are not different by Duncan test ($p < 0.05$).

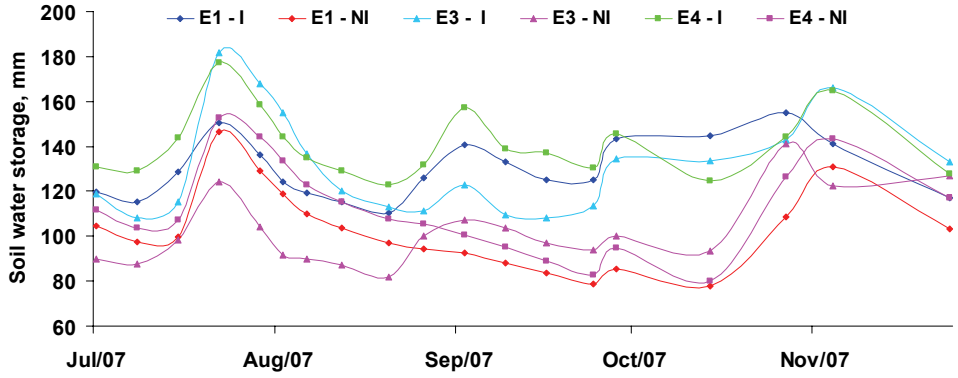


Figure 1. Soil water storage in Mococa, SP, from July to November 2007.

Figure 2 show that the irrigated treatment did not present great variations from July to November 2007. However, an accentuated decrease in the leaf water potential was present on all non-irrigated treatments in September due to the high water demand to the atmosphere associated to a low precipitation level (Table 1).

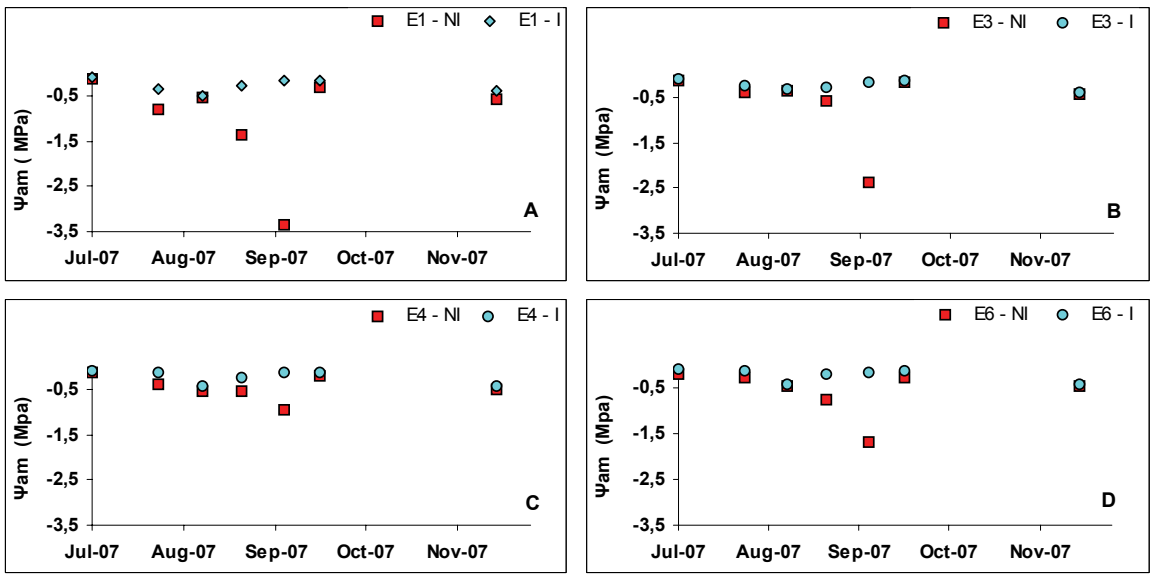


Figure 2. Variation of the predawn water potential in the plant between July and November 2007 for Catuaí plants, in Mococa, SP.

CONCLUSIONS

- The average values of Kc for the period of the study were 0.61; 0.69; 0.48 and 0.25 for E1-I, E3-I, E4-I and E1-NI, respectively;
- The lowest predawn leaf water potentials occurred in September for the non-irrigated treatment, with the lowest value of -3.37 MPa for treatment E1;
- Significant differences of vegetative development among the studied spacing arrangements did not occur, but between the irrigated and non-irrigated treatments. There was an increase of 12% in plant height (h) and 11% in canopy diameter (cd) in the irrigated treatment as compared to the non-irrigated ones.

REFERENCES

Allen R.G., Pereira L.S., Raes D., Smith M. Crop evapotranspiration: guidelines for computing crop water requirements. Irrigation e Drainage, Food and Agriculture Organization of the United Nations, paper 56, Rome: 1998, 300p.

Kobayashi E.S. Consumo de água e produtividade de cafeeiros arábica na região de Mococa, SP. 2007. 64 p. Dissertação (Mestrado) Instituto Agronômico de Campinas (IAC).

Santinato R., Fernandes A.L.T., Fernandes D.R. Irrigação na cultura do café. 2. ed. Uberaba: O Lutador, 2008. v. 1. 483 p.

Silva A.M., Lima E.P., Coelho M.R., Coelho G.S. Produtividade, rendimento de grãos e comportamento hídrico foliar em função da época de irrigação do parcelamento e do método de adubação do cafeeiro catuaí. Engenharia Agrícola, Jaboticabal, v.23, n.3, p.434-440, 2003.

Villa Nova N.A., Favarin J.L., Angelocci L.R., Neto D.D. Estimativa do coeficiente de cultura do cafeeiro em função de variáveis climatológicas e fitotécnicas. Bragantia, Campinas, v.61, n.1, p.81-88, 2002.

Agrometeorological Parameters for Prediction of Sucrose Content in Developing Fruits of Different Cultivars of Arabica Coffee

J.R.M. PEZZOPANE[1*], T.J.G. SALVA[2], V.B. DE LIMA[2], L.C. FAZUOLI[2]

[1]CEUNES, Universidade Federal do Espírito Santo, Rua Humberto de Almeida Franklin, 257, CEP 29933-415, São Mateus, ES, Brazil. [*]E.mail: josepezzopane@ceunes.ufes.br
[2]Coffee Center Alcides Carvalho, Agronomic Institute of Campinas, P.O. Box 28, CEP 13020-902, Campinas, SP, Brazil

SUMMARY

The aim of this study was to establish the relationship between the sucrose content and agrometeorological parameters during the development of coffee fruits. During the growing season of 2004/2005 and 2005/2006, from 150 days after the flowering (DAF) and at weekly frequency, samples of 50 coffee fruits of cv. Mundo Novo, Obatã and Catuaí Vermelho, were picked up from selected branches of the coffee plant of a field trail located at Campinas, São Paulo State, Brazil. Fruit endosperms were freeze-dried, ground and analyzed for sucrose by high pressure liquid chromatography. The climatic data had been collected in weather station located near to the field trail, being obtained, during eighty days from the flowering, the degree-day sum, for base temperature of 10.5 °C, adjusted (DDA) or not (DD) for water deficit. Also, the sum reference (ETo) and actual (ETr) evapotranspiration in ten days base, obtained from the water balance, were considered. Logistic models were carried through to explain sucrose content variation in the coffee beans (dependent variable) as a function of the climatic parameters (independent variable). The results showed that the higher sucrose contents occurred in the yellow-red to cherry phase. Considering the growing season of 2004/2005 and 2005/2006, it was noticed a trend to the maximum sucrose content occur earlier in the first than in the second harvest, mainly for cv. Obatã. When using the climatic variables, similar statistical behavior and even some improvement in the estimated value comparing to the DAF was observed. The Mundo Novo variety presented maximum content of sucrose earlier than the cv. Catuaí Vermelho, which was followed for cv. Obatã, for all analyzed variable.

INTRODUCTION

The coffee quality is influenced by genetic, climatic characteristics, agricultural management and maturity degree of coffee berry at harvest as well as by the processing method. The climatic conditions influence the final coffee quality interfering on the health and fruit development (Ortolani et. al., 2001; Bertrand, 2004).

Amongst the climatic factors affecting arabica coffee beverage quality, air temperature presents great importance since it interferes on the maturation cycle. High temperature implies anticipation of maturation, shorter time to biochemical events and low coffee quality. On the other hand, slow ripening of coffee berry contributes to a longer period of bean filling, larger bean size, better bean biochemical composition and ultimately higher beverage quality (Guyot et al., 1996).

The sucrose content in the coffee beans is one of the most important factors of final beverage characteristics (De Castro and Marraccini, 2006). Recent studies show that fully ripe arabica

coffees grains contain around 9% of sucrose (Ky et al., 2001), and that the sugar content varies with the maturation degree (Geromel et al., 2007).

The objectives of this study were to investigate the influence of climatic conditions upon the velocity of coffee fruit maturation and the upon the potential quality of coffee beans using sucrose content as accompaniment parameter.

MATERIALS AND METHODS

Coffee samples were collected in the field trail of the Centro de Café Alcides Carvalho of the Agronomic Institute of Campinas(IAC), São Paulo State, Brazil. Fifty fruits of the 'IAC Mundo Novo', 'IAC Obatã' e 'IAC Catuaí Vermelho' cultivars were picked up from selected branches of the coffee trees from 150 days after the main flowering until 80 days later, at weekly frequency, during the growing seasons 2004/2005 and 2005/2006. Fruit endosperms were freeze-dried, ground and analyzed for sucrose content. The sugar concentration was measured by HPLC-PAD method using Dionex detector and PA1 column after extraction in hot water as described in Rogers et al. (1999). Isocratic elution with 50 mM NaOH solution, at a flux of 1 mL/min, was performed at 30 °C. The calculations were based on the sample and standard pick areas.

The meteorological data had been collected in weather station located near to the field trail being obtained, from the flowering, the degree-day sum (DD), for base temperature of 10.5ºC, and the degree-day sum adjusted (DDA) for water deficit during eighty days period starting at flowering, as a function of the water availability (Pezzopane et al, 2008).

The sum of reference (ETo) and actual (ETr) evapotranspiration, from the flowering to the sample collection date, were obtained from the water balance in ten days base, calculated for a soil water storage capacity of 125 mm (Thornthwaite and Mather, 1955).

Logistic model ($y = yo + ((1 + ((x/xo)\ b))$ were carried through to explain sucrose content variation in the coffee fruits (dependent variable) in function of the following parameters (independent variable): days after flowering, degree-day, degree-day adjusted for water deficit, reference evapotranspiration (Eto) and actual evapotranspiration (Etr).

RESULTS

Table 1 shows the logistic model coefficients to the analyzed variables and the Figure 1 shows the equations for sucrose content in developing fruits of the three arabica cultivars based on the analyzed variables.

Higher sucrose concentration in the coffee beans had occurred from the yellow-red to the cherry phase phenological stages. Similar statistical performance of the regression models were observed using meteorological variables and days after flowering (R^2 above of 0.93 - Table 1), showing that an influence of the climatic conditions in the sugar accumulation does exist. It is important to point out that the two years of production had presented similar climatic characteristics, and could influence the results.

When using the models to estimate the sucrose content at the final maturation stage the IAC Catuaí Vermelho presented higher value than IAC Mundo Novo and Obatã (9 vs 8%). For all the analyzed variables the maximum sucrose content was reached more precociously in Mundo Novo and Catuaí cultivars than in Obatã.

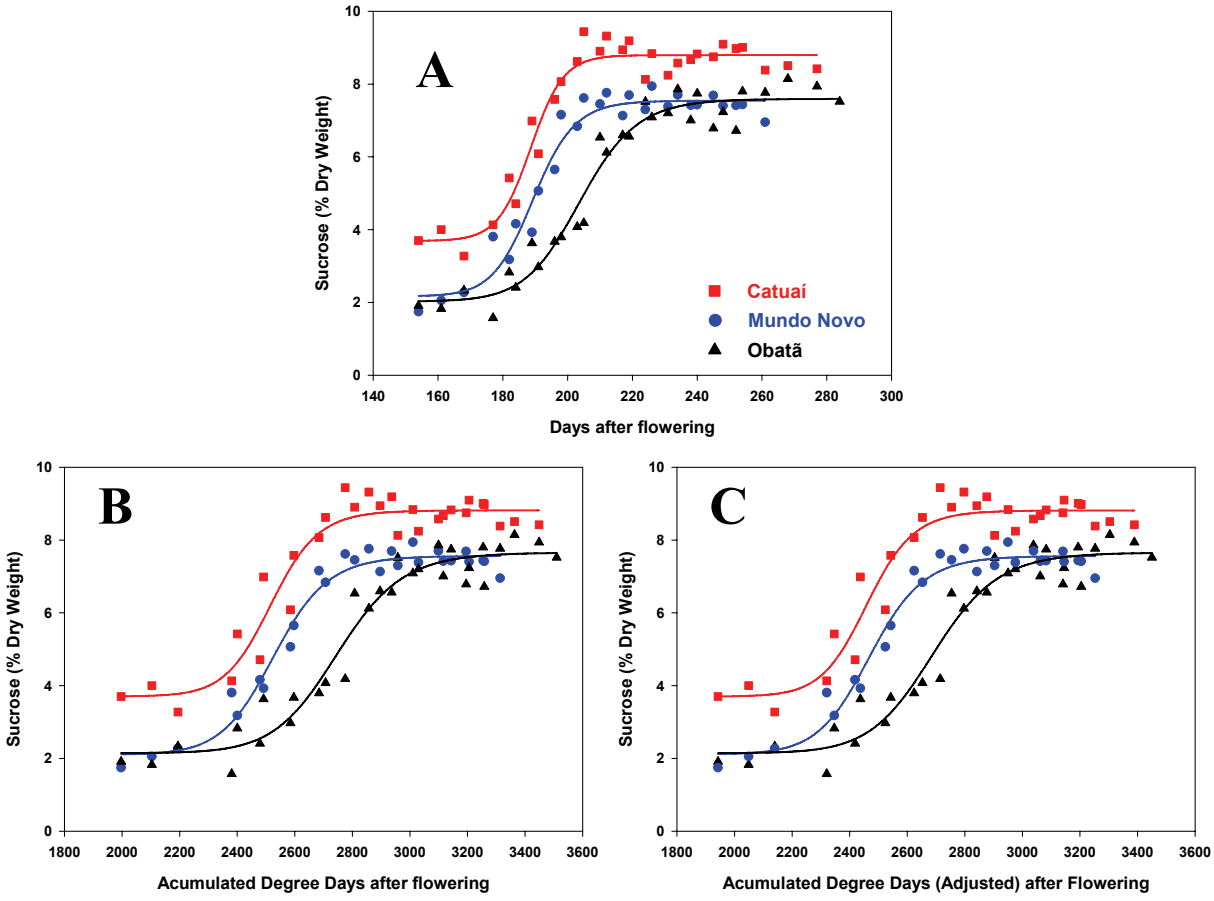


Figure 1. Sucrose content in developing fruits of cultivars of arabica coffee based on days after flowering (A), degree-day sum (B), degree day sum adjusted for water deficit during eighty days period starting at flowering (C).

Table 1. Statistical coefficients of the logistic models for the relationship between the sucrose content (dependent variable) and the independent variables: days after flowering (DAF), degree-day sum (DD), degree day sum adjusted for water deficit (DDA), reference (ETo) and actual (ETr) evapotraspiration for different cultivars of arabica coffee.

Variable			Coefficient		
	a	b	xo	yo	R^2
	Mundo Novo				
DAF	5.3776	-29.8059	189.4888	2.1673	0.96
DD	5.4613	-27.1664	2528.9489	2.1030	0.97
DDA	5.1114	-30.6340	2461.3540	3.7000	0.93
ETo	5.3416	-30.7728	746.0721	2.2221	0.96
ETr	5.4884	-27.5898	690.6975	2.1316	0.97
	Catuaí				
DAF	5.0933	-37.5884	188.5872	3.7042	0.95
DD	5.1094	-30.9328	2519.0868	3.7097	0.93
DDA	5.4520	-26.7021	2472.0048	2.1111	0.97
ETo	5.0868	-36.9509	741.3353	3.7166	0.95
ETr	5.1191	-32.1277	687.3885	3.7160	0.93
	Obatã				
DAF	5.5687	-23.4756	204.0591	2.0260	0.96
DD	5.5125	-24.5240	2749.9218	2.1451	0.95
DDA	5.5135	-24.0798	2692.2101	2.1438	0.95
ETo	5.5171	-28.6023	802.5145	2.1570	0.96
ETr	5.8200	-26.2112	747.2943	2.3830	0.95

REFERENCES

Bertrand B., Etienne H., Guyot B. and Vaast P.. 2004. Year of production and canopy region influence bean characteristics and beverage quality of Arabica coffee. Proc. 20th ASIC Conference (Bangalore, India). ASIC, Paris, France.

De Castro R.D, Marraccini P. Cytology, biochemistry and molecular changes during coffee fruit development. Brazilian Journal of Plant Physiology, 18(1): 175-199, 2006.

Geromel C., Mazzafera P., Marraccini P.R., Ferreira L.P , Vieira L.G., Pereira L.F.P. Açúcares solúveis, sacarose sintase, e sacarose fosfato sintase durante o desenvolvimento do fruto de café, sob diferentes condições de luz e carga. In: IV Simpósio de Pesquisas dos Cafés do Brasil., 2005, Londrina. Anais do IV Simpósio de Pesquisas dos Cafés do Brasil., 2005. v. 1.

Guyot B., Manez J.C., Perriot J.J., Giron J. and Villain L. 1996. Influence de l'altitude et de l'ombrage sur la qualité des cafés arabica. Plantation Recherche Développement 3: 272-280.

Ky C.L. et al. Caffeine, trigonelline, chlorogenic acids and sucrose diversity in wil *Coffea arabica* L. and *C. canephora* P. acessions. Food Chemistry, 75: 223-230. 2001

Pezzopane J.R.M et al. Exigência térmica do café arábica cv. Mundo Novo no subperíodo florescimento-colheita. Ciência e Agrotecnologia, Lavras, in press, 2008.

Ortolani A.A., Pedro Jr M.J., Camargo M.B.P., Cortez J.G., Pallone Filho W.J. Regionalização da época de maturação e qualidade natural de bebida do café arábica no

Estado de São Paulo. In: XII CBA, 2001, Fortaleza, CE. Anais do XII CBA. Fortaleza, CE: SBA, 2001. v. 1. p. 53-54.

Thornthwaite C.W., Mather J.R. The water balance. Publications in Climatology. New Jersey: Drexel Institute of Technology, 1955. 104p.

Certification of Forest Coffee in South-Western Ethiopia: Local Level Performance and Challenges

T. STELLMACHER[1], J. VOLKMANN[2], U. GROTE[3]

[1]Center for Development Research (ZEF, Bonn), Department of Political and Cultural Change
Walter Flex Straße 3, 53113 Bonn, Germany. E-mail: tillstellmacher@gmx.de
[2]Consulting for Sustainable Development, Keltenring 84, 79199 Kirchzarten, Germany.
E-mail: volkmann@evolve-sustainable-development.de
[3]Institute for Environmental Economics and World Trade (IUW), University of Hanover,
Leibniz Universität Hannover, Königsworther Platz 1, 30167 Hannover, Germany.
E-mail: grote@iuw.uni-hannover.de

SUMMARY

Coffee (*Coffea arabica*) has always been Ethiopia's most important cash crop and largest export commodity. Today, with a production of about 250,000 tones, the country is one of the biggest coffee producers in Africa. Coffee export generates about 60% of the total export revenues of Ethiopia. The montane rainforests of south-western Ethiopia are the worldwide origin of the Arabica coffee gene-pool. Until the present time, these forests comprise naturally regenerating coffee populations as an understorey shrub under the coverage of the forest canopy. The local population living in or adjacent to the forests traditionally utilise the coffee for own consumption and for selling. Some peasants transplant coffee seedlings within in the forest and slash competitor plants. In general, though, forest management intensities are minimal with low labour and almost no cash input. Certification of agricultural products in general and non-timber forest products (NTFPs) in particular is a relatively new and rapidly evolving field in Ethiopia. A small number of companies is accredited according to international standards. Most certification takes place under local peasant cooperatives that remained persistent from the former socialist-inspired era in Ethiopia. Until May 2007, 12 forest coffee-producing cooperatives have been certified organic, Fair Trade and Utz Certified respectively. This presentation aims to illustrate the performance and the limitations of existing certification activities for Ethiopian forest coffee. Based on recent empirical findings gathered in an interdisciplinary German-Ethiopian research project, it is argued that certification faces some structural problems and practical challenges. In practice, certification is rather implemented 'top-down'. Members of the coffee cooperatives were found to neither have knowledge nor understanding about the underlying aim or the procedure of certification. Some associate the visit and the examinations of the certification inspector rather with the cooperatives' concern to increase coffee quality than with the monitoring of social or ecological standards. These issues not only undermine the reliability of certifiers and the standards as such but contribute to discontent of cooperative members with the cooperative system as a whole.

INTRODUCTION: FOREST COFFEE IN SOUTH-WESTERN ETHIOPIA

Ethiopia is not just another coffee growing country. The relationship between Ethiopians and coffee is deep-rooted and multifaceted, and coffee production and consumption are closely intertwined with Ethiopian history, culture and economy. Coffee has been cultivated, picked, processed, traded, and consumed over centuries, and still plays a significant role in the daily life of most Ethiopians and – on the macro level - for the state of Ethiopia as a whole.

The highland forests of Abyssinia (present-day Ethiopia) are the cradle of *Coffea arabica,* which is today's most popular coffee species in the world. The accurate 'birthplace', hence the exact area from which the *Coffea arabica* gene pool started its expansion, and the way people begun to utilise and disseminate coffee has not yet been scientifically proven. Nevertheless, legends exist in Ethiopia that explain the 'discovery' of coffee and set the place of its origin. The most complete and appealing Ethiopian coffee legend is 'Kaldi and the dancing goats'. It locates the place of coffee origin in historical Kaffa region, an area now mostly under Kaffa Zone and Bench Maji Zone in South-western Ethiopia. According to the legend, the etymological origin of coffee and its miscellaneous variants in different tongues - Kaffee (German), Koffie (Dutch), café (French, Portuguese), caffè (Italian), Καφές (Greek), Кофеий (Russian), コーヒー (Japanese), etc. - can be traced back to 'Kaffa', its place of origin (Stellmacher 2007a).[1]

Until today, the montane rainforests in historical Kaffa comprise naturally regenerating coffee populations as an understorey shrub under the coverage of the forest canopy. The local population living in or adjacent to the forests traditionally utilise the coffee for own consumption and for selling (see Stellmacher 2007a). Most peasants transplant coffee seedlings within in the forest and slash competitor plants. In general, though, forest management intensities are minimal with low labour and almost no cash input. Forest coffee grows entirely organic, simply because peasants can not afford pesticides, herbicides or other chemical inputs. The yields fluctuate tremendously over time and space. Research results from nine cooperatives in Kaffa and Bench-Maji Zone show an average forest coffee yield of 187 kg of dried coffee per ha/year (n=61). Yields tend to fluctuate tremendously from year to year with even no yield in some seasons due to unfortunate weather conditions. These figures are extremely low in comparison to more intensively managed garden production or plantation systems. In total, it is estimated that only 6-10 % of the total Ethiopian coffee production is gained from forest production systems (Abebaw & Virchow 2003).

However, parallel to the trend in other African countries, the montane rainforests of Ethiopia including the last wild populations of *Coffea arabica* are threatened by rapid deforestation. Forest are gradually depleted and destroyed due to increased extraction of timber and non-timber forest products, and conversed into agricultural land and new settlements. The processes of forest degradation and loss are complex and difficult to assess as there are few reliable primary data. Environmental scientists began their long-term assessments at a point when the ecosystem had already experienced massive human-devised change. Hence, we do not actually know much about the natural 'original' state of the Ethiopian coffee forests. However, data provides evidence that 60 percent of the closed high forests in south-western Ethiopia were lost between 1975 and 1997 (Reusing 1998). This development is not only alarming because of the direct environmental and socio-economic consequences such as land degradation and scarcity of timber and non-timber forest products but of the irreversible loss of the world-wide unique wild coffee gene pool, leading to high consequential costs also for international coffee breeding and production (Gatzweiler 2007).

RESEARCH OBJECTIVE AND DATA COLLECTION

The purpose of the paper is to provide insights into how certification of Ethiopian forest coffee is implemented in local level practice at the birthplace of *Coffea Arabica* and to discuss

[1]Paradoxically, though, most languages of Ethiopia use the idiom *bunna* to express coffee; merely in the Sidama language is coffee named *tukke* (Anbassa Enterprises n.d.).

the question whether certification of forest coffee can combine economic benefit for the producers and conservation of the unique coffee forest ecosystem and biodiversity.

The paper bases on empirical field research conducted in Ethiopia between 2003 and 2007 in the framework of the interdisciplinary research project "Conservation and use of wild populations of coffea arabica in the montane rainforest of Ethiopia" (see: www.coffee.uni-bonn.de). Primary information on forest coffee certification and marketing activities was collected on place, both in the field and in the offices. In total, nine coffee forest sites in Kaffa Zone and Bench-Maji Zone, South-western Ethiopia, were visited. Semi-structured interviews were carried out with 61 local forest coffee farmers in certified and non-certified coffee cooperatives, with cooperative board members, representatives of Forest Coffee Cooperative Unions and certifying agencies. The ecological conditions of the respective coffee forests were assessed. In Addis Ababa, interviews were held with representatives of about 20 concerned agencies from civil society, state and business.

CERTIFICATION AS A MARKETING TOOL

Upgrading of food products by means of certification is a marketing tool that addresses a growing worldwide demand for healthier and more socially and environmentally-friendly products. It is based on the idea that consumers are motivated to pay price premia for products that meet certain precisely defined and assured standards. Additional motivations for certification and justification for price premiums can be given through the assurance of authenticity, such as a designated origin or a specific genetic trait. Being able to label a product as 'organic', 'FairTrade' or 'Ethiopian Wild Coffee' and to protect the label from fraud is considered a valuable marketing advantage in today's consumer market in general and the coffee market in particular. On the production side, the payment of a price premia can be seen as an incentive to maintain the production basis, in the case of forest coffee to use the coffee forest ecosystem in a more sustainable manner.

Over the last decades, a great many of concepts for product certification have been developed and implemented. In principle, forest coffee from Ethiopia can be certified according to three groups of certification concepts, namely: general certification of agricultural commodities (as 'organic' or 'FairTrade'), specific coffee certification (e.g. 'Utz Certified' formerly known as 'Utz Kapeh'), and certification of forest management (as 'Forest Stewardship Council' or 'Rainforest Alliance'). However, each certification scheme was developed by different stakeholders under different agendas and backgrounds with different geographical foci in response to different ecological and socio-economic concerns.

Each certification concept sets up standards and principles, defined with a set of criteria and indicators (classified in major must/minor must or minimum/progress requirements) that serve as parameter for verification. The FairTrade concept, for example, essentially sets up a set of social standards following several internationally recognized conventions - particularly those of the International Labour Organisation (ILO) - but also involve some basic environmental concerns. FairTrade certification can only be granted to smallholder coffee producers when they organise themselves in peasant organisations (cooperatives/associations) "which are able to contribute to the social and economic development of their members and their communities and are democratically controlled by their members" (Fairtrade Labelling Organizations International, 2003).

Utz Certified is a package of farm-level, brand-level and financial tools to bring social and environmental performance to the mainstream coffee market. It bases on a Code of Conduct developed in 1999 ago by a consortium of Guatemalan grower-exporters together with the

Dutch Ahold Coffee Company. Its social component implies, inter alia, chapters and criteria from ILO conventions and the Universal Declaration of Human Rights. The environmental criteria of the Utz Certified Code of Conduct are based on the Eurepgap code, an initiative of the Euro-Retailer Produce Working Group (Eurep) that agreed on standards and procedures for good agricultural practices (GAP). While FairTrade concentrates all its efforts on smallholder farmers, the Utz Certified code of conduct is more directed towards medium and large-scale coffee plantations. Both, FairTrade and Utz Certified certificates are only issued for one year. After that period the certified body has to be visited and inspected again (Slob and Oldenziel, 2003).

In Ethiopia, certification of agricultural commodities in general and non-timber forest products (NTFPs) in particular is a relatively new phenomenon. First certification of forest forest coffee started in 2002. Activities and structures have continuously evolved ever since, but are still at their infancy stage. In the first years, only one certifier in the whole country was accredited/registered simultaneously by EEC (Europe), NOP (USA), and JAS (Japan) to issue concerning certificates. This monopoly fell in 2006 with other certifiers opened branches in Ethiopia. Some of them started to certify forest coffee for the German market. Simultaneously, Ethiopia increasingly attracted attention of international standard holders, and 'Rainforest Alliance', 'Utz Certified' and 'Forest Stewardship Council' opened own branch offices in Addis Ababa.

CURRENT FOREST COFFEE CERTIFICATION ACTIVITIES IN SW ETHIOPIA

In the following section, we will discuss local level performance of forest coffee certification in Ethiopia exemplified on current certification activities undertaken within the coop-union system in Kaffa Zone and Bench Maji Zone.

Currently, the bulk of Ethiopian forest coffee harvest is sold and traded via conventional coffee market structures in Ethiopia and abroad. Thereby, peasants collect the coffee from the nearby forest tracts, dry it in their homesteads and sell it to local merchants, the *sebsabies.* They resell the dried coffee to the wholesalers, the *akrabies*, who facilitate the processing (de-hulling) and take the beans to Addis Ababa where they are inspected by the state-run 'Coffee and Tea Quality Control and Liquoring Unit' at the national processing and liquoring centre. Coffee suitable for export is send to the national coffee auction, where exporters with a corresponding license can bid on it. The exporters sell the coffee to international importers, who then sell it on to the roasters in the destination countries (GTZ 2006, Petit 2007).

The conventional coffee value chain in Ethiopia involves a large number of intermediaries and is largely state-controlled. Exporters must be Ethiopian nationals and are not allowed to cup taste the coffee before buying it at the auction. Licenses are required for every function in the market chain. They are, however, difficult to obtain and can be arbitrarily withdrawn or not renewed. The auction put emphasis on keeping consignments from different coffee growing regions separate (Limu, Sidamo, Yirgacheffe etc.). However, a separation of forest coffee against other coffees is not foreseen in this market chain. Forest coffee from South-western Ethiopia is rather blended with semi-forest and garden coffee, and sold at the national auction for the most part under the classification Jimma 5 (Petit 2007).

In the last years, however, an alternative coffee value chain – the so-called 'coop-union system' – developed parallel to the conventional market chain. It is essentially based upon local Agricultural Service Cooperatives (ASC) established in the 1970s by the then military government in the framework of the enactment of the "Proclamation to Provide for the

Nationalization of Rural Land No. 71/1975". By the End of the 1990s, as many as 4,000 ASCs with no less than 4.5 million members were established in Ethiopia.

However, the cooperatives widely served as a vehicle for the government's mass collectivisation policy and were to a large extent characterised by corruption and mismanagement. In 1990, with the *Derg*'s reign drawing to a close, all ASCs were formally dismantled and numerous cooperative offices and shops looted and destroyed. Particularly in remote rural areas, though, the change was not sudden and definite like that. The organisational and infrastructural skeleton of many cooperatives continued to exist, however, often in a status of bankruptcy (McCarthy 2001, Stellmacher 2007a, Stellmacher 2007b).

Since the End of the 1990s, the new government of the "Ethiopian People's Revolutionary Democratic Front" facilitated the restructuring of the cooperative system and the formation of cooperative umbrella associations, the coffee cooperative unions. Unions buy the coffee from their member cooperatives and take over the processing and transport to Addis Ababa. Since 2001 the unions are legally allowed to by-pass the coffee auction and directly negotiate with and sell to international exporters, although their coffee must also be inspected at the national processing and liquoring centre (GTZ 2006, Petit 2007). In 2007, six Coffee Unions were operative in Ethiopia. Out of them, two are specialized on forest coffee. The Forest Coffee Unions have been established only recently and are the countries' smallest Coffee Unions in terms of total production. Both are geographically located in former Kaffa region in the "Southern Nations and Nationalities Peoples' Regional State", Ethiopia's most ethnically heterogeneous province.

In the last years, concerned stakeholders highlighted the coop-union system as a chance to create an alternative 'shorter' coffee value chain in order to promote efficiency, transparency, traceability, and ecological and social responsibility as well as to facilitate product upgrading by certification and quality improvement in the Ethiopian coffee sector. A lot of money was invested by NGOs and international donors in physical and institutional infrastructure and training. This is particularly remarkable as cooperatives until recently had a bad reputation with donors due to their political use during the *Derg* regime (Dempsey, 2006).

In this context, the two Ethiopian Forest Coffee Unions started to engage in certification. The unit of certification were the local cooperatives. They were visited by accredited certification inspectors from Addis Ababa who checked the fulfilment of the respective standards. The resulting information was filled in certification forms and then sent for evaluation to the certifiers' headquarters in Europe. The headquarters issued the Master Certificates for the union. In May 2007, 12 forest coffee-producing cooperatives were certified in both unions, according to organic, FairTrade and Utz Certified standards respectively. However, certification has considerable built-in costs - directly (e.g. certification fees, costs for annual re-evaluation) and indirectly (e.g. investment in training, infrastructure and marketing) - that can not be afforded by cooperatives nor by the unions (Shanley et al 2005). Hence, the whole process was facilitated and subsidised from 'outside' i.e. by international donors, NGOs, certifiers, and concerned coffee industry and trade.

STRUCTURAL PROBLEMS AND PRACTICAL CHALLENGES

However, with particular focus on the connection between economic benefits and conservation of the forest ecosystem and biodiversity, scepticism seems justified. The first concern relates to the compatibility of economy and ecology. The actors involved into certification of Ethiopian forest coffee cooperatives do this on the basis of different goals and interests. The leading objective of the Forest Coffee Unions as main local actors is an

economic one, namely to "to increase farmer/member income through the selling and exporting of their products." (KFCFCU, 2008). Accordingly, they foremost try to produce larger quantities of forest coffee and to obtain higher prices. This is, however, likely to have negative impacts on the forest. Forest coffee is a part of the natural ecosystem and its yields can not be expanded beyond limited thresholds without degrading its natural habitat. Higher prices provide an incentive to produce more forest coffee by increased forest management at the expense of the forest ecosystem and biodiversity. Forest management not only disturbs the forest structure, but also modifies the original plant species composition of the forest (Gole 2003, Schmitt 2006). The objectives of other stakeholder, donors and NGOs in particular, are more related to the concept of environmental sustainability and the conservation of the forest ecosystem and biodiversity. However, in practice, the question needs clarification whether the general undertaking is more concerned on forest coffee or the coffee forest. The certification standards have to be chosen accordingly.

The second problem concerns the structural and organisational capacity of the coop-union system. In contrast to the cooperative system in the Latin Americas, the Ethiopian cooperatives are not 'grown' bottom-up but have been state-enforced by a communistic-inspired authoritarian regime within a rigid command economy. Despite considerable steps towards market liberalisation since the 1990s, this is a path dependent burden. Until the present time, the coffee cooperatives and unions are de facto para-statal entities. Positions are assigned by intransparent considerations and/or on the basis of political or ethnical affiliation, rather than by pure qualification.

However, the system is fed by international money. To be sarcastic, one may say that the coop-union system is more dependent on and part of the political and development arena in Ethiopia than of the market and its needs and constraints. This might not only imply negative impacts on efficiency and transparency but also creates a situation that may be untenable in the long term (see also Shanley et al., 2005). In addition, the coop-union system in South-western Ethiopia tends to be weakened by a strong ethnical heterogeneity in this area, exaggerated by decades-long state enforced immigration. The 61 interviewed cooperative members, for instance, were affiliated to 10 ethically diverse groups, with different linguistic, religious and cultural background. The cooperatives' internal structure consists of 'core' decision makers and less integrated 'peripheral' members. Lower respected ethnicities such as the Mandjah people in Kaffa Zone are proportionately underrepresented in the cooperatives. This setting brings about local level tensions and low levels of trust.

The third concern is lack of know-how and expertise on modern business management in general and certification in particular. Relevant knowledge is extremely unequally spread along the value chain, being highest among the actors in Addis Ababa and lowest among those in the remote rural areas. Understanding of goals and concepts of certification and its standards ends latest at cooperative committee board's level. The overall majority of coffee-producing coop members does actually not know whether their cooperative is certified and what the respective standards are. When being asked what certification of a cooperative means, none of the interviewed 61 cooperative members could give a somewhat reasonable answer. 25 answered frankly with "I don't know". The others associated certification with ideas such as "certification permits the cooperative to buy and sell coffee" (#28) or "certification means that the coop gets loan from government to give it to its members" (#10). The visit of the certification inspector and his/her examinations was rather associated with the cooperatives' concern to increase coffee quality than with the monitoring of ecological or social standards. These findings reveal basic deficiencies in knowledge management and participation. However, one has to be aware that schooling is a relatively new phenomenon in rural Ethiopia. The educational level of the rural population in rural Ethiopia is still

considerably low and broad levels of the adult population - also among the so-called 'decision makers' – are illiterate. 45% of the interviewed cooperative members did never go to school; 53% obtained only few years of primary education (n = 56).

The forth challenge concerns the economy of scale. As mentioned beforehand, the forest coffee production system implies considerable low yields and high fluctuations from year to year and from one forest plot to another. The coffee cooperatives in South-western Ethiopia consist of around 100 to 300 – in one case of more than 500 – members. The average production per individual member was assessed with 596 kg of dry forest coffee per year. After processing and the quality check in Addis Ababa, the final end product, hence the amount of coffee that can be actually exported, is much lower. In the last years, each of the two Forest Coffee Unions from South-western Ethiopia sent overseas an annual maximum of 3 or 4 container of certified coffee with high annual fluctuations. Given these relative low amounts, the costs with certification, investment and advertisement per kg of coffee are disproportionately high. Accordingly, both Forest Coffee Unions face problems to reach the economy of scale and to provide clients with guaranteed amounts of certified forest coffee over a period of several years.

CONCLUSIONS

'Wild' forest coffee from Ethiopia is a fashionable product with a number of particularly positive features. It is worldwide unique with a specific flavour and an authentic and positive image. Since 2002, a growing number of stakeholders engage in upgrading of forest coffee by means of certification within the cooperative-unions system. Para-statal coffee cooperatives and unions, certifiers, coffee industry and trade, as well as environmentally and socially-concerned donors and NGOs 'discovered' forest coffee certification as a sphere of activity – though, under diverging - and party conflicting – agendas and interests, and sometimes stiff competition.

This paper, however, illustrates that current modes and activities to certify forest coffee in Ethiopia face practical performance problems and structural dilemmas. FairTrade and Utz Certified certification standards have been developed for and most widely applied in intensive agricultural production systems like coffee plantations in the Americas. These standards can not simply be 'transferred' to the Ethiopian coffee forests, describing an entirely different ecological, institutional and socio-economic situation with different contextual needs.

Certification needs for capable organisational and institutional arrangements, market structures and expertise. First of all, Ethiopia's coffee market in general and the coop-unions system in particular is highly state controlled and regulated. Secondly, on the local level in the remote forest areas, the certification process is not actively promoted and understood by those who are to be certified, and difficult to assess and control by certification inspectors. Forest coffee is produced in badly accessible large forest tracts by smallholders. Certification of centrally organised coffee plantations is indeed much easier and cheaper. Thirdly, the overall institutional framework lacks independent control devices. Ethiopia has no national certification agency. The country has no or very limited experience with a free press/media as a 'watchdog' against personal or organisational abuse of certification. In this context, the booming worldwide speciality coffee market together with a specific market advantages of the Ethiopian forest coffee (the image of 'wild', 'natural', 'unique', 'exotic' or 'good for the poor'), may provide incentives for some actors to (mis-)use certification for their own benefit disregarding the (negative) impact on the coffee forest ecosystem and biodiversity.

The marketing of forest coffee does not contribute per se to forest conservation in the strict sense. A fundamental structural dilemma concerns the fact that certification price premia paid to forest coffee producers provide an incentive to degrade the forest ecosystem. In view of long term better prices, producers tend to increase production quantities by means of intensified management of the forest coffee including the surrounding forest. Slashing of competing undergrowth several times a year, cutting of unnecessary trees and stand thinning and/or transplanting of coffee trees are the most prevailing activities. All contribute to the degradation and loss of the natural forest ecosystem and biodiversity.

One recommendation to overcome the illustrated bottlenecks addresses the market chain. After several years of experience with the coop-union system, stakeholders may consider alternative chains for the marketing and certification of forest coffee. An innovative and promising approach may be the organisation of forest coffee producers in Public Limited Companies (PLCs) in the context of Participatory Forest Management projects and their direct linkage to small exporters. Another recommendation concerns the certification standards. To better balance the contradiction between economic benefits and biodiversity conservation, the development of a consistent and distinctive standard for forest coffee from Ethiopia is recommended, perhaps in the framework of international forest certification standards already existing. The implementation of a new certification and marketing concept is, of course, not an easy task. To be effective and efficient, public-private partnerships in cooperation with local stakeholders are of fundamental importance. Nevertheless, certification can only be one tool in this regard. Beyond, functional and product upgrading through institutional restructuring and coffee quality enhancement are required.

REFERENCES

Abebaw D., Virchow D. (2003): The Microeconomics of Household Collection of Wild Coffee in Ethiopia: Some Policy Implications for In-situ Conservation of Coffea arabica Genetic Diversity. Presented at Workshop Economy Analysis of Policies for Biodiversity Conservation. Venice

Anbassa Enterprises (n.d.): Coffee in Ethiopia. www.telecom.net.et/~ambassa/page6.html

Dempsey J. (2006): A Case Study of Institution Building & Value Chain Strengthening to Link Ethiopian Cooperative Coffee Producers to International Markets, ACDI7VOCA. Addis Ababa

Fairtrade Labelling Organizations International (2003): Fairtrade Standards for Coffee. Version January 2003. Bonn

Gatzweiler F.W. (2007): Deforestation of Ethiopia's Afromontane rainforests. Reasons for Concern. ZEF Policy Brief No. 7; Center for Development Research. Bonn

Gole T.W. (2003): Vegetation of the Yayu forest in SW Ethiopia: impacts of human use and implications for in-situ conservation of wild Coffea arabica L. populations. Cuvillier Verlag. Göttingen

GTZ (2006): Value Chains for the Conservation of Biological Diversity for Food and Agriculture. Potantoes in the Andes, Ethiopian Coffee, Argan Oil from Marocco and Grasscutters in West Africa, GTZ. Division Agriculture, Fisheries and Food. Sector Project Food, People & Biodiversity. Eschborn

KFCFCU (2008): Kaffa Forest Coffee Farmers Cooperative Union. http://www. kafacoffeeunion. com/union.htm

McCarthy S. (2001): The History of Agricultural Cooperative Development in Ethiopia. In *Cooperative Business Today*, pp. 5. Voca-Ethiopia. Addis Ababa

Petit N. (2007): Ethiopia's Coffee Sector: A Bitter of Better Future? Journal of Agrarian Change 7 (2): Blackwell Publishing Ltd. 225-63

Reusing M. (1998): Monitoring of Natural High Forests in Ethiopia, Federal Ministry of Agriculture (MoA), Gesellschaft für Technische Zusammenarbeit (GTZ). Addis Ababa

Schmitt C.B. (2006): Montane rainforest with wild Coffea arabica in the Bonga region (SW Ethiopia): plant diversity, wild coffee management and implications for conservation. Cuvillier Verlag. Göttingen

Shanley P., Pierce A., Laird S. (2005): Beyond Timber: Certification of Nontimber Forest Products, Forest Trends, Washington D.C.

Slob B., Oldenziel J. (2003): Coffee & Codes. Overview of Codes of Conduct and Ethical Trade Initiatives in the Coffee Sector. SOMO. Amsterdam

Stellmacher T. (2007a): Governing the Ethiopian Coffee Forests: A Local Level Institutional Analysis in Kaffa and Bale Mountains. Shaker Publishing. Aachen

Stellmacher T. (2007b): The historical development of local forest governance in Ethiopia. From the imperial times to the military regime of the Derg. Afrika Spektrum 3 (42): GIGA - Institute for African Affairs. Hamburg

Commercialization Analysis of Organic and Traditional Coffee Farming in Londrina – PR, Brazil

R. MIGLIORANZA, J. MASSARUTTI, E. MIGLIORANZA

Center of Applied Social Sciences, Dep. of Business and Administration, Universidade Estadual de Londrina, Rodovia Celso Garcia Cid PR 445, Km 380, Londrina, Brazil

SUMMARY

Organic and traditional coffee farmers have different market appeal. In this study, it was possible to evaluate the market agents usually involved in commercializing organic and traditional coffee in Londrina. The influence of market agents in prices was also quantified. Organic coffee growers showed to sell more coffee using traders than traditional coffee farmers. Market speculators are especially important for producers with no access to market (due to: lack of information; green coffee selling; high cost of transportation; or small quantities), being the second most used commercialization channel. Even though the price paid for organic coffee in Londrina is higher than traditional coffee, the standards are less than gourmet coffee selling prices.

INTRODUCTION

In agribusiness, most of the products commercialized are in the stage of maturity, considering its product life cycle. With maturity, comes the concept of commodity. Commodity means that the products have little differentiation and there is a large quantity of sellers. Producers concern with maintaining profits, among other factors, show a new perspective to mass markets with growing niche market, specialties market and growth in segmentation. An organic coffee farm has as objective of not only attaining niche market, but also gaining sustainability by conscious management.

To reach a market, there are different institutions related to the process that transforms a coffee grain into a coffee drink. Globalization imposes the choice of professional business conduct and mindset to coffee producers, with the ability to make good decisions over commercialization methods. Considering this, this study aims on understanding the current channels used by organic and traditional coffee producers to sell and the influence in coffee sales price.

MATERIALS AND METHODS

The object of this study is coffee farmers from Londrina, Paraná. This region is 590 meters over sea level, has rainy winters and inclination to uncontrolled fermentation. The study happened between March and June of 2006, and considered a sample from the 400 coffee producers, 58 being organic coffee growers in the second year of conversion.

The authors used a structured questionnaire to obtain data, regarding commercialization channels, post-harvest handling and sales prices obtained. Social and demographical characteristics, cost and financing methods was also collected. The sample was non-probabilistic stratified, were the population of coffee growers was divided in two groups:

organic and traditional. The criteria used for the selection was accessibility. The error margin considered for this study was 5%.

LITERATURE REVIEW

For the comprehension of where coffee growers stand in economy, it is important to acknowledge the agents that complement the supply chain. Coffee market has different agents, related to a phase or function in coffee production. Cramer (1991) suggests there are three basic types of segmentation in agribusiness: functional, institutional and structural. For this study, institutional approach was chosen to classify and determine channels used.

Institutional approach examines the activities of organizations or people involved in agribusiness supply chain. Besides the producer and the capital inputs producers there are other institutions that may be involved, such as facilitators. Facilitators provide general data of the industry sector, or offer physical trading facilities, such as stock exchange, share market or bourse. In the stock exchange brokers represent sellers, and it is a place to negotiate future or present sales contracts. Contracts determine the quantity and price of sales (Zanotti, 2005).

Coffee producers may choose to sell directly to consumer, coffee industry, and export. There are also different types of intermediates, classified as the following:

1) Market dealers (wholesale or retail) – wholesale is the chain between the production stage and retail. The main purpose of wholesaling is to have inventory, pack in smaller portions, prepare lots to shipment and organize transportation. Retailing consists of sale for direct consumption by the purchaser.
2) Agents (brokers and sales representative) – can be differentiated from market dealers or speculators, because they do not obtain good's ownership. They help transfer the title of ownership from seller to buyer. Brokers are responsible for engaging sellers and buyers. Sales value normally base brokers' fee. Sales representative have more authority than brokers do, any product consigned to them is sold for the best price found. They receive usually when the sales occur, retain their commission and give the final amount to the seller.
3) Speculators – assume ownership and stock commodities, assuming risk of loss due to unfavorable prices fluctuation. They are also called scalpers or day traders.
4) Cooperatives – are associations of people united voluntarily through a jointly owned and democratically controlled organization, which can offer advantage to producers gaining scale in negotiation with input sellers and buyers, and stock the production.

COMMERCIALIZATION CHARACTERISTICS OF ORGANIC AND TRADITIONAL COFFEE FARMING IN LONDRINA

Coffee growers from Londrina, as showed bellow, sell their production most of the times (42%) in stock market by brokers. That guarantees a high value for the coffee, eliminating intermediate speculators. The organic coffee growers sell, in 55% of the times, in stock market, as well as 37% of the traditional coffee growers. Market speculators are especially important for producers with no access to market (due to: lack of information; green coffee selling; high cost of transportation; or small quantities), being the second most used commercialization channel.

Table 1. Coffee buyers in Londrina.

Buyer	Organic coffee	Traditional coffee	Total
Cooperative	-	26%	18%
Broker	55%	37%	42%
Speculator	27%	33%	32%
Export	-	4%	3%
Coffee Industry	9%	-	3%
Industry and Broker	9%	-	3%

A problem to organic coffee growers is the control of the internal market by one buyer (monopoly). Even though the price paid for organic coffee in Londrina is higher than traditional coffee, the standards are less than gourmet coffee selling prices and the payments are scheduled for about 60 days after the sale. The strategy used by organic coffee growers with higher payments is to find a buyer before converting the farm.

Analyzing the commercialization strategy, 70% of the traditional coffee producers do not wait for best market price, but sell the production as soon as they need money, as 55% of the organic coffee growers. Organic coffee producers tend to speculate coffee prices more often than traditional coffee growers do.

Table 2. Sales strategy in Londrina.

Selling strategy	Organic coffee	Traditional coffee	Total
After the harvest	18%	15%	16%
As soon as necessary	55%	70%	66%
Speculate	27%	15%	19%

Applying χ^2 test, to verify if the commercialization channel is related to prices paid to producers, it was possible to identify that the correlation between the two variables was 94.28%, in other words, that coffee sales prices are directly influenced by the commercialization channel chosen by the producer. Table 3 demonstrates coffee sales prices for 60 kg in both of the most often used channels (dollar value from 31/07/2006).

Table 3. Coffee Prices for 60 kg by commercialization channel in Londrina.

Sales price	Organic coffee	Traditional coffee
Broker	US$ 121.95	US$ 93.14
Speculator	US$ 70.89	US$ 56.78

Conclusion

Organic coffee growers in Londrina sell to brokers and speculate more than traditional coffee producers speculate. The choice of a commercialization channel by coffee producers also showed direct affects on the prices obtained and the influence is not only in one or in the other production method. Coffee producers that sell through brokers tend to have better gains than producers who sell to speculators. This study indicates that in Londrina organic coffee farmers use more lucrative commercialization channels than traditional coffee growers do.

REFERENCE

Cramer G.L., Jensen C.W. Agricultural Economics and Agribusiness. 5ª ed. Nova Yorque: Wiley, 1991.

Zanotti P.D., Sousa Neto E.M. O Café no Espírito Santo, no Brasil e no Mundo. Vitória, 2005. In: <http://enetovix,tripod.com/cafe-final.pdf>. Access in: May, 2006.

Implementation of an Institutional Coffee Quality Assurace Strategy at the Farm Level in the Quindío Region of Colombia

O. JARAMILLO, J. DUQUE

The National Federation of Coffee Growers of Colombia – The Quindío Coffee Growers Committee. Armenia-Quindío- Colombia

SUMMARY

This work has been developed in the Quindío Region, an important coffee producer area located in the Central coffee zone of Colombia, with 43,083 hectares devoted to coffee plantations, distributed in 6,820 coffee farms, most of them owned by small growers. Mountain ranges allow coffee to be grown between 3700 and 6500 feet (1200-2000 meters) above sea level. Annual precipitations averages of rainforest patterns from 100 to 200 inches (1800-3200 millimeters) with well-defined rainy season. The Quindío coffee area has more than 45 different soil profiles. Coffee production is about 60% of the gross domestic product for the region. The National Federation of Coffee Growers, acting through The Quindío Coffee Growers Committee has been focusing on improving important aspects for coffee marketing and consumer´s trust including Quality, Safety, and Health to increasing the coffee value. Quality Coffee indicators in 2005, obtained through Almacafé, the logistic operator in charge of monitoring quality at the national level, showed that while the national average of rejected lots was 10,6%, the average for Quindio was 18,5%. In order to track cup quality from the farms, and to involve directly the coffee growers in the quality control process, a quality coffee assurance strategy was implemented by the Coffee Growers Committee through its Extension Service. Since February 2005, 2195 coffee farms (35% of the region total) were surveyed by taking 5,138 one kg samples of parchment coffee until June 2008, applying a standard check list on the farm, with emphasis in harvesting, milling, drying, storage and transportation. At the end of the day, we hope that Quindío Coffee Growers and their coffees will be recognized as an important quality source for exporters, importers, traders, roasters and retailers who are willing to recognize an added value for coffee originated from this region.

INTRODUCTION

Farmers have been producing coffee in Colombia for over 200 years, but most of them, if not all, hardly taste their coffee. As a first step, regional lab facilities focused on flavor identification and improvement can help to strength this aspect of coffee production. The labs bring coffee tasting and evaluation tools into the coffee growing regions to help growers develop their craft, compete for honors, be rewarded for their attention to detail, and to reap immeasurable rewards derived from pride and workmanship. The labs also serve as a reception area for international coffee buyers.

At the coffee farm level, an extension package on how to harvest and handle coffees has been formulated and disseminated to organized farmers through their local office of the National Coffee Growers Federation (FNC) in Quindío. The entire chain focuses on production, giving value to domestic consumption and creating awareness of the high quality of Quindío Coffees. All of these will end up improving the quality of life of the producers and in turn will

satisfy the consumer with higher quality coffee products, finding quality coffee directly through cupping.

For the specialty coffees movement, it is the cup taste that matters, rather than the appearance and the size of the coffee bean. Growers therefore need to learn to appreciate the factors that affect the quality of their coffee. The goal of our educational program rooted from the farms is to build up the capacity of coffee growers to understand quality as it relationship to cup quality.

The Quindio Region

Quindío Region, important is an coffee producer area located in the Central coffee zone of Colombia (Figure 1), with 43,083 hectares devoted to coffee plantations, distributed in 6820 coffee farms, most of them owned by small growers. Mountain ranges allow coffee to be grown between 3700 and 6500 feet (1200-2000 meters) above sea level. Annual precipitations average rainforest patterns from 90 to 200 inches (1500-3200 millimeters) with well-defined rainy seasons. Quindío coffee area has more than 45 different soil profiles. Coffee production is responsible for 60% of the gross domestic product for the Region

"El Agrado" cupping lab facilities

"El Agrado" is a 43 hectare coffee farm owned by the Quindio Coffee Growers Committee, with 35 Ha planted with coffee of the Caturra, Colombia and Castillo varieties. It has several production systems as fully sun exposure, associated with plantain and under the shade of forest trees. The committee decided to build an analysis and cupping facility, which opened on february 26, 2005. Its two purposes were to cup the coffee produced in all of the Quindio farms, and to educate all the people involved in the production chain: owners, administrators, operators and pickers, in addition to the personnel working in the lab, technical assistants and social workers of the Extension Service. The facility has the necessary equipment to develop this strategy, besides the equipment for coffee preparation as espresso and clover machine, among common way to brew a delicious cup of coffee. Although this kind of infrastructure is usually present only in urban areas, "El Agrado" is located in the middle of the country side. It has 4 permanent cup testers, certified by the SCAA Start Cupper, with three of them recognized as Q-Graders.

The specific purpose of this project is to integrate a strong quality strategy from the farm, improving their harvest and milling facilities, avoiding waste and dirty surfaces, getting a healthy and clean product, illustrating the advantages of good farming practices and the different taste of coffees, and illustrating how these best practices relate to end up with the best cup for the market. In order to track cup quality from the farms, and to involve directly the coffee growers in the quality control process, a quality coffee assurance strategy was implemented by the Coffee Growers Committee through its Extension Service.

MATERIALS AND METHODS

General strategy

For every farm involved, an *in-situ* sensory evaluation was carried out together by the Extension Service technician and the farm owner, and a first set of observations were shared with the farmer. One kilogram coffee sample was taken, labeled and shipped to the cupping lab facilities located at "El Agrado", a commercial coffee farm that belongs to the Quindío

Committee. Following the ISO9001 protocol, the samples were received and cup tested by a 4 person cupping panel certified by the Specialty Coffee Association of America. Average cup test qualifications and additional information were returned by internet to the Extension Service agronomists, who finally used the result to feedback the farmer in person (Figure 2), with suggestions on how to improve their coffee and remove defects to correct quality problems or keep going producing a clean cup looking for a consistency process and result. Simultaneously, a detailed database was fed and linked to the National Coffee Information Database (SICA), and made accessible using the FoxPro environment.

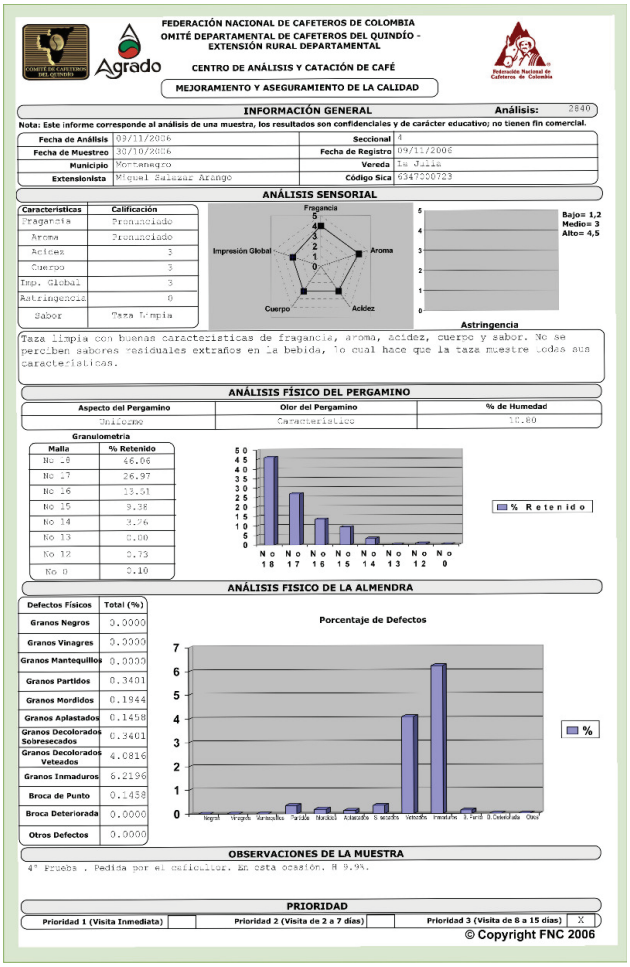


Figure 2. Flow diagram of the procedure implemented to assure better coffee quality at the farm level in the Quindío region of Colombia

1. Farm visit by extensionist.
2. Check list with the farm owner
3. Sampling of 1 kg of dry parchment coffee
4. *In situ* sensory analysis: parchment odor and color
5. First set of recommendations are given
6. Shipment of samples to the cupping center
7. Sensory and physical analysis
8. A result is produced
9. Diagnostic is sent via internet to the extensionist with copy to the farmer
10. The extensionist visits the farm again to search for the problem or congratulate the farmer for the clean cup
11. The farmer's copy of the diagnostic is delivered

12. The recommendation and the result are reviewed at the lab
13. The Extension Service receives feedback in group activities

Software development

A computer program named CACC (standing for Coffee Analysis and Cupping Center) was developed in a team work fashion with the inputs from an agronomist, a food technologist and a systems engineer, making continuous improvements for 2 years to reach the current version 1.3. The development was done in a FoxPro environment to organize more than 200,000 alphanumeric data entries, with the purpose of serving as a tool for administrating. The output results are given in pdf format without modification permissions (Figure 3). The program has currently restricted use inside the lab facilities, with remote updates from the Committee offices in Armenia.

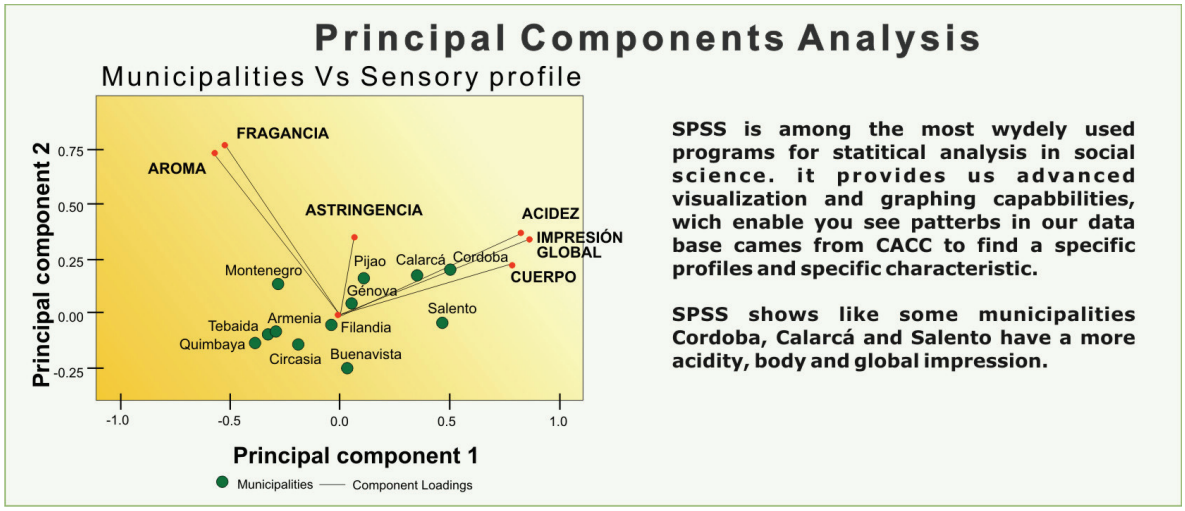


Figure 3. Facsimile of the pdf report delivered to the Extension Service technician in charge of visiting the farms and carry on the recommendations from the lab to the farmer, who also receives a hardcopy of the file.

Teaching program

Free teaching programs were implemented in order to develop a model for technology transfer based on the education of the personnel involved in the coffee production chain. At the beginning of the Project the education activities were oriented towards those people in charge of directing the lab facility. Later, the activities were expanded to members of the Extension Service, including agronomists, technologists, farm administrators and social workers, to develop finally activities according to the several coffee farmer types. By 2005 a single workshop in sensory and physical analysis was carried out. Today, 3 workshops are offered for every teaching period, only with the coffee farmers, administrators, managers and pickers. Every course has a time intensity of 6 hours.

RESULTS AND DISCUSSION

After three years, more than 11.100 advising visits farms on quality assurance were Accomplished, 3215 people around coffee chain have been trained in 210 workshops of physical and sensory analysis carried out, 2195 coffee farms (35% of the region total) were surveyed by taking 5,138 one kg samples of parchment coffee.

One of the most important benefits has been the construction of an organized data base that allows the application of statistical analyses using the variable "coffee profiles" that later provide competitiveness indexes over other coffee regions. Additionally, a robust software was built to follow-up on cup quality in the farm, as well as on the technicians' activities at the local level.

Summary of cup quality changes in coffee plantations between 2005 and 2008

Compare Clean and Faulty Cups

Item	2005	2007
# Farms	1144	1272
# Samples	1426	1468
# Clean cups	242	664
# faulty cups	1014	775

Source: Coffee Analisys and Cupping Center

National vs. Quindío Average of Rejected Lots

Year	National %	Quindío State %
2005	10.6	18.5
2006	15.3	18.9
2007	10.9	6
2008	10	4,6

Source: Almacafé Logistic Operator of FNC

CONCLUSIONS

Besides providing a detailed indicator for monitoring coffee quality in the Quindío region, which is being implemented in other coffee growing regions, our strategy has guaranteed both a higher quality and a safer product for the consumer.

At the end of the day, we hope that Quindío Coffee Growers and their coffees will be recognized as an important quality source for exporters, importers, traders, roasters and retailers who are willing to recognize an added value for coffee originated from this region.

Coffee Seed Conservation in Penta Packing

L. SANTANNA[1], G. GUIMARÃES[1], M. REIS[2]

[1]Daterra Atividades Rurais Ltda, Av. Faria Pereira, 20, Patrocínio, Minas Gerais, Brasil.
E-mail: daterracoffee.ptc@daterracoffee.com.br
[2]Topex Agro Serviços Ltda, R. Osório Afonso, 784, Patrocínio, Minas Gerais, Brasil.
E-mail: topex@oi.com.br

SUMMARY

The experiment evaluated the germination capacity of coffee seed in different situation of storage in "Penta Packing" process with the purpose of seedling one year after harvesting. The objective is to anticipate the planting, in brazilian "cerrado", starting until October/November when the rainy season begins, by anticipating the seedling.

INTRODUCTION

Usually, in Brazil, the right maturation period for the seeds preparation is May/June.

This is when most of the Brazilian producers start to prepare the seeds to be planted. Due to the climate conditions, the seedlings will be ready to go to the fields in January or February.

In the Penta Packing method, the coffee is also picked in May and then packed through the Penta System. The coffee is kept in this packing until March/April when it is planted at the nursery. The seedlings will then be ready to go to the field in October/November where the weather promotes much more vitality to the plant than when it goes to the field in January or February for instance.

MATERIALS AND METHODS

The seed used were produced in Boa Vista Farm, field BV14, variety Yellow Catuaí, lineage 62. The harvest was on the 3rd of June 2005, and dried on the shed until 11,6% humidity and then packed in aluminum plastic bags under different concentration of: Vacuum and introduced Nitrogen. The bags were stored in warehouse, with reduced lighting, 60% air humidity and average temperature of 26 °C.

Before sowing the seed was immerse in running water during 72 hours with the purpose of stimulate the germination. The seeds were treated with 3 g/kg de Pencycuron before sowing.

The Figure 1 shows the "Penta Packing".

Figure 1.

TREATMENTS

Table 1. Description of treatments (date of packing 7-7-2005).

Treatment	Description		Humidity (%)	Weight (kg)
	Vacuum (%)	Nitrogen (%)		
A	1	0	11,6	6,0
B	20	0	11,6	6,3
C	45	25	11,6	6,0
D	90	55	11,6	6,4
T	-	-	11,6	6,0

GERMINATION TESTS

Tests for germination potential was made in the Seed Analysis Laboratory of Universidade Federal de Lavras, and the results obtain were 89% germination viability.

After the lab test the seed were submit to germination viability under field conditions in two different moments: before packing and when seedling. That made possible measure the percentage of germination was lost during storage. Table 2 show the percentage of germination in different treatments:

Table 2. Germination test 7-7-2005.

Treat.	Initial Date	Final Date	Germination (%)			
			Rep. 1	Rep. 2	Rep. 3	Average
A	21-3-2006	1-7-2006	83,34	86,70	85,56	85,20
B	21-3-2006	1-7-2006	81,27	81,63	91,26	84,72
C	21-3-2006	1-7-2006	82,50	87,42	83,13	84,35
D	21-3-2006	1-7-2006	85,23	84,70	80,39	83,44
Control	7-7-2005	10-9-2005	81,12	92,93	83,00	85,68

SUMMARY AND CONCLUSIONS

The seed were storage for 291 days and did not decrease germination rates.

It made clear that the treatments with addition of Nitrogen and with higher amounts of air extraction out of the package did not show significant difference on the germination level. Treatment A show that with only 1% of vacuum, is sufficient to the seed be preserved in satisfactory condition for sowing, respecting the environment conditions of light control, temperature average of 26ºC and humidity of 60%.

The following conclusion can be drawn

1. The germination potential has not significantly changed in any treatment.
2. Under the operational and cost point of view treatment A is the most recommended.
3. The humidity of 11,6% for storage in the Penta Packing was satisfactory.
4. It is viable to storage coffee seed with the purpose of early planting on the rainy season.
5. The objective of anticipate seedling was reached.
6. It is necessary to anticipate all activity before sowing, in the nursery, so that the rainy season does not compromise efficiency.
7. The ideal period to prepare the soil for sowing is September/October. Then is necessary to protect with plastic so the rain does not let it muddy. Fill up the little plastic bags in March and sow in April.
8. Make sure that the preparation of the field, that will be planted with early, is ready early as well.

REFERENCES

Braccini A., Scapim C., Braccini M., De Basto C. e Vidigal P. 1999. Incidência de microorganismos em sementes de café robusta durante o armazenamento. Bragantia. 58(2): 305-315.

Calvin Ch., Bible B. e Yoon H. 2002. Germination and Emergence. IN: Handbook of Plant and Crop Physiology. Ed: Mohammad Pessaraki. Second Edition. p. 57-115.

Dias M. e Barrios A. 1993. Conservação de sementes de café (*Coffea arabica* L.) em diferentes embalagens. Bragantia. 15 (2): 197-202.

Levy F., Fasuoli I. e Gallo P. 1989. Estudos de germinação de sementes de coffea canephora armazenadas em diferentes condições. 15° Congresso Brasileiro de Pesquisas Cafeeiras. Ministério do Desenvolvimento da Industria e do Comercio. Instituto Brasileiro do Café. Diretoria de Produto. 159-160.

Lima W., Díaz D., Alvarenga E., Reis M. e Cecon P. 2001. Preconditioning of Coffea (*Coffea arabica* L.) seeds effects on germination, vigour and storability. Seed Sci. and Technol. 29: 549-555.

Oliveira D., Da Silva, De Miranda W. 2001. Grau de umidade e temperatura na conservação de sementes de café. Bragantia. 60(1): 53-64.

Oliveira D., Da Silva, De Miranda W. 2001. Grau de umidade e temperatura na conservação de sementes de café. Bragantia. 60(1): 53-64.

Morphological Aspects of *Coffea arabica* L. cv. *Mundo Novo* Seed Development†

P. CRISAFULLI[1], F. SILIZIO[2], J.C. MISTRO[3], F. MICALI[1], A. PALLAVICINI[1], E. DENTAN[2], G. GRAZIOSI[1]

[1]University of Trieste, Dept. of Biology, Genetics Lab, Trieste, Italy
[2]illycaffè S.p.A., Via Flavia 110, Trieste, Italy
[3]Centro de Cafè "Alcide Carvalho", IAC, Campinas, SP, Brazil

†In memory of Dr. Ernesto Illy (July 18, 1925 - February 3, 2008)

SUMMARY

Studies investigating coffee seed development are very scarce in scientific literature. The present work is an attempt to fill the knowledge gap on this neglected topic by means of different experimental techniques and resorting to recent findings on *Coffea arabica* botanical aspects. Seed sampling was carried out in the period from September 2006 to June 2007 on plants belonging to cv. *Mundo Novo* of the IAC in Campinas (SP, Brazil). About 60 samples for each development phase were collected every two weeks until the 34th week after anthesis, AA), from blooming up to full fruit ripening. Collected material was rapidly fixed and sent to Trieste (Italy) for downstream analysis. Samples have been studied by means of standard microscopy techniques (optical, SEM and TEM) as well as resorting to hystochemical techniques performed under strict controlled protocols and adapted to the tissue to be examined. During the initial phases, the young seed is mainly constituted by perisperm, a tissue of maternal origin; in the first month, the zygote is quiescent and the endosperm slowly starts to grow. The exponential growth period occurs between 8th and 16th week AA. Seed reaches his definitive size on 18th week, when both silver skin and parchment are completely formed and the endosperm tissue is still soft. Starting from 20th week, the endosperm cell walls are thick and the cellular content shows several protein and lipid clusters.

INTRODUCTION

Seed morphology has been an interesting subject for some authors since early forties. Houk (1938) described the *Coffea* ovule like unitegmic and tenuinucellate, and noticed the presence of a developed obturator and an extensive perisperm; but in Mendes' work (1941) we can find a first detailed description of the developing endosperm up to the maturation of the bean: during the first embryonic stages, he observed the endosperm nuclear type, almost one month after the flower fertilisation and the zygote in quiescence for the first 70 days. Later on, Dedecca (1957) and Roth & Lindorf (1971) gave their essential contribute about the anatomy and morphology of fruit and seed. Dentan (1985) studied new protocols for the observation of the ultrastructure of the bean, using also the first histochemical dyes in the coffee seed tissues. Besides, de Castro et al. (2002) correlated seed development with histodifferentiation, organogenesis, germinability, desiccation tolerance and cell cycle events.

This study is a contribution to previous works on the development of the *Coffea arabica* seed through different microscope techniques, suggesting new specific protocols. The aim of researchers was to produce good images in order to develop this subject.

MATERIALS AND METHODS

The material was collected from September 2006 to June 2007 from coffee plants cv. *Mundo Novo* (IAC, Campinas, SP, Brazil). Approximately 60 samples were collected every two weeks for each developmental stage, from flowering to complete fruit ripening, for a total of 34 weeks after anthesis (AA). Samples were immediately fixed and sent to Trieste laboratory (Italy).

Size analysis was performed on a subset of 30 random chosen cherries per each developmental stage. A digital gauge was used to measure length, major axis, minor axis of the cherry and of the bean.

After the standard dehydration in increasing scale of ethanol (50, 70, 90, 95 and 100%) samples were embedded in Technovit 7100 resin (Kulzer®, Germany)from the anthesis to the 20th week AA and then cut by a rotary microtome (6 µm sections). A low-temperature cryostat was used to slice samples older than 22 weeks AA, due to the hardness of the material (10-12 µm sections). The samples were stained with Toluidine blue-O 0.05% in acetate buffer (pH 4.4) for metachromatic stain, Periodic Acid Schiff and UV-Schiff, DAPI, Nile Blue 1%, then observed under microscopic light and under UV light (filters H3, D, Leica DMRXE). Some samples underwent SEM and TEM observation (protocol not reported).

RESULTS AND DISCUSSION

We identified three main developmental stages from the cherries size analyses (Fig. 1): a first slow growing stage, until 4 weeks AA; a second rapid expansion stage, from 6 to 14 weeks AA; a last ripening stage, from 16 weeks AA onwards.

The second stage is closely related to the climatic conditions of the period and particularly to the increase in rain frequency (data from CIIAGRO not reported), especially between November and January.

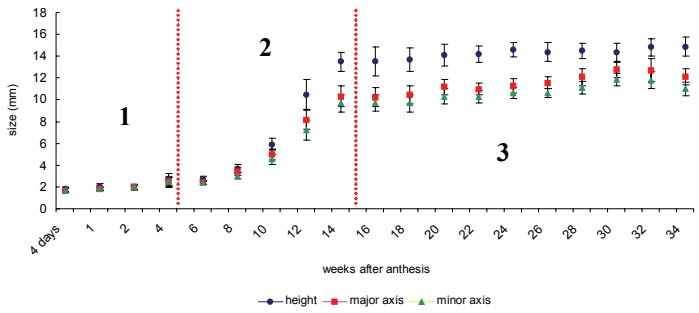


Figure 1. Cherries size analyses (mm): 1. slow growing stage; 2. rapid expansion stage; 3. ripening stage.

During the first *slow growing stage*, the longitudinal section of the ovary showed the typical structure of the coffee drupe with two locules with an anatropous ovule each (Figure 2b). The ovule consisted in a funiculus, one single integument deriving from the maternal tissue and a small area occupied by an undeveloped embryo sac (Figure 2a). An obturator – which in *Coffea* has a funicular derivation – linked the pollen tube to the micropile. The ovary thickness was 0,4-0,5 mm until the first two weeks of development, the seed size was 0,7 x 0,4 mm while the embryo sac had 100 x 70 µm and he accumulated inside many starch grains.

After approximately one month (4-6 weeks AA), no relevant seed growth was observed.

During the second *rapid expansion stage* the mesocarp grew to a thickness of 0,6 mm. At 8 weeks AA, the seed was 2 mm and the fertilized embryo sac started to develop the endosperm. At 100 x magnification some endosperm nuclei could be seen in the embryo sac (Figure 2c; size: 150 x 100 µm by the TBO dye).

At 10 weeks AA the seed was 3 mm. The embryo sac was larger (0,2 mm) and was composed by approximately 20 endosperm cells. At 12 weeks AA the seed was 10 mm. The embryo was about 1 mm, growing within the endosperm. At this stage, two different tissue layers were noticed outside the embryo: the first layer was the new endosperm; the second layer was the perisperm from which would originate the silver skin, stained with the blue TBO dye (Figure 2d). During the 14 weeks AA the embryo was surrounded by the mucilage (Figure 2e), the silver skin with some living and nucleated cells was visible (Figure 2f), as well as the parchment deriving from the fruit endocarp. The seed had almost reached its definitive size.

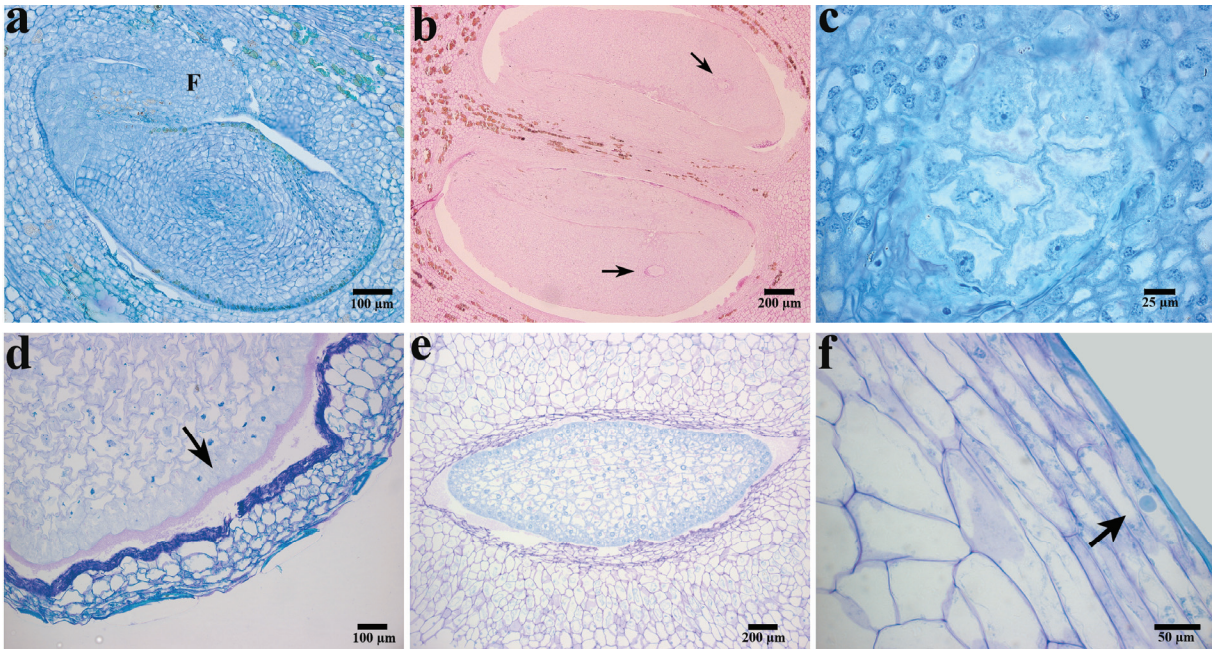


Figure 2. a) coffee anatropous ovule and his funiculus (F) (longitudinal section, TBO, 20x); b) two ovules in their locules with partial embryo sac (arrows) (longit. sect., PAS, 5x); c) 8 weeks AA: some cells of embryo sac (transv. sect., TBO, 100x); d) 12 weeks AA: endosperm in growth (arrow), mucilage (violet), developing silver skin (blue) (TBO, 20x); e) embryo (transv. sect., TBO, 10x); f) nucleated cell of silver skin (arrow, TBO, 63x).

At the *ripening stage* the growth in size was modest, while chemical and structural changes were observed. The 16th week was characterised by the almost complete formation of the silver skin. Many endosperm cells were in mitotic activity; two weeks after this stage the parchment was completely formed and showed the typical thick-walled fusiform fibres, that in TBO dye presented a smart blue colour and a strong polarisation at optical microscope.

From the 20th week AA onwards the recently formed endosperm had thin-walled cells (3 µm) and the bean was moderately hard; the vacuolar content of the endosperm cells was rich in protein bodies (Figure 3d,e) and sacchariferous structures, differentially stained by different dyes. The embryo was completely developed and its cells were rich in protein bodies. During the 26th week the vacuolar system was rich in proteins and cell walls were thick (4-6 µm)

(Figure 3a,b). The formation of some cell wall areas with irregular thickness ('membrana perladas', Roth and Lindorf, 1971 or 'nodular cell wall', Dentan, 1980) in the endosperm cell walls near the embryo were observed. The seed and embryo development was completed at the 30th week AA (Figure 3c). The cell walls reached their definitive thickness (from 6 to 10 µm). The small embryo could be described like spatulate type (Martin, 1946), erect with hypcotyle and two heart-shaped cotyledons. Finally, at the last stage (34 weeks, Figure 3f) cells were somewhat empty and most part of the cell walls were 'nodular'.

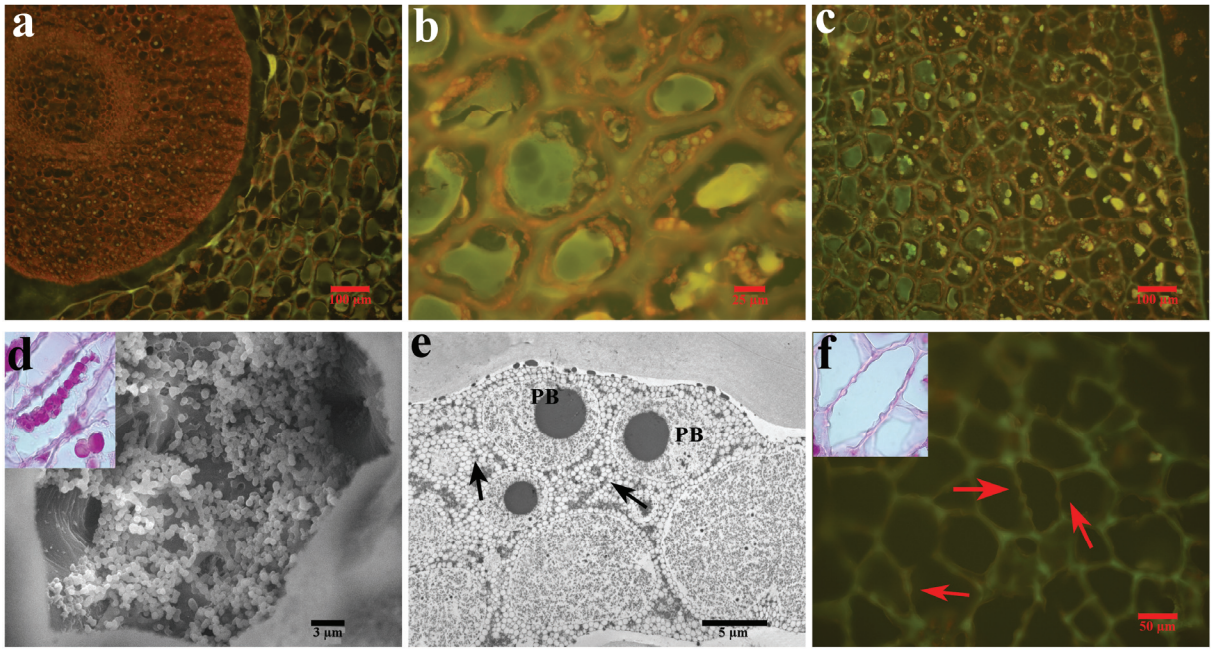


Figure 3. a) 26 weeks AA: hipocotyle of embryo (red) and endosperm cells (UV-Schiff, 20x); b) 28 w. AA: full endosperm cells, lipids in red, protein bodies (PB) in yellow (UV-Schiff, 63x); c) 30 w. AA: endosperm cells in the mature seed (UV-Schiff, 20x); d) PB (SEM, 9800x) and in fuchsia (PAS, 100x); e) PB with electron-dense core, and lipids (arrows) (TEM, 4000x); f) endosperm cells almost empties and 'nodular' cell walls (arrows) (green in UV-Schiff, 40x; pink in PAS, 100x).

CONCLUSIONS

After the fertilization, the embryo sac compresses the internal integument cells and the perisperm occupies entirely the locules of the ovary. The twentieth week sees the complete formation of the silver skin, but it would be interesting to know the chemical changes it entails and the meaning of the presence of initial perisperm.

Furthermore, the presence of the endosperm was observed after one month. Nevertheless, it is still difficult to determine when the first zygotic division took place, because the first embryo cells could be seen only after two months. Finally, our technique for analysing the seeds at the ripening stage (20 weeks and more) should be improved in order to have a better picture of the developing tissues. Beside the morphological aspects the ability to dissect coffee seed tissues during development can be of pivotal importance for a biomolecular analysis of genes expression.

REFERENCES

Clark G., 1981. Staining procedures. Williams & Wilkins (eds.)

de Castro R.D., Estanislau W.T., de Mesquita P.R. and Hilhorst H.W.M., 2002. A functional model of coffee (*Coffea arabica*) seed development. Salamanca 2002 International Workshop on seeds.

Dedecca D.M., 1957. Anatomia e desenvolvimento ontogenético de *Coffea arabica* L. variedade Typica Cramer. Bragantia 16:315-366.

Dentan E., 1977. Structure fine du grain de café vert. ASIC, 8^{e} colloque, Abidjan, 1977

Dentan E., 1985. The microscopic structure of the coffee bean. In: Clifford M.N. and Wilson K.C. (eds.). Coffee botany, biochemistry and production of beans and beverage. The AVI Publishing Company. Westport, Connecticut, pp. 284-304.

Houk W.G., 1938. Endosperm and Perisperm of Coffee with Notes on the Morphology of the Ovule and Seed Development. American Journal of Botany, Vol. 25, No. 1 (Jan., 1938) , pp. 56-61.

Johri B.M., 1970. Symposium on comparative embryology of angiosperms. Bullettin of the Indian National Sciences Academy 41: 1-385.

Martin A.C., 1946. The comparative internal morphology of seeds. The American Midland Naturalist 36: 513-660.

Mendes A.J.T., 1941. Cytological Observations in *Coffea*. VI. Embryo and Endosperm Development in *Coffea arabica* L. American Journal of Botany, Vol. 28, No. 9 (Nov. 1941) , pp. 784-789.

Roth I. and Lindorf H., 1971a,b. Anatomia y desarrollo del fruto y de la semilla del café. Acta Botanica Venezuelica, 6: 197-238.

Wintgens J.N., 2004. Coffee: growing, processing, sustainable production. Wiley - VCH

Coffee Quality Assessment: the Case of Two Kenyan Cultivars, Ruiru 11 and Sl 28

C.O. OMONDI

Coffee Research Foundation (CRF), P O Box 4, 00232 Ruiru, Kenya.
E-mail: ddr@crf.co.ke

SUMMARY

Coffee quality is an important determinant of coffee prices in the international market. The prominence given to Kenya coffee world-wide is derived from the fine quality coffee it supplies to the world market. Quality parameters of two Kenya coffee cultivars Ruiru 11 and SL 28 believed to be similar in their major quality attributes were assessed to determine factors that influence the final beverage quality. The parameters assessed were bean quality, beverage quality and overall class. The study revealed that the growing environment had a strong effect on the expression of quality parameters exhibited by Ruiru 11 and SL 28. The study also evaluated the consistency of cup tasters to assess similar samples and arrive at similar results. It was revealed that there was significant difference among cup-tasters. The study recommends proper management of the growing environment to obtain the desired quality attributes. Where preferences differ in favour of certain coffees from distinct origins, brands could be developed to meet the preferred taste of different consumers.

INTRODUCTION

Kenya produces coffee that is classified within the Colombian milds known for balanced acidity and body with pleasant distinctive aroma. The reputable quality of Kenyan coffee is a result of favourable climatic conditions, good agronomic practices, rigorous harvesting and post-harvest practices, appropriate processing and storage conditions and cultivation of varieties with proven genetic constitution. Kenya has also positioned itself to take advantage of the emerging specialty market, which though small, pays even higher premiums. The challenge that remains is whether the supply of the reputable quality coffee in the quantities that is desired by the traditional and emerging markets can be sustained. Production has already suffered a major reduction with only 50% of the crop being realized from over 130,000 metric tons that was recorded in 1989. Studies have further shown that the decline in production have simultaneously been followed by decline in the proportion of premium classes (1-3) which have fallen from about 40% in 1988/89 to about 10% currently.

It is with this background that this study has focused on assessing the contribution of critical parameters determining coffee quality as a strategy to meet the demand for fine quality coffee in the world market. The objective of the study was to assess the contribution of the following parameters on overall expression of quality; (1) varietal effects with main focus on two arabica coffee cultivars (SL 28 and Ruiru 11), (2) effect of growth environment on expression of quality, (3) consistency of cuppers in assessing quality and (4) opportunities for value addition.

MATERIALS AND METHODS

The experiment was established as a 3-replicate randomized complete block design with 55 lines of Ruiru 11 at Kisii and 51 lines at Kitale. Two treatments of SL 28 in sprayed and non-sprayed plots were included in the trial. The recommended spray programme for control of coffee berry disease (CBD) and coffee leaf rust (CLR) were used in the SL 28 sprayed plot (Anon 2006). Samples were obtained from Kisii and Kitale and transported to CRF laboratories for assessment of a range of quality parameters namely; single berry weight, %AA, % AB, %AA+AB, % PB, %T, %C, %TT, %outturn. Evaluation of cup quality was done by SOCFINAF Co Ltd, Kenya Planters Cooperative Union (KPCU) and CRF quality laboratory for Kisii samples. Samples from Kitale were submitted to Kenya Coffee Traders Association (KCTA) and two testers at Coffee Board of Kenya (CBK) for similar assessment. A two-stage analysis was performed with the data from Kisii and Kitale as follows; (a) multivariate hierarchical cluster analysis based on the Euclidian Distance Matrix (b) analysis of variance to evaluate consistency of cuppers.

RESULTS AND DISCUSSION

The results shown in Figure 1 indicate that, at Kisii, Ruiru 11 lines 91 and 92 clustered with sprayed and un-sprayed SL 28 at a Euclidian distance of 8% and 18% respectively. The shorter the Euclidian distance, the more closely related are the treatments. Spraying SL 28 against CBD and CLR has an effect on quality that appears not only to differentiate the unsprayed SL 28 treatment from the sprayed treatment but also to increase similarity with the two Ruiru 11 lines whose quality characteristics are closest to SL 28. The remaining Ruiru 11 lines formed clusters with the two treatments of SL 28 variety at a distance of 25%. At Kitale, a tight cluster was formed between sprayed SL 28 and Ruiru 11 lines 80, 137, 73 and 113 (Fig 2). Although these Ruiru 11 lines were also assessed at Kisii, they were divergent from SL 28 at a Euclidian distance of 25%. This is an indication that the growing environment has a strong effect on the expression of quality parameters exhibited by Ruiru 11 and SL 28. It can also be observed that SL 28 formed a cluster with 27 genotypes of Ruiru 11 at a Euclidian distance of 5%. This indicates that there was higher similarity in quality of Ruiru 11 genotypes with SL 28 at Kitale than at Kisii where the shortest Euclidian distance with only two Ruiru 11 genotypes was 8%. Ruiru 11 genotypes with quality characteristics similar to SL 28 in a given location can change in another environment. This calls for proper management of the growing environment to obtain the desired quality.

The consistency of cup tasters to evaluate similar samples and arrive at similar results was also tested for samples from Kisii and Kitale. The analysis of variance results indicate that there was significant difference among cuppers ($P< 0.05$) (Table 1). In other words, when cuppers were presented with the same samples for assessment, the results varied from one cupper to the other. This is a common phenomenon due to the subjectivity of the test. To achieve uniformity of cup quality assessment, analytical parameters based on the compounds responsible for certain quality attributes require to be considered. Another consideration was the comparison between the treatment samples representing the different genotypes of Ruiru 11 and the traditional SL 28 variety which revealed no significant difference. This was an indication that the differences revealed by cluster analysis were not significant to be detected by the cup tasters and therefore Ruiru 11 genotypes were to a large extent similar to SL 28. The overall mean class for all the samples was 4.04 at Kisii and 4.26 at Kitale. SL 28 samples scored classes 4.67 and 4.33 for sprayed and non-sprayed treatments respectively at Kisii. At Kitale, the score was class 3.78 for sprayed treatment.

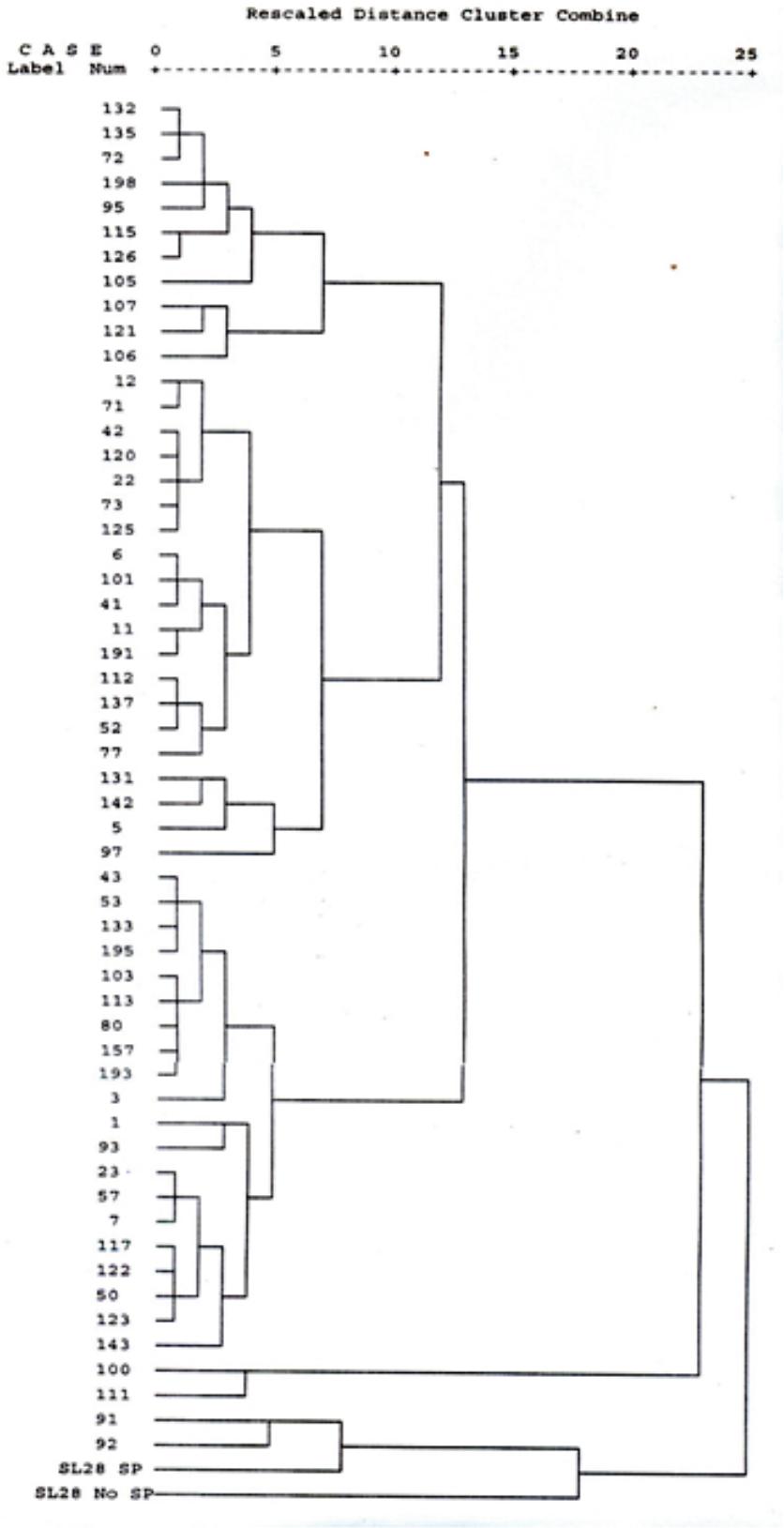


Figure 1. Dendrogram depicting the the genetic distance between Ruiru 11 genotypes and SL 28 check variety on the basis of coffee quality parameters at Kisii.

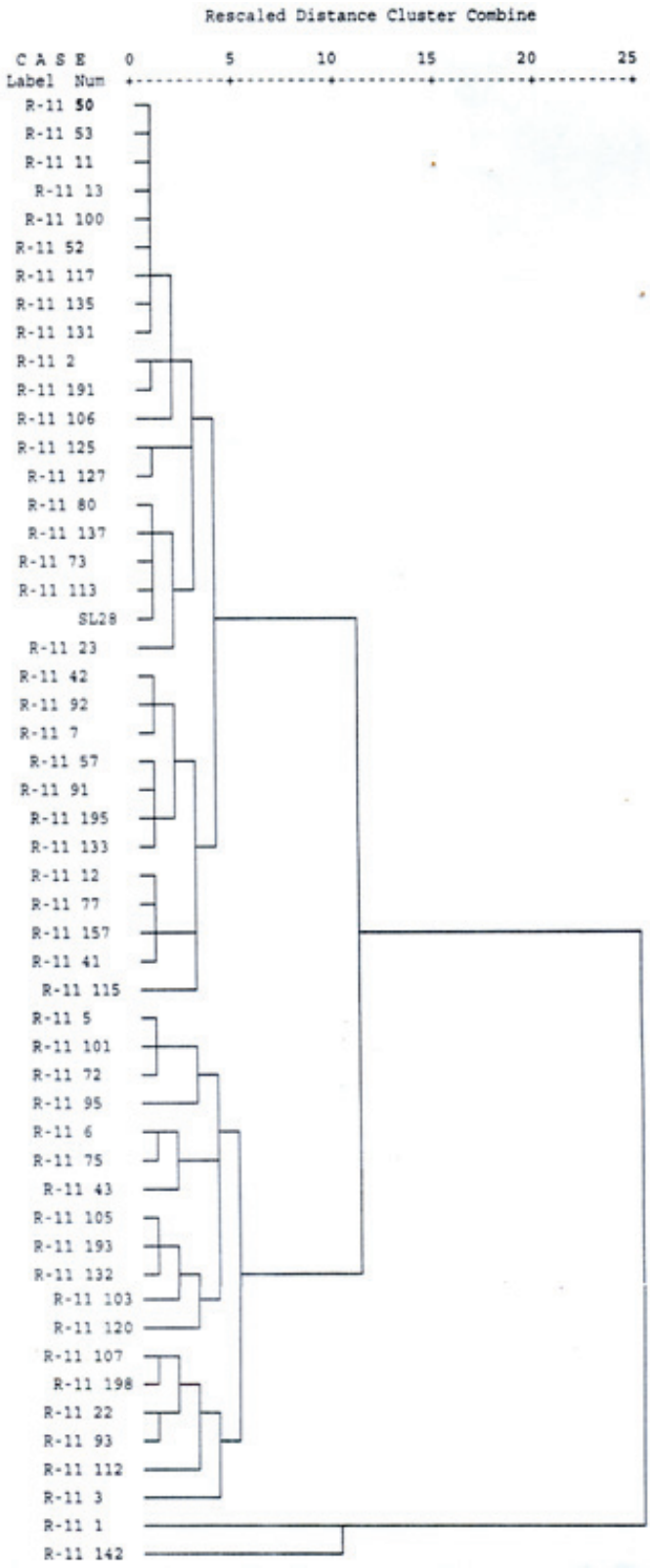


Figura 2. Dendrogram depicting the the genetic distance between Ruiru 11 genotypes and SL 28 check variety on the basis of coffee quality parameters at Kitale.

As growing environments differentiate coffee quality into different brands, there is potential that the brands can be used as the basis for value addition. Value addition at production level is more tenable than local roasting which may appear attractive but have many impediments (Gitonga, 2005). Such impediments include tariff and non-tariff barriers of entry to key consumer markets, segmentation and dispersion in these markets, high investment costs and reduced shelf life for processed coffee. The scope for this route in value addition may be limited to partnerships with the dominant multinationals, and local roasting that is targeted at the domestic markets, hotels and air travel.

Table 1. Analysis of Variance results for overall class of Ruiru 11 genotypes and traditional variety SL 28 at Kisii and Kitale.

Kisii				Kitale		
Source of variation	d.f	Mean Square	Prob	d.f	Mean Square	Prob
Cuppers	2	2.54	**	2	10.39	**
Genotypes	56	0.23	NS	38	0.16	NS
Error	112	0.22		76	0.18	
Total	170	-		116	-	

*** Significant at P < 0.05.*
NS Not significant.

ACKNOWLEDGEMENT

This paper is published with the permission of the Director of Research, Coffee Research Foundation. Assistance of CRF staff in Plant Breeding and Chemistry Sections, and cupping panelists is highly appreciated.

REFERENCES

Anon, 2006. Coffee Production Recommendations (Handbook). L W Njeru (Ed). 3rd Edition pp 31.

Gitonga K.T.K., 2005. Value addition for coffee in Kenya. A Report prepared for the Ministry of Agriculture, Kenya.

Recent Advances in Genetic and Biological Solutions to Reducing Biotic Stress Factors to Coffee Production in Latin America

A.G. BUSTAMANTE

Colombian National Coffee Research Center (CENICAFE), Chinchiná, Caldas, Colombia

SUMMARY

For almost 200 years coffee cultivation thrived in Latin America as a consequence of propitious weather and soil conditions, and in the absence of limiting diseases and pests. The adoption of intensive coffee cultivation practices brought along a greater productivity but also the alteration of the relative importance of the existing diseases, and together with the arrival and dispersal of the Coffee Berry Borer (CBB) and the Coffee Leaf Rust, they meant a change in the landscape of coffee production in several aspects: onset of new disorders after crop migration towards less optimum environments; mandatory implementation of disease and pest management practices in areas where the economic impact of biotic stresses was significant, which ecologically coincided with the most favorable land for cultivation; human health and pest resistance problems originated by the new experiences with chemical control and its inadequate use; reduced competitiveness in the international free market due to costs of chemicals and their application; limited range of control products permitted by global regulations; and finally, modified practices to meet an increased demand for healthier goods from society. Under this scenario, genetic and biological control methods become major players for preserving the sanitary quality of the plantations in the continent. Although more efficient and easier to implement by the farmers, genetic control in coffee is very limited due to the narrow diversity among cultivated varieties and the lack of significant resistance in the germplasm collections. For more than three decades rust resistance has been successful through the use of major genes present in the Timor Hybrid, a genotype promissory also for its incompatibility reactions against the Coffee Berry Disease. Nematode and Stem Canker resistance have been introduced into elite varieties where disease incidence is important. Sources for insect resistance inside the *Coffea* genus, especially against CBB, have been harder to find and more uncertain to use in conventional breeding. On the other side, application of biological control in coffee has a more recent history and development. This has required not only the search and evaluation of antagonists and parasites, but also formulation research, implementation of industrial infrastructure, and government regulations that as a whole result in reliable recommendations for producers. It also implies a change of mind in the coffee farmers to adopt Integrated Pest and Disease Management strategies. Currently, biological solutions are implemented for the control of soil born diseases (damping-off, root rots, stem canker) with antagonist fungi, and for CBB using entomopathogens, parasitoids and entomonematodes. The current progress depends on the finding of new resistance sources and on the development of genetic maps and gene discovery achievements that allow the manipulation of qualitative traits in the incorporation and selection of new varieties with multiple resistance. Biological control must compete in efficiency with traditional chemical control, exploiting the agents' diversity towards the generation of improved isolates, genetic mixtures with better field performance and a greater involvement of the private sector in their formulation and production. For coffee growers in Latin America, these advances will supply the agronomic tools for a sustainable and certified production, with an economic advantage that guarantees them an adequate income and their continuity in the business.

INTRODUCTION

America was one of the final destinations of the *Coffea* genus after a very large journey it underwent around the world by the hand of men. This trip took almost 1200 years, beginning with shepherds moving seeds from the Ethiopian plateau to the lands of Yemen, and concluding with the arrival of a French ship bringing plantlets sent by the king of France to be cultivated in the Caribbean colonies. In between, coffee had a profound impact on producer and consumer societies along the way, with implications for both its own biology and its further developments as a commodity, especially in the new world.

The historical highlights of the geographical dispersal of coffee must include the smuggling of seeds by Baba Budan in the 1600 from Yemen to India, the shifting of plants by Nicolas Witzen from Moka to Batavia (Java), and the transplanting of trees from Malabar to Java in 1699 by Dutchmen. In 1714 the Jardin des Plantes de Paris got a healthy Typica coffee plant from the botanical garden in Amsterdam, and a little later (1718) the French were introducing coffee plants into the Bourbon island. The American continent comes into the story when Gabriel Mathieu de Clieu introduced an offspring from the Paris plants into Martinique in 1723, followed by Dutch and French introductions into Guiana. From there, Francisco de Mallo Palheta brought seeds and plants to Brazil, and others anonymously took care of dispersing materials to Central and South America (Chalarca, 1998; Pendergrast, 1999). On the other side, the biological highlights in coffee distribution were defined by the few individuals that represented the species in their way to the new world, that for *C. arabica* implied in the first place an accelerated process of reduction of its genetic diversity, as seeds from an autogamous plant were easily obtained and sown, and the bias for agronomically attractive individuals determined the movement of a limited set of gene combinations. This historical dispersal had also an effect from the pathological point of view, when an unintended quarantine occurred determined by two facts: the lack of a major limiting diseases along the places where coffee plantations were exploited out of Africa, including the Indian subcontinent and the southeastern lands of Asia, and the selection of healthy plants that this movement brought along, as only good seeds and plantlets probably moved easier and prospered in the colonization efforts.

In the Americas, coffee plants faced a variety of environments defined by the differences in latitude, from Mexico to Brazil, the diversity of altitudes due to the Andes and the mountain systems in Central America, and the rain distribution established by the Inter Tropical Convergence Zzone. On top of that, cultivation practices determined as well the nature of microclimate conditions that range from traditional plantations with low tree densities (2,500 or less tress per Ha), densely shadowed by trees and with fertilization based on decomposed organic material, to technified areas with high densities (up to 10,000 trees per Ha), heavy fertilization with synthesized chemicals and full sun exposure. In those places coffee plants met with a variety of uncommon pathogens (either as genera, species or as populations) which had been developing for over thousands of years without this new host, and along a large set of biological control agents

LIMITING COFFEE STRESSES IN AMERICA

The interactions between host, pathogen and environment have been represented in plant pathology as the disease triangle, where the simultaneous occurrence of these three factors is an absolute requirement for disease onset. When biological control agents are included, a new triangle forms, in which the host is now the pathogen, but both organisms share the environmental conditions. For the first 200 years in America, the interactions with coffee-pathogens were weak and can be considered as local an non-limiting. In addition, plant

pathology was still far from becoming an independent science since many principles required to understand disease were in development including the absence of spontaneous generation of microorganisms (Pasteur, 1861), the role of microbes in plant disease (de Bary, 1861), and the rules for disease diagnosis (Koch, 1887). The first report of a new disease in the Americas came from the Colombian university professor Nicolas Saenz who in 1875 sent leaf samples to England suspecting the presence of the coffee leaf rust; they turned out to contain the fungus *Mycena citricolor*, the causing agent of the "American Leaf Spot", as it was called to differentiated it from the rust (Castaño, 1978). For this new set of pathogens, disease occurrence was highly dependent on the environmental conditions and on the ecological equilibrium provided by the populations of biological controllers. They had patchy distributions, did not develop at epidemic levels and economically did not turn out in significant losses, at a point where no control measures were routinely taken.

The arrival of pests and pathogens from Africa meant a radical turn of events for coffee growers and scientists in the Americas. Three of the most serious biological limiting factors to coffee made their entry to the continent through Brazil, partially because of the predominant role of this country as world coffee producer, and also due to the volume of trade in its ports and the lack of scientific tools to protect plant health efficiently and with certainty. In 1900, the coffee leaf miner (*Leucoptera coffeicola*), a butterfly whose larvae feeds and develops inside coffee leaves, was reported. In 1913 it was the turn for the coffee Berry borer (*Hypothenemus hampei*), a beetle that also in the larval stage feeds only from the coffee endosperm. Finally the Coffee Leaf Rust (*Hemileia vastatrix*) a fungus with a life cycle that takes place exclusively in the coffee leaves. The interactions between coffee and these organisms had been developing for over hundreds of thousands of years, to a point of adaptation where the parasites can not live without coffee plants. Under natural conditions, the genetic diversity of the host and the parasites, together with the environment and the populations of biological control agents play a stabilizing role in the relationship, and disease never reaches threatening levels for coffee populations. Under man-made conditions, however, and especially in America, the interaction takes different proportions. Low genetic diversity of both the host and the pathogen due to genetic bottlenecks, interacting in an environment that resembles the best possible conditions found at their place of origin, results in a geographically wide (global) dispersal, that has no breaks in time and therefore conducts to an epidemic that affects a significant part of the plantation and generates significant losses for the growers.

This new situation creates also a conflict between the interests of growers and those from scientists. The former see all the problems as local and desire an immediate response, the later want to focus on global problems and look for long term solutions. Balancing this act, enter into consideration the costs of the problems vs. the solutions, the compatibility of the solution with the market requirements, the feasibility to transfer a solution to a growers community that on average has not finished the primary school, and the effect of the measures taken on the environment, in particular the immediate impact on the people living in the plantations, that in many cases houses the family of the grower. As a consequence pest management strategies had to be implemented. The two main objectives of disease control are the reduction of the inoculum potential, especially at the beginning of the epidemics, and the lengthening of the life cycle of the pathogen, which will take longer to cause damage to initiate a next generation of infection. For a long time the option immediately available was the use of chemicals, that brought along the unpleasant consequence of disturbing at a larger scale the biological equilibrium reached over the years in coffee plantations and in some cases worsening the losses caused by problems that were mostly local and non-limiting. Currently, pest management is considered as an integrated operation with a rational participation of chemical control, and including cultural control activities that limit the conditions for disease

onset, strengthening biological control measures either by augmenting the controllers populations or introducing new controllers in the environment, and finally, making use of the plants natural ability to defend themselves, applying genetic control.

The adoption of Integrated Pest Management practices in Latin America has been very slow slow, in part because of the necessity to break with the legacy of the green revolution, that was very practical but did not consider the sustainability of the practices after using large amounts of pesticides and chemical fertilizers. The IPM adoption has also been affected by the scarce understanding of diseases, which for the last 30 years was mainly focused on the coffee leaf rust. However, significant achievements have been made in the application of genetic control for the global problems, taking into account the long cycles of selection required to obtain a competitive new coffee variety using conventional breeding. More recently, biological control measures have been replacing mandatory chemical applications complying with a world market every day more severe in the protection of consumers health and the environment. Under this scenario, genetic and biological control methods become major players for preserving the sanitary quality of the plantations in the continent. These advances will be reviewed next.

GENETIC CONTROL

Perhaps the easiest way to adopt a disease control strategy is by getting seeds of an improved resistant variety. For the farmers it means no change in the daily practices applied in the plantation and more importantly, it does not imply additional costs in the activities of every year. However, genetic control in coffee is very limited due to the narrow diversity among cultivated varieties and the lack of significant resistance in germplasm collections. For *C. arabica* in Latin America, the sources of resistant characteristics have been outstanding genotypes of the Bourbon variety, the Timor Hybrid, Ethiopian germplasm, and other species of the *Coffea* genus. Breeders work has been focused on the two major limiting pests, the coffee rust and the coffee leaf miner, since they are the ones with known resistance sources.

Coffee leaf rust

Development of coffee rust resistant varieties has a long history that goes back to the 60s, when accessions of the so-called Timor Hybrid were distributed by the Coffee Leaf Rust International Center (CIFC), located at Oeiras in Portugal. For more than three decades the introduced rust resistance has been successful through the use of major genes present in the Hybrid. Three accessions named 1343, 832 and 2252 were used in Central America, Colombia and Brazil to produce a wide set of varieties, some based in F1 progenies that could be distributed very quickly to the farmers, some produced after advancing the generations to F4 up to F6 that takes a longer time but resemble the adaptation, architectures and agronomical characteristics of the accepted susceptible genotype, and other based on composed varieties that make use of the genetic diversity to face the problem of resistance break up.

After the release of the first bred varieties, adoption has been slow in part because chemical control with copper based fungicides does not exerts a selection pressure that promotes the onsent of fungicide resistance rust genotypes, and also because when available, coffee plantations moved up in the mountains to heights over 1500 m above sea level, where the coffee rust does not cause economic damage. Complete resistance, the one that impedes completely the reproduction of the pathogen, was enjoyed for over 15 years, but eventually new pathogen genotypes were able to overcome this resistance and susceptibility was observed among the bred varieties, with some lines being completely attacked, but with many

others showing an unexpected background of incomplete resistance (Alvarado, 2004), one that allows the reproduction of the pathogen but at a lower rate and in lesser amounts (reduced uredospore density in sorus). Although the progenies derived from the Timor Hybrid have showed susceptibility, the resistance of the original accessions is still standing, even under heavy rust pressure. This has led to strategies to stack rust resistance genes from related progenies in order to select for acceptable levels of incomplete resistance. The purpose now is to get durable resistance and to understand the mechanisms of pathogen variation and plant interaction under the expectations of new aggressive physiological races of the *H. vastatrix*, a situation that based on known cases in other crops (for instance, ug99 Wheat Stem Rust in Africa) must be taken into consideration (Zambolin et al., 2005).

Coffee leaf miner

For the oldest African pest present in America, resistance was identified in the diploid species *Coffea racemosa* (Carvalho and Monaco, 1968). By interspecific crosses, resistance was transferred from *C. racemosa* into *C. arabica* at the Campinas Agronomical Institute in Brazil. Several levels of resistance have been identified, starting with materials where there is no attack of *L. coffeicola* larvae, up to reduced formation of galleries in the parenquimatous mesophyll cells together with an extended larval life cycle. Further studies indicated that two complementary and dominant genes, named Lm1 and Lm2, are responsible for the dominant resistance found in *C. racemosa* (Guerreiro Filho, 2006). Coffee leaf miner resistance has been also the subject of the first application of transgenic technology to improve the resistance of a coffee variety. Using *Agrobacterium* transformation, a synthetic version of the cry1Ac gene of *Bacillus thuringiensis* was introduced into *C. canephora* and tested in French Guiana (Leroy et al., 2000), showing stability of the transformation and in the biological activity of the introduced gene (Perthuis et al., 2005).

Coffee Berry Borer

One of the major challenges for breeders in Latin America is the development of varieties with some resistance against the beetle *Hypothenemus hampei*, known in Spanish as Broca. The coffee berry borer has a detrimental effect in production either because the weight of the endosperm is consumed by the larvae, or the berries fall off after infestation. The borer´s attack also affects quality since perforated berries are colonized by fungi and suffer a degradation process that generates undesirable odors and flavors. Chemical control is difficult since the insect reduces exposure remaining most of the time inside the fruits, and products like Endosulphan are very toxic, banished in many areas and tested at entry points in many countries.

Unfortunately no significant sources of genetic resistance has been found after screening several accessions and germplasm collections from Africa. Only recently a reduced susceptibility has been observed in materials from Ethiopia and in the species *Coffea liberica*. Development of the CBB in these materials results in a 30% reduction in the number of eggs laid, affecting the fecundity of the insect when compared to traditional cultivars (Romero and Cortina, 2004). These findings still require further characterizations in terms of heritability, stacking and durability before they can be considered for a long term conventional breeding program, but are currently the only hope at the moment for the use for genetic control in places where rain distribution results in the absence of dry periods and therefore provides the berry borer with an adequate environment for continuous reproduction.

Coffee root knot nematodes

Nematodes have become a major cause for concern for an American disease, in particular for coffee root knot nematodes (*Meloidogyne spp.*) that have proved to be very limiting when root infestations are abundant, soils are conductive to infection and inoculum potential is high (secondary infections from plants carrying the nematodes for the nursery). Sources of resistance have been found in the Timor Hybrid as well as in accessions of *C. canephora*. Using the Timor Hybrid CIFC 832/2, the Honduran Coffee Institute released IHCAFE 2004 which came from crosses with *C. arabica* Villa Sarchi. The work of several institutes of Central America using graftings of productive but susceptible scions of *C. arabica* on top of a selected rootstock of *C. canephora* from the CATIE collection in Costa Rica resulted in the new Robusta variety Nemaya, effective against several *Meloidogyne* species (*M. arenaria*, *M. exigua*, *M. arabicida* and *M. incognita*) as well as tolerant against *Pratylenchus spp* (Bertrand et al., 2000). By using *C. canephora* rootstocks, it has been also possible to reduce mortality in the field and the number of plants afected by corky-root, a disease complex caused by *Meloidogyne arabicida* and *Fusarium oxysporum* (Bertrand et al., 2002).

Coffee Stem Canker

Another biological stress factor that has been present in Latin America is the Coffee Stem Canker, also known as Macana, that is a direct consequence of the wounds caused to the tree during the labor practices in slope plantations, that become entry points for the pathogenic fungus *Ceratocystis fimbriata*. In Colombia, for example, 55% of the planted areas are on slopes with gradients of 75% or more, which oblige the farm workers to step on the stem and grasp for support, wounding the tree. Also, renovation practices such as stumping, cause large wounds close to the ground where the fungus lives as a saprophyte. The fungus inevitably kills the plant and after a while the plantation has spots without production, or the trees are replaced with new ones, causing a loss of uniformity, both conditions resulting in losses to the farmer. Management practices are mandatory since losses can reach between 30 and 50% of the trees after stumping. An outstanding Bourbon phenotype was discovered in Colombia by 1960 (Castillo, 1965) that had a quick reaction to fungal invasion creating a cork tissue around the wound and blocking the pathogen from advancing. The plants eventually recover themselves from the attack. This Macana resistant Bourbon was later crossed with either the Caturra variety or elite Colombia variety components to generate Stem Canker resistant cultivars, that are now in the F4-F5 generation of back crossing (Castro and Cortina, 2007). Selection of resistant progenies was performed after characterizing the genetic and pathogenic variability of *C. fimbriata* isolates present in Colombia (Marin et al, 2003), ensuring a stringent challenge of the materials to be used under diverse conditions and populations.

Coffee Berry Disease

Although CBD has not been reported yet in America, risk scenarios must be considered due to trade, movement of materials and people, and especially because of the similarity of the conditions present in the continent that could be adequate for the dispersion and colonization of *Colletotrichum kahawae*, the causal agent. Incomplete resistance has been observed in Timor Hybrid accessions, among them the ones used to generate Leaf Rust resistant varieties (Castillo J., Alvarado, 1987; Moreno et al., 1991), therefore screening tests using hypocotyl inoculations with 4 African isolates have been carried out at the CIFC in Oeiras in order to select materials that could mitigate the effect of the disease if it shows up in America, in particular in mountain plantations. Field tests are still required in order to guarantee the resistance observed under green house conditions, and this requires the collaboration of African countries and the partnership of Research Centers there.

Biological control

As stated earlier, the balance among biological controllers, the native pathogens, the semi-perennial coffee plantations and the nature and distribution of the surrounding landscape is critical to reduce the importance and economical effect of diseases in Latin America. Unexplored for many years, the rich biodiversity present in coffee plantations has been revealed after studies in the 80s and 90s, following a global trend in Bioprospection to reduce the use of synthesized chemicals. The discovery of multiple parasitic relationships on pathogens affecting coffee makes contrast with the scarce adoption of biological control into every day practices. On retrospective, this probably has to do with the overwhelming information on bioprospection compared to key developments in crucial aspects such as formulation technology (including UV and dehydration resistance), improvements in extended shelf life, and assimilation by the farmers of the concepts behind the application of biological products, including efficiency and the importance of timing.

A major change in recent years came from the implementation of quality control standards, with the participation of the private sector and the government. This effort has led to cost effective production, the implementation of formulation technologies, and the legislation to regulate the market for the benefit of farmers. These actions make possible today to find biological alternatives along others in the consumables market, at competitive prices and with guaranteed quality. Consistent results have been obtained when Biological control is used in controlled environments, such as the germination beds and the nursery, where temperature, UV radiation, water availability and substrate conditions can be completely manipulated, therefore favoring the colonization and activity of the products used. This approach has enabled the development of alternatives to previously unavoidable uses of synthesized chemicals in the practices described next.

Damping off

Caused by *Rhizoctonia solani*, damping-off affects germination beds made either with soil of with river sand. It requires preventive control that traditionally has been based on the use of thiabendazole or pencycuron molecules. Research on the application of *Trichoderma harzianum* right before seeding generated an alternative to the use chemicals and permited root colonization by the biocontroller, which can last into the field (Castro and Rivillas, 2005).

Root knot

Caused by nematodes of the genus *Meloidogyne*, this disease must be controlled during nursery in order to avoid inoculum increases in the field, which in turn reduce the ability to assimilate fertilizers and result in low production trees during their life time. Abundant field populations of nematodes create contaminated fields from which inoculum is difficult to remove and can affect many other plant species. Using a commercial formulated mixture of three fungi: *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces lilacinus*, infections under severe inoculum pressure in the nursery can be significantly reduced. This offers an alternative to chemical products such as Carbofuran (an inhibitor of the nervous system) that can be highly toxic to humans.

Iron spot

Caused by *Cercospora coffeicola*, Iron Spot can be a major limiting factor in nurseries, especially in association to poor nutrition and excessive shade. For endemic conditions,

colonization of coffee roots with Mycorrhizae of the *Glomus* genus reduces the incidence and severity of the fungus attack and also initiates a symbiotic relationship that can continue into the field for years (Guzman and Rivillas, 2007).

The application of biological products has been taken into field practices and in the case of the Coffee Stem canker caused by *Ceratocystis fimbriata* this involved the preventive management of the wound after stumping. Benzimidazole, Carbendazim and Benomyl have been the active molecules effective against the pathogen, and now a formulation based on *Trichoderma harzianum* can be used alternatively with the same efficiency, taking into consideration that it must be applied late in the afternoon to avoid the damaging effects of the sunlight (Castro and Rivillas, 2003).

As part of the integrated pest management of the Coffee Berry Borer (CBB), there have been recommendations for optional applications of the entomopathogen *Beauveria bassiana*, complemented with chemical and cultural practices. The use of this fungus opened the door to large scale biological control and introduced adjustments in production and spraying technologies in Colombia (Bustillo and Posada, 1996). It works very efficiently when humidity remains high, and maintains the biological equilibrium when applied openly in the field since the fungus preferentially acts against coleoptera, and the augmented populations go back to low levels in short time. Commercially it meant a success for many small and medium size companies for its reliability when growers used it. For CBB it is of great importance the control of the inoculum sources for the next epizootic, which are located in the berries that fall to the ground and are eventually infested by the females of *Hypothenemus hampei*. Although *B. bassiana* can be applied in those cases, alternatives such as entomonematodes have been in development. Entomonematodes of the genus *Steinernema* have been widely recognized as efficient parasites, since they can move relatively long distances and can infect and replicate abundantly in CBB larvae (Molina and Lopez, 2002). Their use however still requires further developments in mass production and formulation to make them cost effective.

Parasitoids were another alternative to control CBB populations, that implied the introduction of these insects from their native Africa, where they originally developed the specific parasitic relationship. *Prorops nasuta, Phymasticus coffea* and *Cephalonomia stephanoderis* are parasitic wasps released by the millions in Colombia during the first half of the 90s. The economic crisis of coffee that affected the second half of that decade stopped the implementation of this alternative in part, again, because a cost effective way to increase populations under lab conditions was never found. Surprisingly, after almost 10 years of the last release of the parasitoids, populations of *P. nasuta* were found in the southern regions of Colombia, exerting a percentage of the natural control of CBB in the area (Maldonado and Benavides, 2007).

Besides providing a set of biological control alternatives for most of the mandatory chemical applications required to maintain an efficient and healthy coffee plantation, there are currently developments to improve the efficiency under field conditions, where it reaches at most 70%, compared to a chemical spray that stays around 85% or more. Based on the genetic characterization of the *B. bassiana* collection maintained at Cenicafé (Gaitan et al., 2002), mixtures of genotypically diverse isolates have been tested indicating improvements in the performance of the mixtures over single isolates even when the pathogenicity is low (Cruz et al., 2006). On the other hand, genetic modifications by Protoplast transformation have enabled the over-expression of protease and stearase genes in high and low virulence strains indicating the importance of these two enzymes to select pathogenic isolates (Rodriguez and Gongora, 2005).

THE CHALLENGE AHEAD

In order to continue improving the availability of genetic control for current and future biological stress factors, the development of a series of tools is needed to accelerate and facilitate the work of plant breeders. One tool that is missing for *C. arabica* is a genetic map that enables the identification of quantitative trait loci (QTLs), where several minor genes are responsible for the phenotypic characteristic. An approach to a map is the use of genomics to characterize directly the genes involved. Advances are being made towards the identification of genes in the *Coffea* genus (Lin et al., 2005; Vieira et al., 2006), but the association of sequences to functions is still required. Even with the structural and functional information, validation of the proposed role of genes as well as of the phenotypic characterization will involve large screening of materials under pathogen challenge in conductive environments. Still a large percentage of the germplasm collections has not been evaluated for disease resistance against other pathogens. The use of these resources will depend on the heritability and mayor impact of these genes, and on the impact of the diseases. In addition, new sources of resistance that provide genes to widen the genetic pool must be identified, especially from the wild populations of *C. arabica* that still remain in the secondary woods of the central Ethiopian plateau, as well as from interespecific crosses produced naturally such as the one recently found in New Caledonia (Mahé et al., 2007). In the long term, new resistance traits must be incorporated into elite varieties, resulting in multiple resistance.

In addition to tool development, management of biological stress requires the elaboration on concepts of Population biology (for both pathogens and Biocontrolers, using genotyping and phylogenetics, to understand mechanism of pathogen variation, regulation of populations, changes in time and among zones, and to make better use of the diversity present. For the host, durable resistance for a wide range of pathogens can be explored, in particular the field application of induced resistance, which has been repeatedly observed acting against the coffee leaf rust, more recently with inducers such as fungal secondary metabolites (Arboleda and Gaitan, 2005) or commercial inducers (Cristancho and Diaz, 2006). Finally, the extreme relationship with biological control agents represented by endophytes, fungal organisms that interact at the interior of the host tissue and that have been found in coffee, must be taken into account (Santamaría and Bayman, 2005). Biological control must compete in efficiency with traditional chemical control, exploiting their diversity towards the generation of improved isolates, genetic mixtures with better field performance and a greater involvement of the private sector in formulation and production. Deeper considerations must be done on the way the landscape and the diversity around the coffee plantations affect the balance that results in observed disease or pest control events, and the tradeoffs required to conserve biodiversity and keep sustainable production goals.

FINAL REMARKS

For coffee growers in Latin America, advances achieved by scientific research in local and global issues will supply the agronomic tools for production with an economic advantage that guarantees them an adequate income and their continuity in the business. The recommendations to reduce the effect of the biological stress must comply with local and international regulations, more strictly when they define the nature of certified and specialty coffees, but also according to the market demands in quality and price. For both producers and consumers, responsibility with the environment and the sustainability of land use impose additional pressure on the development of genetic and biological control alternatives to be applied in the continent.

REFERENCES

Alvarado G. Colombia, cultivar compuesto de café con resistencia durable a la roya - atributos actuales. Fitotecnia Colombiana 4:18-27. 2004.

Arboleda J., Gaitan A. Actividad antifúngica de metabolitos del biocontrolador *Beauveria bassiana* sobre la roya del café *Hemileia vastatrix*. Fitopatología Colombiana 29:59-64. 2005

Bertrand B., Peña G.M.X., Anzueto R..F., Cilas C., Etienne H., Anthony F., Eskes A.B. Genetic study of *Coffea canephora* coffee tree resistance to *Meloidogyne incognita* nematodes in Guatemala and *Meloidogyne sp.* nematodes in El Salvador for selection of rootstock varieties in Central América. Euphytica 113:79-86. 2000.

Bertrand B., Ramirez G., Topart P., Anthony F. Resistance of cultivated coffee (*Coffea arabica* and *C. canephora*) trees to corky-root caused by *Meloidogyne arabicida* and *Fusarium oxysporum*, under controlled and field conditions. Crop Protection 21:713-719. 2002.

Bustillo A., Posada F. El uso de entomopatógenos en el control de la broca del café en Colombia. Manejo Integrado de Plagas, 42:1-13. 1996.

Carvalho A. Monaco L.C. Relaciones genéticas de especies seleccionadas de *Coffea*. Cafe, Lima, 9:1-19. 1968.

Castaño J.J. Trayectoria de la fitopatología en Colombia : 1571-1974. Letras. Medellin. 164p.1978.

Castillo J. Algunas características morfológicas de una selección resistente a la llaga macana. Cenicafé 16:31-41. 1965.

Castillo J., Alvarado G. Selección por resistencia a la enfermedad del fruto del cafeto causada por *Colletotrichum coffeanum* Noack. In: Congreso de la Asociación Colombiana de Fitopatología y Ciencias Afines, 8. Manizales, Mayo 26-29, 1987. Memorias: Conferencias y Resúmenes. Manizales (Colombia), ASCOLFI, 1987.

Castro B., Cortina H. Selección de progenies de café resistentes a llaga macana *Ceratocystis fimbriata* Ellis & Halst. Fitotecnia Colombiana 7:51-62. 2007

Castro A., Rivillas C. Manejo sostenible de la llaga macana en cafetales renovados por zoca. Avances Técnicos Cenicafé, 312:1-8. 2003.

Castro A., Rivillas C. Biorregulación de *Rhizoctonia solani* en germinadores de café. Avances Técnicos Cenicafé: 336:1-8. 2005.

Chalarca J. Vida y hechos del café en Colombia. Comun Presencia eds. 265p. 1998

Cristancho M., Diaz A. Inducción de resistencia con tres productos de origen biológico en plantas de café contra el ataque de la roya. Fitopatología Colombiana 30:19-24. 2006.

Cruz L., Gaitan A. Gongora C. Exploiting the genetic diversity of *Beauveria bassiana* for improving the biological control of the coffee berry borer through the use of strain mixtures. Applied Microbiology and Biotechnology 71:918-926. 2006.

Gaitan A., Valderrama A., Saldarriaga G., Velez P., Bustillo A. Genetic variability of *Beauveria bassiana* associated with the coffee berry borer *Hypothenemus hampei* and other insects. Mycological Research, 106:1307-1314. 2002.

Guerreiro Filho O. Coffee leaf miner resistance. Braz. J. Plant Physiol., 18: 109-117. 2006

Guzman O., Rivillas C. Relación de *Glomus manihotis* y *G. fasciculatum* con el crecimiento de plantas de café y la severidad de la mancha de hierro. Cenicafé 58:236-257. 2007.

Leroy T., Henry A., Royer M., Altosaar I., Frutos R., Duris D., Philippe R. Genetically modified coffee plants expressing the *Bacillus thuringiensis* cry1Ac gene for resistance to leaf miner Plant Cell Reports, 19: 382-389. 2000

Lin C., Mueller L.A., McCarthy J., Crouzillat D., Petiard V., Tanksley S.D. Coffee and tomato share common gene repertoires as revealed by deep sequencing of seed and cherry transcripts. Theoretical and Applied Genetics, 112:114-130. 2005.

Mahé L., Várzea V., Le Pierrès D., Combes M., Lashermes P. A new source of resistance against coffee leaf rust from New-Caledonian natural interspecific hybrids between *Coffea arabica* and *Coffea canephora*. Plant Breeding 126: 638-641. 2007

Maldonado C., Benavides P. Evaluación del establecimiento de *Cephalonomia stephanoderis* y *Prorops nasuta*, controladores de *Hypothenemus hampei*, en Colombia. Cenicafé 58:333-339. 2007.

Molina J., Lopez J. Desplazamiento y parasitismo de entomonematodos hacia frutos infestados con la broca del café *Hypothenemus hampei* (Coleoptera: Scolytidae). Revista Colombiana de Entomología, 28:145-151. 2002

Moreno L., Castillo J., Alvarado G. Advances in the genetic improvement of coffee in Colombia by combining resistence to "Leaf Rust" and to "Berry Disease". In: Colloque Scientifique International sur le Café, 14. San Francisco (Estados Unidos), July 14-19, 1991. Abstracts. Editado en París (Francia), ASIC, 1991.

Pendergrast M. 1999. Uncommon Grounds. Basic Books.458p

Perthuis B., Pradon J., Montagnon C., Dufour M., Leroy T. Stable resistance against the leaf miner *Leucoptera coffeella* expressed by genetically transformed *Coffea canephora* in a pluriannual field experiment in French Guiana. Euphytica 144:321-329. 2005

Rodriguez M., Gongora C. Transformación de Beauveria bassiana Bb9205 con genes pr1A, pr1J y ste1 de *Metarhizium anisopliae* y evaluación de su patogenicidad sobre la broca del café. Revista Colombiana de Entomología 31:51-58. 2005.

Romero J., Cortina H.A. Fecundidad y ciclo de vida de *Hypothenemus hampei* (Coleoptera: Curculionidae: Scolytinae) en introducciones silvestres de café. Cenicafé 55:221-231. 2004.

Santamaría J., Bayman P. Fungal Epiphytes and Endophytes of Coffee Leaves (*Coffea arabica*) Microbial Ecology 50: 1-8. 2005

Vieira L.G.E., Andrade A.C., Colombo C.A., Moraes A.H. De A., Metha A., Oliveira A.C. De, Labate C.A., Marino C.L., Monteiro V.C. De B., Monte D. De C., Giglioti E., Kimura E.T., Romano E., Kuramae E.E., Lemos E.G.M., Almeida E.R.P. De, Jorge E.C., Alburquerque E.V.S., Silva F.R. Da, Vinecky F., Sawazaki H.E., Dorry H.F.A., Carrer H., Abreu I.N., Batista J.A.N., Teixeira J.B., Kitajima J.P., Xavier K.G., Lima L.M. De, Camargo L.E.A. De, Pereira L.F.P., Coutinho L.L., Lemos M.V.F., Romano M.R., Machado M.A., Costa M.M. Do C., Grossi De Sa M.F., Goldman M.H.S., Ferro M.I.T., Tinoco M.L.P., Oliveira M.C., Sluys M.A. Van, Shimizu M.M., Maluf M.P., Eira M.T.S., Guerreiro Filho O., Arruda P., Mazzafera P., Mariani P.D.S.C., Oliveira R.L.B.C. De, Harakava R., Balbao S.F., Tsai S.M., Mauro S.M.Z., Santos S.N., Siqueira W.J., Costa G.G.L., Formighieri E.F., Carazzolle M.F., Pereira G.A.G. Brazilian coffee genome project: an EST-based genomic resource. Brazilian Journal of Plant Physiology, 18:95-108. 2006.

Zambolin L., Zambolin E., Varzea V. Durable Resistance to Coffee Leaf Rust.Vicosa UFV, DFP. 540p. 2005

White Stem Borer Resistance in Coffee: Perspectives on Breeding, Management and Consumption

A.S. RAM[1], R.K. SABIR[1], S.R. MYTHRASREE[1], H.G. SEETHARAMA[2], R.V. RAO[3]

[1]Division of Plant Breeding and Genetics, Central Coffee Research Institute, Coffee Research Station 577117, Chikmagalur District, Karnataka, India
[2]Division of Entomolgy, Central Coffee Research Institute, Coffee Research Station 577117, Chikmagalur District, Karnataka, India
[3]Institute of Wood Science & Technology, Malleswaram, Bangalore

SUMMARY

Coffea arabica, the plant species that provides the non-alcoholic stimulant beverage coffee, is susceptible to a variety of diseases and pests. Most prominent among the adversaries of this important beverage crop in India are the disease coffee leaf rust (CLR, *Hemileia vastatrix*) and the pest white stem borer (WSB, *Xylotrechus quadripes*). The main thrust of coffee breeding programme in India has been in the direction of achieving higher levels of resistance to CLR since its inception. The pest, WSB became very prominent during the coffee crisis and efforts devoted to identify possible sources of resistance to this insect revealed that on some Indian coffee selections the WSB damage is considerably less. Histo-anatomical studies on the stems and enzyme assays on the bark tissues revealed the presence of three main components of resistance in these selections. These are: presence of more layers of lignified sclerotic parenchyma cells in the bark, the presence of abundant tannins in the sclerotic parenchyma cells and the higher levels of endogenous chitinase activity in the green (phloem and cambium) tissues of the bark. Even though the histo-anatomical features were observed to be constant in the following generation, the enzyme activity levels are variable in the exploited materials; possibly because of the selection in the direction of manifested resistance towards the coffee leaf rust rather than the insect resistance in the evolution of these selections. Present study outlines a possible breeding protocol to consolidate the observed insect resistance in different selections in order to improve the WSB resistance of future commercial populations. The study also proposes to plant refugia of susceptible coffee selections to harbour the insect that is expected to dissuade or prolong the evolution of the insect to overcome the consolidated resistance in the multi-line composite that can be commercially exploited to advantage.

INTRODUCTION

Coffee is a non-alcoholic beverage consumed all over the world for the mild stimulation that it provides. The stimulant property of coffee is attributed to the presence of caffeine, a purine alkaloid in the beverage. Besides stimulation coffee also provides aroma, flavour and taste rewards to the consumers and it is the totality of all these properties that makes up the quality (Leroy et al., 2006). Consumed coffee is mainly derived from two species of the genus *Coffea* L. viz. *C. arabica* L. and *C. canephora* Pierre ex Froehner. Of these two, *C. arabica* is known to produce coffee with all the quality attributes while *C. canephora* produces a beverage deficient in flavour and aroma.

Also, *C. arabica* is the species that is highly susceptible to diseases and pests. Whereas, *C. canephora* and the many other related species of the genus are relatively more resistant/tolerant to diseases and pests. There are also other biological differences between *C. arabica* and *C. canephora* that make it difficult to transfer resistance from other species or *C. canephora* to *C. arabica*; the most important being the tetraploidy and self-compatibility of *C. arabica* and diploidy and self-incompatibility of *C. canephora* and other species. However, pest resistance becomes a very important criterion in breeding new varieties or improving old ones on account of two important aspects. The first is the health conscience of the consumers who prefer to drink a residue free coffee and the other is the general concern over the accumulation of pesticide residues in the environment. Present study presents the observations on the resistance of some Indian coffee hybrids against the dreaded pest white stem borer that became very prominent in the recent coffee crisis and outlines the possible breeding protocol for consolidating it.

PEST RESISTANCE/TOLERANCE IN *COFFEA*

Coffea arabica is the most susceptible to a majority of diseases and pests and thus far there was no known recording of resistance/tolerance against pests in this species (Van der Vossen, 2001). Tetraploidy and autogamy of this species, combined with the cultivation of materials with narrow genetic base that are multiplied through inbreeding would have led to this situation of large genetic homogeneity (Lashermes et al., 1996) and consequent vulnerability (Anonymous, 1972). In the center of origin and diversity of this species in Ethiopia, where coffee is grown in its natural environment, a situation of many pests existing in equilibrium with parasites and predators was reported so that their numbers seldom reach epidemic proportions (Mitchell, 1988). Comparatively larger genetic base of *C. canephora* under cultivation, primarily due to its obligate out-breeding behavior appears to be the main reason for its higher resistance to a variety of diseases and pests (Ram et al., 1994). However, *C. canephora* has specific pests of its own. Branch and berry boring insects do cause some damage and possibly considerable crop loss, but never known to lead to the death of the infested plants. While white stem borers of *C. arabica* (*Xylotrechus quadripes* Chevrolat and *Monochamus leuconatus* Pascoe) are known to infest the main stem of the plant and gradually lead to the death of the plant. Tolerance to this pest is not known to exist in the different strains of *C. arabica*. This pest is much less prevalent on *C. canephora* and the many diploid species of *Coffea* that is probably non-preference (Antixenosis?) as reported against the leaf miner (Matos et al., 2002; Guerreiro-Filho, 2006).

Artificial infestation studies revealed that this insect lays eggs on the seventeen materials of *Coffea* including several species exposed to the insect for oviposition. Tunneling of various orders up to “extensive” was recorded in the various species. However, an examination of the stems revealed that all the tunneling is only superficial between the green tissues of the bark and the outer layers of wood. The insect could not complete its life cycle in any of the materials tested indicating the presence of a powerful mechanism of resistance (Anonymous, 2000; Ram, 2003).

CONTROL OF COFFEE WHITE STEM BORERS

Coffee white stem borers were controlled by the laborious application of BHC solution as a scrub on the main stem of Arabica coffee plants two times in a year just prior to the flight periods in India and a similar practice with Dieldrin and Aldrin in Africa (Anonymous, 2000; Kutywayo, 2002). With the banning of these pesticides in most of the coffee growing countries on account of their residual effects on environment and human health, control of this pest became very difficult. At present, it is controlled by the application of Chlorpyriphos or

Lindane that have comparatively short residual life. Application of lime solution (10%) on the main stem to control the pest is under trials in India that might lead to an eco-friendly alternative to chemical control. The time tested method of tracing the infested plants and destroying them constitutes the most practiced ecologically benign mode of controlling this deadly pest.

Price crisis of 1999-2004 and the neglect of plantations during the crisis resulted in an unprecedented upsurge in the populations of this pest and the devastation of huge numbers of Arabica coffee trees in the producing countries. This has led to a serious compromise in the production of Arabica coffee and consequent revenue loss.

SOURCES OF COFFEE WHITE STEM BORER TOLERANCE

During this period of upsurge of the borer, it is noticed that some selections developed in India manifested a much lower level of damage that is an indication of resistance (Kiggundu et al., 2003). These selections, Sln.5A, Sln.5B (Devamachy hybrids), Sln.6 (Robarbica hybrids), Sln.8 (Hibrido de Timor) and Sln.11 (Ligenioides) were developed with the primary objective of achieving a high level of resistance against the devastating disease, coffee leaf rust.

ORIGIN OF TOLERANT SELECTIONS

All these selections were developed with the aim of transferring coffee leaf rust (CLR) resistance genes from *C. canephora*, *C. liberica* and *C. eugenioides* to *C. arabica* by using different plant breeding techniques as described below.

Sln.5 (Devamachy hybrids)

These are derived from the putative Robusta-Arabica hybrids discovered in Devamachy forest of Kodagu district in the state of Karnataka in India. Original Devamachy hybrids are unstable in their reproductive behavior and produced a crop with large preponderance of small beans (Ramanathan et al., 1951; Ram et al., 1990). These were improved by crossing with Arabica strains S.881 (Rume Sudan) and S.333 (Doobla hybrid 2) and S.26 (Doobla hybrid 1) and came to be known as Sln.5A, Sln.5B and Sln.5C respectively. Sln.5A and Sln.5B are exploited for commercial coffee production in India.

S.881 (Rume Sudan), the primitive Arabica from Anglo-Egyptian Sudan is known to be a source of many resistance genes including those against CLR and CBD (coffee berry disease). This genotype is involved in the derivation of Sln.5A that manifests a high level of resistance against CLR. This manifested resistance is attributed to the contribution of genes for horizontal resistance (HR) against CLR by Rume Sudan Arabica (Srinivasan and Vishveshwara, 1980, Varzea et al., 1985).

S.333 (Doobla hybrid 2) is known to carry the gene S_H3 that conditions resistance against races I and II of CLR. This gene is reported to have been incorporated in Arabica from *C. liberica* by a process of natural introgression (Rodrigues and Bettencourt, 1965). Thus, it is possible that some other genes conditioning resistance to white stem borer from *C. liberica* are also present in this genotype that is involved in the derivation of Sln.5B that manifests considerable resistance to CLR and relatively lower damage by the white stem borer.

Sln.6 (Robarbica hybrids)

These hybrids are derived by the hybridization of Robusta (S.274) and Arabica (Kents) and backcrossing the hybrid to the Arabica parent. Third generation progenies developed by pedigree selection from second backcross manifested a high level of resistance to CLR and are exploited as Sln.6 in commercial coffee production (Sreenivasan, 1987). In the lineage of these hybrids, Robusta (S.274) was involved as the ♀ parent and thus possibly contributed the cytoplasmic-genetic endowments. Borer damage on this selection is also relatively much less than that on pure Arabica strains.

Sln.8 (Hibrido de Timor)

This is a spontaneous hybrid of putative Arabica-Robusta parentage (Bettencourt, 1973). The Timor Hybrid is well known for its highest level of resistance against CLR. It is used extensively in many coffee-breeding programmes as a source of rust resistance genes (Bettencourt and Rodrigues, 1988). Pedigree lines from this hybrid are exploited as Sln.8 in India and are observed to be much less damaged by the white stem borer.

Sln.11 (Ligenioides)

Ligenioides is an amphidiploid isolated from an interspecific hybrid of *C. liberica* and *C. eugenioides* and manifests a high level of resistance against CLR. Even though it was released as a selection earlier on account of its resistance to CLR, it has not become very popular because of its small bean size. New selection lines are now being developed from the cross of this amphidiploid with Hibrido de Timor with the objective of improving bean size and retaining the resistance character of both parents. These lines are still in a development phase in India (Ram et al., 2004). On account of the involvement of *C. liberica* as the ♀ parent in the evolution of Ligenioides these lines are expected to carry the cytoplasm of that species. Second generation lines manifesting high level of resistance are selected and slated for increase and field trials. Incidence of white stem borer on these lines is very low.

POSSIBLE NATURE OF TOLERANCE

Insect resistance in crops is reported to be “non-host specific”, meaning that generally certain plant species are not attractive to given species of insects which leads to immunity (Krattiger, 1997). This also means that the observed resistance of plants is a result of insect behavior in choosing its host. When a preferred host is not available the insect can choose the next best. Thus, new host-insect relationships continue to appear in nature. There are three well-known mechanisms of plant defense against insects. Antibiosis is the mechanism involving toxic substances in the cells of the host tissue that deter the insect from attacking those plants. Antixenosis is the mechanism involving surface texture of the plant (smooth bark, trichomes etc.) or even the cell contents of the plants that inhibit the development of insect (e.g. proteolytic enzyme inhibitors and hydrolytic enzymes) because of which the insect does not prefer the given plant as a host (Al Ayedh, 1997). It is reported that antixenosis and antibiosis often act together and are difficult to separate; both are genetic attributes of the plant and can be manipulated by man (Hooker, 1984). Tolerance is the ability of the plant to withstand the insect attack and continue its life relatively unaffected. Tolerance is said to exist for several diseases and insects and is a genetic, manipulative trait of a plant (Maxwell and Jennings, 1980). Finally, parasites and pests also vary in their fitness (Nelson, 1973) that can lead to a lower damage of the crop plant. In this study, an attempt is made to understand the possible presence of any of the above mechanisms in the coffee selections of India.

A singularly common feature of all the Indian coffee selections described above is their interspecific hybrid origin involving *C. canephora* or *C. liberica* either directly or indirectly. Considering the non-infestation of these species by the insect in nature and the observed hypersensitivity of diploid species to this pest (Anonymous, 2000; Ram, 2003) it is plausible to infer that introgressed genes from the diploid species cause the manifestation of lower damage of borer on these selections as in the case of leaf miner resistance in the Arabica hybrids carrying introgressed genes from *C. racemosa* (Guerreiro Filho et al., 1999).

The main thrust of Indian coffee breeding programme has been achieving resistance against CLR and all the above selections were developed in that process (Srinivasan and Vishveshwara, 1980). In the field populations of these selections, it is observed that the CLR incidence is very low (~ 6%) and even some of the susceptible plants carry very few disease lesions with sparse sporulation. Full leaf retention by most of the plants in the populations was noticed. Masking of the main stem and thick primary branches by the leaf canopy is a possible escape mechanism protecting the plants in the field against stem borer. In the CLR susceptible selections, it is generally observed that the entire leaf complement is lost due to disease, if not protected with prophylactic/ systemic fungicide application. It is possible that these defoliated plants are more vulnerable to adversaries because leaves are the seat of a variety of biosynthetic activities that produce all the constituents needed for survival, fitness and defense. Thus, full retention of leaves by all the plants of above-mentioned selections is possibly contributing significantly to their protection against the white stem borer.

THE RESISTANCE STUDY- METHODOLOGY

In a bid to understand the lower damage of borer to the plants in the above selections of interspecific hybrid descent, studies were carried out including Cauvery/Catimor as susceptible control and S.274 (Robusta) as resistant control. Initially, the populations available at CCRI (F_2 and F_3 generations) and Sub-stations (next generation) were surveyed to understand the damage pattern. It is observed that the plants of these selections also show ridges on the main stem that is considered important in assessing the infestation and removal of infested plants. However, in the above selections, this appears to be only the result of an attempted attack, in that the insect lays its eggs on the stems of these selections also and the neonates start feeding on the green tissues of the bark just as they do in all other coffee plants. This feeding damage leads to the development of callus from the green tissue, which is also meristematic (cambial portion). In turn, this leads to the development of ridges that become visible with advanced healing of the damage caused by the larvae. However, it is observed that a large frequency of these plants do not manifest any other symptoms associated with the presence of an active borer in the stem, i.e. yellowing of leaves, wilted appearance of the canopy, high frequency of empty locules etc. in these selections. A recent report on the incidence of borer on these selections indicated that there are more dead stages of the insect in the stems of these selections that are removed on the basis of the presence of ridges as an indicator of infestation (Anonymous, 2006). On the basis of these observations and reports, plants sampled in the survey of these selections were classified into three main classes viz. HP (healthy plant not showing either ridges or symptoms), RN (plants showing ridges but no other symptoms of infestation, hypersensitive plants) and RM (plants showing ridges as well as mild symptoms indicating the presence of an active borer in the stem). A survey of the field populations at CCRI revealed variable frequencies of these three types of plants (Table 1).

White stem borer eggs were inoculated on selected healthy plants of Sln.5A, Sln.5B, Sln.6 and Catimor/Cauvery and the various changes in the level of presence of food ingredients like starch, proteins and lipids in the stem tissues of these selections was monitored over a period of one year including the two flight periods. Analysis also involved testing the presence and

intensity of tannins in the bark and wood tissues of these selections to understand the role of these plant constituents in defense against the stem borer. Samples were analyzed at monthly intervals, using histo-anatomical, histo-chemical and quantitative techniques to capture changes (Jensen, 1962; Gahan, 1984; Hagerman, 1987). Samples from a few plants of diploid tree coffee species were also analyzed to perceive the differences between them and tetraploid Arabica. Studies were also carried out on the activity of hydrolytic enzyme chitinase (Isaac and Gokhale, 1982) in the bark tissues of these selections in order to understand its possible involvement in the defense against stem borer.

Table 1. Frequency of different WSB resistance classes in Indian Coffee Selections.

Variety	HP	RN	RM	CLR*
Sln.5A	59.60	29.40	11.00	14.20
Sln.5B	93.30	4.60	2.10	1.20
Sln.6	87.10	12.90	0.00	6.50
Sln.8	77.90	21.10	1.00	3.00
Sln.11	70.70	19.50	9.80	2.40
Catimor/Cauvery**	88.00	9.60	2.40	1.10
S.274 (Robusta)	96.00	4.00	0.00	2.00

** CLR incidence was scored to understand the frequency of likely pre-disposed plants.*
*** Well maintained plants in CCRI plot.*

OBSERVATIONS AND INFERENCE

Visual scoring in the stained sections of stems of these selections indicated that there are no large differences in the presence and/or density of food ingredients between the stems of healthy (HP), hypersensitive (RN) and susceptible (RM) plants. The tannin test, however, presented large differences between Arabica selections and diploid species as well as between healthy, hypersensitive and susceptible plants of the selections.

In our various observations, the bark tissue of the coffee plants is found to possess thin-walled green parenchyma cells (phloem and cambium) and thick-walled sclerotic parenchyma cells configured into alternate layers. Susceptible Catimor/Cauvery plants were found to possess one to three layers of the sclerotic parenchyma cells whose walls are thickened, while the diploid species and the Selections of interspecific hybrid descent possess up to five or more layers of sclerotic parenchyma with strongly thickened cell walls that are interspersed with green phloem and cambium parenchyma cells. These additional layers of sclerotic parenchyma could be a strong barrier protecting against stem borer. Relatively lower number of sclerotic parenchyma layers in the stems of Catimor/Cauvery may be a possible predisposing factor in its large susceptibility to the pest in the field. Interestingly, the sclerotic parenchyma cells are positively and intensely stained with Lugol's Iodine, the stain for tannins, indicating that these cells could also be a powerful barrier for deeper penetration of the stem borer, in that the tannins present in these cells when consumed can complex with the proteins and render them indigestible by the insect (Koukoura and Nastis, 1994). Another observation is that the vascular tissue of the wood of diploid species and the Selections of interspecific descent is also laden with tannins that can also lead to the same situation for the insect larva even if it enters the central wood portion of the stem. The importance of plant tannins in defense was elucidated in studies on other perennial plant species such as *Lespedeza capitata* (Springer et al., 2002) and *Populus tremuloides* (Mansfield et al., 1999). Synthetic as well as tannins of plant origin were shown to be inhibitory to insect development

and fitness in other studies also (Nomura and Itioka, 2002; Ayres et al., 1997; Reed, 1995) and support the present observations.

Assays to quantify tannin in the bark tissues of the above selections indicated that a considerable frequency of plants in the resistant selections possess a higher level of tannin in the bark cells while in the susceptible Cauvery selection most of the assayed plants possess low tannin in this tissue (Table 2). However, Sln.5A manifesting considerable resistance in the field appears to possess low tannin in a large frequency of plants, indicating that the number of layers of sclerotic tissue and tannin together constitute the defense. This draws support from the observations on S.274 (Robusta) that is not showing large differences from Arabica selections in tannin content pattern but possess larger number of sclerotic cell layers in bark (Table 2).

Table 2. Tannin in Bark Tissues of Indian Coffee Varieties.

Variety	Quantity of Tannin (in 1g of bark tissue)(in % Plants)				S.L**
	0 – 1.99	2.00 – 3.99	4.00 – 5.99	6.00 – 7.99 >	
Sln.5A	76.00	22.00	2.00	0.00	6-7
Sln.5B	40.00	46.00	12.00	2.00	6-7
Sln.6	14.00	72.00	12.00	2.00	6-8
Sln.8	68.00	28.00	4.00	0.00	6-7
Sln.11	56.00	30.00	12.00	2.00	5-6
Catimor/Cauvery*	70.00	28.00	2.00	0.00	2-3
S.274 (Robusta)	50.00	38.00	12.00	0.00	6-8

** well maintained plants at CCRI*
*** No. of sclerotic cell layers in bark*

Chitin is the major component of the exoskeleton (cuticle) and peritrophic membrane (midgut lining) of a majority of insects and acts as a physico-chemical barrier to environmental hazards and predators. Entomopathogenic fungi like *Beauveria bassiana*, *Metarrhizium anisopliae* etc., overcame this barrier by producing multiple extra-cellular degradative enzymes with chitinolytic and proteolytic activities (Nahar, 2004; St. Leger et al., 1993; Nitoda et al., 2003). Chitinases were shown to degrade insect gut peritrophic membranes. Brandt et al. (1978) proposed that chitinase causes perforations in the peritrophic membrane facilitating the entry of pathogenic fungi into the insect tissues. Chitinases with chitinolytic activity occur widely in plants, fulfilling a possible defense role (Karasuda et al., 2003; Botha et al., 1995, 1998). In the present study, constitutive endogenous chitinase activity is considered an important indicator of insect resistance.

In the Chitinase assays, it is generally observed that the healthy coffee plants have a high level of activity of this enzyme in the extracts from green tissue of bark of the diploid species (data not shown) as well as the Selections of interspecific descent. Even in the Catimor/ Cauvery variety that is known for high susceptibility to white stem borer, the chitinase activity in the green tissues of bark was observed to be considerably high, in the plants that are well protected and healthy with full leaf complement. Large, random samples of plants from all the above selections indicated that the Chitinase activity varies in the populations, but there is a higher frequency of plants with higher level of endogenous activity of this enzyme in the resistant selections (Table 3) that can be attributed to the selection background in the evolution of these selections towards CLR resistance.

From the foregoing, the observed lower damage to the Coffee Selections of interspecific hybrid descent can be substantiated as due to three factors. First, the anatomical presence of sclerotic parenchyma in larger number of layers in the bark of interspecific hybrid derived selections and the configuration of these layers in interspersion with green parenchyma constitutes a strong barrier for the pest to be able to cause serious damage. Second, the abundant presence of tannins in the sclerotic parenchyma cells could inhibit the protein digestive process of the insect by the formation of protein-tannin complexes. Third, the high level of endogenous chitinase activity in the green tissues of bark interspersed with the sclerotic parenchyma also constitutes a powerful barrier for the development of the borer beyond the early stages, resulting in its not completing the life cycle. The structural pattern of sclerotic and normal parenchymatous cell layer alternation was maintained from generation to generation with good preservation of the number of layers, indicating that this anatomical structure is possibly determined by the epigenetic mechanisms.

Table 3. Specific Activity of Chitinase in the Green Bark Tissue of Coffee Varieties.

Variety	Chitninase Specific Activity (n mol/min/mg protein) (in % Plants)			
	1.00 – 5.99	6.00 – 9.99	10.00 – 15.99	16.00 – 20.00>
Sln.5A	27.71	42.17	15.66	14.46
Sln.5B	31.37	22.55	17.65	28.43
Sln.6	28.57	16.67	28.57	26.19
Sln.8	26.40	33.33	19.44	20.83
Sln.11	17.05	44.32	23.86	14.77
Catimor/Cauvery*	28.57	23.81	28.57	19.05
S.274 (Robusta)	0.00	53.33	26.67	20.00

** well maintained plants at CCRI.*

INHERITANCE OF BIOCHEMICAL MARKERS

An examination of the data on variation of both biochemical markers in the studied materials indicates that these are possibly quantitative traits. However, when the class-intervals are grouped together into low and high classes of expression of these traits a pattern close to the Mendelian inheritance emerges as shown in tables 4 and 5, although there are a few variations that cannot be explained only with Mendelian models. Thus, larger sampling of the populations for study reveals real patterns of inheritance that can be effectively used in plant breeding. Mendelian patterns reveal key major genes that cause resistance and superimposing the selection for the quantitative value of expression of the biochemical traits is expected to lead to the development of populations with long lasting resistance as they combine the Mendelian and Biometrical aspects of selection (Robinson). Combination of vertical and horizontal elements of resistance conditioned by the Mendelian and Biometrical genes are known to impart long lasting resistance against the leaf rust disease in coffee (Sreenivasan et al., 1984; Ram, 2000).

Table 4. Inheritance of Bark Tannin Content in Indian Coffee Selections.

Selection	Bark Tannin (Frequency of Plants %)			
	High	Low	Ineritance	χ^2 (P value)
Sln.5A	24	76	1:3 (Monohybrid)	0.103 (0.70-0.80)
Sln.5B	60	40	1:1 (Backcross)	2.00 (0.10-0.20)
Sln.6	86	14	1:2:1 (Monohybrid)	1.28 (0.20-0.30)
Sln.8	32	68	1:3 (Monohybrid)	0.935 (0.30-0.50)
Sln.11	44	56	1:1 (Backcross)	1.44 (0.20-0.30)
Catimor/Cauvery	30	70	1:3 (Monohybrid)	0.415 (0.70-0.80)
S.274	50	50	1:1 (Backcross)	0.08 (0.70-0.80)

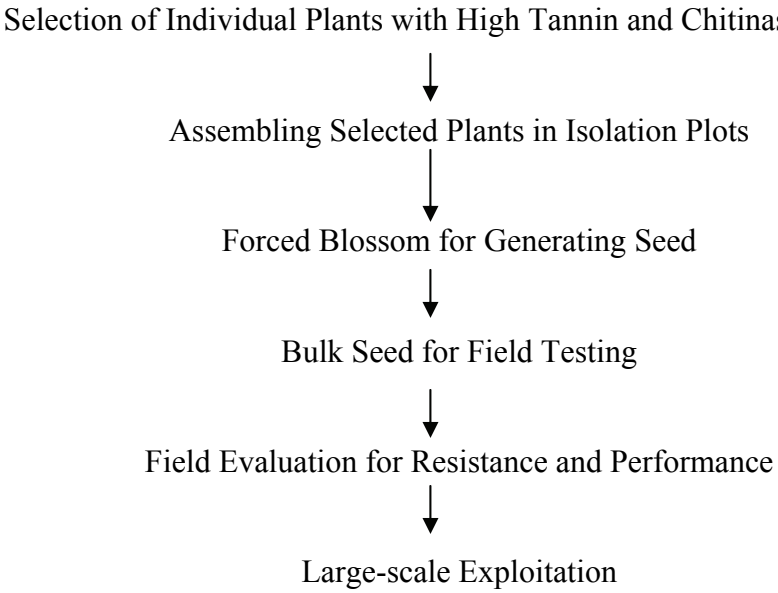
Table 5. Inheritance of Bark Chitinase Activity in Indian Coffee Selections.

Selection	Bark Chitinase Activity (Frequency of Plants %)			
	High	Low	Ineritance	χ^2 (P value)
Sln.5A	73	27	3:1 (Monohybrid)	0.255 (0.50-0.70)
Sln.5B	69	31	3:1 (Monohybrid)	2.605 (010-0.30)
Sln.6	71	29	3:1 (Monohybrid)	0.571 (0.30-0.50)
Sln.8	74	26	3:1 (Monohybrid)	0.073 (0.70-0.80)
Sln.11	83	17	3:1 (Monohybrid)	2.965 (0.05-010)
Catimor/Cauvery	71	29	3:1 (Monohybrid)	0.123 (0.70-0.80)
S.274	0	30	--	--

Selection and Breeding

As all the above described selections are tetraploids, it is possible that they can be intercrossed to consolidate the genes responsible for the manifested resistance in genotypes that can be assembled in isolation plots for commercial exploitation as shown schematically below. Control of pollination process through isolation is very important to maintain the resistance in these genotypes on account of the negative effects of natural selection leading to a loss of resistance genes with the advancement of generations in hybrid selections of interspecific descent (Sreenivasan et al., 1994). An important aspect of all these selections is that all of them carry genes introgressed from the diploid species *C. canephora* and/or *C. liberica*. The direction of selection and evolution is towards the type of *C. arabica* and all the selections manifest Arabica features. A composite of these selections is expected to resist the insect very strongly without compromising the characters under selection (such as quality, yield etc.) due to the gene pyramiding effect in this multi-line (Ram, 2001). Such multi-line composites can be exploited to advantage in commercial cultivation.

Selection and Breeding for Consolidation of Pest Resistance-Arabica Hybrids



MANAGEMENT OF RESISTANCE

On account of the pest resistance being imparted by genes of alien origin, in that they are introgressed from related species, the resistance requires to be managed and maintained by a strong operational mechanism. Selection and consolidation of plants expressing these traits in strength is the first step. Seed production in isolated plots of such plants is an important mode of maintaining resistance genes in these populations. Creating multi-line complexes or gene pyramids to forestall the insect attacks is a desired pathway for managing the resistance in favour of the plant (Ram, 2001). The possibility of evolution of insect biotypes that can defeat the resistance should be countered by providing refugia of susceptible plant clusters within the plantations that harbour the pest and reduce the natural selection pressure on the insect, as prescribed for some transgenic crops (Anonymous, 1999, 2008). The size of refugia required to reduce the spread of the insect should be studied and then only recommended. Application of broad-spectrum bio-pesticides is also important to ensure that the available resistance is managed in an eco-friendly manner.

IMPLICATIONS FOR CONSUMERS, PRODUCERS AND ENVIRONMENT

The impact of pesticide residues on the quality was reviewed earlier (Ongeri, 2002). The consolidation and management of inherent resistance reduces the chemical in-puts for controlling the pest. In turn, this leads to a reduction or elimination of pesticide residues in the coffee that reaches the market. For the health conscious consumers this is a strong incentive to continue with the consumption of coffee that is residue free. For the producers, the main incentive is reduced cost of managing the pest and consequent reduced cost of production when multi-line composites are in cultivation. Another major incentive is that their coffee meets the minimum residue levels (Codex Alimentarius; http://www.itscb.com) specified by various importing countries ensuring disposal of the produced coffee profitably. A final but not the least important implication is that this mode of pest control is the most benign to the environment as it does not involve chemical pesticides and consequently there is no concern of residue loads in the crop and the soil.

REFERENCES

Al Ayedh H.Y. 1997. Antixenosis: The Effect of Plant Resistance on Insect Behavior. http://www.colostate.edu/depts/entomology/courses/en507/papers_1997/alayedh.html.

Anonymous, 1972. Genetic vulnerability of major crops. National Research Council, NAS, Washington D.C.

Anonymous, 1999. EPA and USDA Position Paper on Insect Resistance Management in Bt-Crops. 5/27/99 (minor revisions 7/12/99). http://www.agbios/docroot/articles/epa_irm. pdf

Anonymous, 2000. Fifty Third Annual Report – Central Coffee Research Institute, Balehonnur, India.

Anonymous, 2006. Fifty Ninth Annual Report. Central Coffee Research Institute, Balehonnur, India.

Anonymous, 2008. Insect resistance management when planting Bt-Corn hybrids. Wisconsin Crop Manager News Letter, 24 June 2008. http://ipcm.wisc.edu/default.aspx?tabid= 53&entryID=218

Ayres M.P., Clausen T.P., MacLean S.F., Redman A.M., Reichardt P.B. 1997. Diversity of structure and antiherbivore activity in condensed tannins. Ecology 78(6): 1696-1712.

Bettencourt A.J. and Rodrigues Jr. C.J. 1988. Principles and practice of coffee breeding for resistance to rust and other diseases. In: Coffee (Vol.4) Agronomy (Eds. R.J.Clarke and R. Macrae) pp 199-234. Elsevier Applied Science, London, New York.

Botha A-M., Nagel M.A.C., Botha F.C. 1995. Effect of exposure to the Russian Wheat Aphid on the expression of chitinase. Plant Physiol. 108 (Suppl): 93.

Botha A-M., Nagel M.A.C., Van der Westhuizen A.J., Botha F.C. 1998. Chitinase isoenzymes in near-isogenic wheat lines challenged with Russian Wheat Aphid, exogenous ethylene and mechanical wounding. Bot. Bull. Acad. Sin. 39: 99-106.

Brandt C.R., Adang M.J., Spence K.D. 1978. The peritrophic membrane: ultrastructural analysis and function as a mechanical barrier to microbial infection in *Orgyia pesudotsugata*. J. Invertebr. Pathol. 32: 12-24.

Codex Alimentarius. http://www.itscb.com.

Gahan P.B. 1984. Plant Histochemistry and Cytochemistry. Academic Press, London, New York.

Guerreiro Filho O. 2006. Coffee leaf miner resistance. Braz. J. Plant Physiol. 18: 109-117.

Guerreiro Filho O., Silvarolla M.B., Eskes A.B. 1999. Expression and mode of inheritance of resistance to leaf miner. Euphytica 105: 7-15.

Hagerman A.E. 1987. Radial diffusion method for determining tannin in plant extracts. J. Chem. Ecol. 13: 437-449.

Hooker A.L. 1984. The pathological and entomological framework of plant breeding. In: Gene Manipulation in Plant Improvement (Ed. J.P. Gustafson), pp. 177-207. Plenum Press, New York.

Isaac S., Gokhale A.V. 1982. Autolysis: A tool for protoplast production from *Aspergillus nidulans*. Trans. Br. Mycol. Soc. 78: 389-394.

Jensen W.A. 1962. Botanical Histochemistry. Freeman, San Fancisco.

Karasuda S., Tanaka S., Kajihara H., Yamamoto Y., Koga D. 2003. Plant chitinase as a possible biocontrol agent for use instead of chemical fungicides. Biosci. Biotechnol. Biochem. 67(1): 221-224.

Kiggundu A., Pillay M., Viljoen A., Gold C., Tushemereirwe W., Kunert K. 2003. Enhancing banana weewil (*Cosmopolites sordidus*) resistance by plant genetic modification: A Perspective. Afr. J. Biotechnol. 2: 563-569.

Koukoura Z.S., Nastis A.S. 1994. Tannin content of selected fodder trees and shrubs and their effect on in vitro digestibility. In: Fodder Trees and Shrubs (Eds. V.Papanastasis and L. Stringi) pp. 117-127. CIHEAM, Palermo, Italy.

Krattiger A.F. 1997. Insect resistance in crops: A case study of *Bacillus thuringiensis* (*Bt*) and its transfer to developing countries. The International Service for the Acquisition of Agri-biotech Applications (ISAAA), New York, USA.

Kutywayo D. 2002. Observations on the life history of the coffee white stem borer, *Monochamus leuconatus* Pascoe (Coleoptera, Cerambycidae): Adult emergence patterns in Chipinge, Zimbabwe. In: XIX International Colloquium on Coffee Science. ASIC, Paris. (Electronic Publication).

Lashermes P.,Combes, M.C., Trouslot, P., Anthony, F., Charrier, A. 1996. Molecular analysis of the origin and genetic diversity of *Coffea arabica* L: Implications for coffee improvement. Proc. Eucarpia. pp. 23-29.

Linthorst H.J.M. 1991. Pathogenesis related proteins of plants. Critical Rev. Plant Sci. 10: 123-150.

Mansfield J.L., Curtis P.S., Zak D.R., Pregitzer K.S. 1999. Genotypic variation for condensed tannin production in Trembling Aspen (*Populus tremuloides*, Salicacease) under elevated CO_2 and in high- and low-fertility soil. Amer. J. Bot. 86 (8): 1154-1159.

Matos J.W., Ramiro D.A., Goncalves W., Guerreiro-Filho O. 2002. *Coffea canephora* and *C. congensis* have antixenosis resistance to the coffee leaf miner. In: XIX International Colloquium on Coffee Science. ASIC, Paris. (Electronic publication).

Maxwell F.G., Jennings P.R. 1980. Breeding Plants Resistant to Insects. John Wylie & Sons, New York.

Mitchell H.W. 1988. Cultivation and harvesting of the Arabica coffee tree. In: Coffee (Vol.4) Agronomy (Eds. Clarke, R.J., Macrae, R.) pp. 43-90. Elsevier Applied Science, London, New York.

Nahar P. 2004. Development of biocontrol agents for the control of pests in agriculture using chitin metabolism as target. Ph.D. Thesis, University of Pune, India, 137 p.

Nelson R.R. 1973. The meaning of disease resistance in plants. In: Breeding Plants for Disease Resistance: Concepts and Applications (Ed. R.R. Nelson), pp.13-25. Penn. State Univ. Press, University Park.

Nitoda T., Usuki H., Kanzaki H. 2003. A potent insect chitinase inhibitor of fungal origin. Z. Naturforsch. 58C: 891-894.

Nomura M., Itioka T. 2002. Effects of synthesized tannin on the growth and survival of a generalist herbivorous insect, the common cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). Appl. Ento. Zool. 37(2): 285-289.

Ongeri D.M.K. 2002. Pesticide residues in coffee beans and their impact on coffee quality in Kenya – A review. In: XIX International Colloquium on Coffee Science. ASIC, Paris. (Electronic Publication).

Ram, A.S. 2001. Breeding for rust resistance in coffee: The gene pyramid model. J. Plantn. Crops. 29: 10-15.

Ram A.S. 2003. Plant Defense: The Case of Coffee. Study Report, CCRI, India.

Ram A.S., Ganesh D., Reddy A.G.S., Srinivasan C.S. 2004. Ligenioides – A source of new genes for Arabica coffee breeding. J. Plantn. Crops 32 (Suppl.): 5-11.

Ram A.S., Sreenivasan M.S., Naidu R. 1994. Exploitation of coffee germplasm in India II: Diploid species. J. Coffee Res. 24: 53-60.

Ram A.S., Sreenivasan M.S., Ramaiah P.K. 1990. A study of peaberry development: Its implications in coffee breeding. J. Coffee Res. 20: 69-76.

Ramanathan V., All V., Thomas K.M. 1951. A review of the past experimental work on coffee in South India with suggestions for the future. Evaluation Committee Report, CCRI, pp.56.

Reed J.D. 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes. J. Anim. Sci. 73: 1516-1528.

Rodrigues Jr., C.J., Bettencourt, A.J. 1965. Routine screening for resistance to Hemileia vastatrix B. et Br. on *Coffea arabica* L. accessions from different coffee producing regions of the world. In: Progress Report 1960-1965. pp. 47-99. Oeiras, Portugal.

Rodrigues C.J. Jr., Bettencourt A.J., Rijo L. 1975. Races of the pathogen and resistance to coffee rust. Annu. Rev. Phytopathology 13: 49-70.

Sahai A.S., Manocha M.S. 1993. Chitinases of fungi and plants: their involvement in morphogenesis and host parasite interaction. FEMS Micobiol. Rev. 11: 317-338.

Springer T.L., McGraw R.L., Aiken G.E. 2002. Variation of condensed tannins in Roundhead Lespedeza germplasm. Crop Sci.42: 2157-2160.

Sreenivasan M.S. 1987. Cyto-Embryological Studies of Robusta x Arabica Coffee Hybrids. Ph.D. Thesis. University of Mysore. 234p.

Srinivasan C.S., Vishveshwara S. 1980. Selection in coffee: Some criteria adopted and results obtained in India. J. Coffee Res. 10: 53-62.

St. Leger R.J., Staples R.C., Roberts D.W. 1993. Entomopathogenic isolates of *Metarhizium anisopliae*, *Beauveria bassiana* and *Aspergillus flavus* produce multiple extracellular chitinase isozymes. J. Invertebr. Pathol. 61: 81-84.

Van der Vossen H.A.M. 2001. Agronomy I: Coffee Breeding Practices. In: Coffee: Recent Developments (Eds. R.J. Clarke, O.G. Vitzthum), pp. 184-201. Blackwell Science, Oxford, London.

Varzea V.M.P., Rodrigues Jr., C.J., Mexia J.T. 1985. Evaluation of horizontal resistance in some Arabica plants of different physiologic groups when confronted with virulent races. XI International Colloquium on Coffee Science. pp.625-633. ASIC, Paris.

Characterization of Variability on *Colletotrichum kahawae* Isolates from Angola and Search for Resistance on Angolan Coffee Germplasm

L. MANUEL[1], J.M. NEVES-MARTINS[2], P. TALHINHAS[2,3], V.M.P. VÁRZEA[3]

[1]Instituto Nacional do café, Luanda, Angola
[2]Departamento de Botânica e Engenharia Biológica, ISA, Lisboa, Portugal
[3]Centro de Investigação das Ferrugens do Cafeeiro, IICT, Oeiras, Portugal

SUMMARY

Coffee Berry Disease (CBD) is the main limitation for the cultivation of arabica coffee in high altitudes in Africa. With the goal of obtaining resistance to CBD, a study was carried out on the variability of the pathogen, the fungus *Colletotrichum kahawae* Waller & Bridge, from Angola and other tropical regions and on the disease resistance of coffee plants from Angola. Morphological and molecular diversity was found in a collection of CBD pathogens from Angola. In morphology, variability was found for growth rate in culture medium. Molecular diversity was recorded from the analysis of the nucleotide sequence of the rDNA-ITS region and by ISSR molecular markers, but with high levels of similarity (99% and 97%, respectively). Resistance to CBD was registered on seven *Coffea canephora* genotypes, mostly from Cazengo (Kuanza Norte, Angola), and on three *Coffea arabica* genotypes, from Kuanza Sul, Angola. These genotypes were selected on the field in Angola as resistant to local *C. kahawae* isolates and showed resistance in the laboratory (pre-selection test of hypocotyls) to Kenya and Camaroon isolates. Genetic diversity found, both among the pathogen and host resistance, is important for defining breeding strategies for coffee, with the purpose of selecting resistant plants, an important step on the larger effort of re-launching coffee cultivation in Angola.

INTRODUCTION

Coffee production is a major agro-industrial activity with high socio-economic importance in the world, particularly in tropical and subtropical areas in over 60 countries. It is responsible for a worldwide social beverage, creation of work posts, and national and family income.

In Angola, before the independence and before the development of oil and diamond extraction, all economic and social development was based on coffee production. Angola was the fourth largest coffee producer in the world and the first or second in Africa alternating with Ivory Coast (over two hundred thousand tonnes exported coffee, during the nineteen seventies). Currently coffee production in Angola is a minor activity (below five thousand tonnes) due to various sorts of reasons, among which are the problems related to technic and phytosanitary assistance to farmers and plantations, the aim of this research.

Coffee berry disease (CBD), caused by *Colletotrichum kahawae*, affects all coffee species, but with higher incidence on arabica coffee. The most economically important damages are due to the infection of green fruits. Typical symptoms are slightly sunken dark or brown necroses with various shapes. In a more advanced stage, fruits can become entirely necrotic, with dark points corresponding to acervuli. On this stage, fruits will drop or rather mummify and remain on the tree (Muller, 1980) (Figure 1).

Figure 1. Coffee Berry Disease caused by *Colletotrichum kahawae.*

This research project aims to promote the initial steps of a genetic coffee breeding programme against CBD with the purpose of supporting a sustainable coffee cultivation in Angola.

MATERIAL AND METHODS

Coffee and *C. kahawae* germplasm were collected in Angola (Figure 2). Genetic diversity of the pathogen was assessed by morphology studies (colony colour in Malt Extract and Potato Dextrose Agar culture media and colony size at 15 °C, 22 °C and 30 °C in Malt Extract), DNA-based methods (ISSR markers and rDNA-ITS nucleotide sequences) and pathogenicity tests (inoculation of spore suspensions on 'Caturra' detached green fruits). Resistance screening of *Coffea* spp. germplasm was conducted with CIFC *C. kahawae* isolates from Kenya (Que2) and Cameroon (Cam1) on hypocotyls observed 30 days after inoculation according to the 0-4 van der Graaff (1981) scale and results were analysed by calculating a Disease Intensity Index **(DII)**:

$$\mathbf{DII} = \frac{\Sigma \text{ (number of hypocotils in each class x class numeric value)}}{\text{number of hypocotils in all classes x 4}}$$

Disease susceptibility of germplasm was rated according to DII result: **R**esistant **(R)**: **DII** ≤ 0.25, **M**oderately **R**esistant **(MR)**: 0.25 <**IID** ≤ 0.50, **M**oderately **S**usceptible **(MS)**: 0.50 <**IID** ≤ 0.75, **S**usceptible **(S)**: **IID**> 0.75.

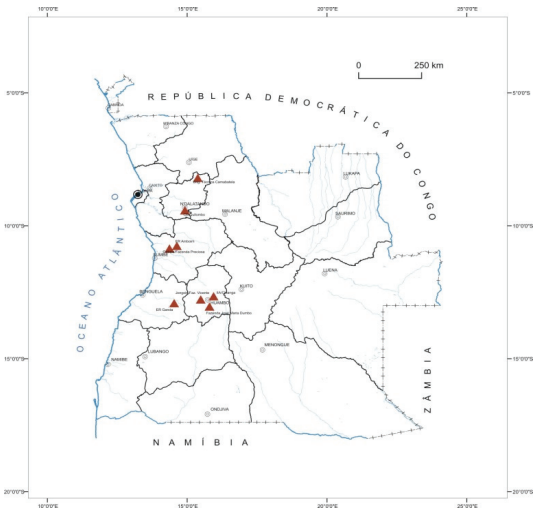


Figure 2. Germplasm collection locations (▲) in Angola.

RESULTS AND DISCUSSION

One hundred and twenty three samples coffee germplasm samples were collected in Angola (Benguela, Kwanza Norte, Kwanza Sul and Huambo), from which 55 *Colletotrichum* spp. isolates were obtained, 55% of which were identified as *C. kahawae* (all from Benguela and Kuanza Sul; Figure 3).

Figure 3. Experimental layout of pathogenicity test on *Coffea* detached fruits.

Colony colour varied among olive (44.4%), cream (33.3%) and grey (22.2%) on Malt Extract and between grey (88.8%) and cream (11.1%) on Potato Dextrose Agar.

Colony diameter ranged 1.82-4.17 cm at 15 °C, 3.52 - 7.48 cm at 22 °C and 0.76-4.5 cm at 30 °C. For most isolates, the largest colony diameter was registered at 22 °C (Figure 4).

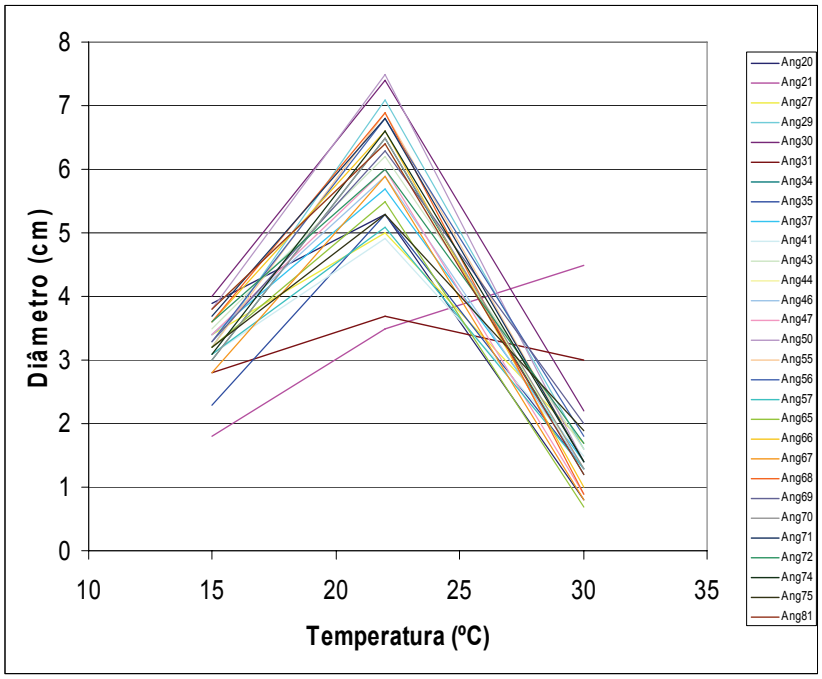


Figure 4. *C. kahawae* colony diameter in Malt Extract at different temperatures.

The nucleotide sequence of rDNA-ITS region revealed differences between *C. kahawae* and *C. gloeosporioides* isolates, but nevertheless showing a high degree of similarity between these species. Also, a high degree of similarity (97%) among Angola *C. kahawae* isolates was evident upon ISSR analysis. However, small differences found among these isolates show the existence of variability in this population, suggesting that it is not entirely clonal (Figure 5).

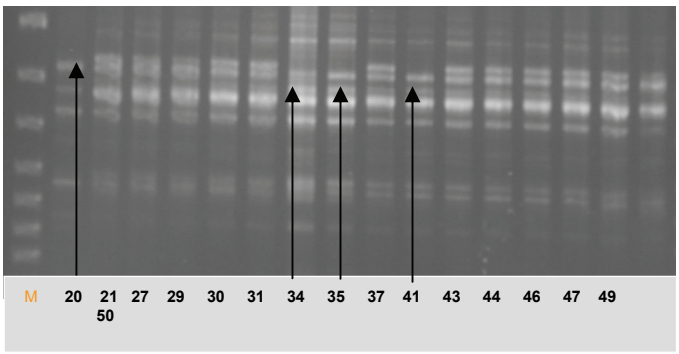


Figure 5. ISSR profiles among *C. kahawae* isolates, using primer TCC5.

Germplasm screening revealed 10 *Coffea* spp. genotypes showing resistance to *C. kahawae* from Kenya and Cameroon, among which six *Coffea canephora* (LM51, LM84, LM85, LM87, LM40 and LM83) genotypes are resistant to *C. kahawae* from Kenya, one *Coffea canephora* (robusta amboim) genotype is resistant to *C. kahawae* from Cameroon and three *Coffea arabica* (LM27**,** LM35 and a 'Híbrido de Timor'-derived) genotypes are resistant to *C. kahawae* from Kenya. No resistance was detected among these accessions to *C. kahawae* isolates collected in Angola (Figure 6).

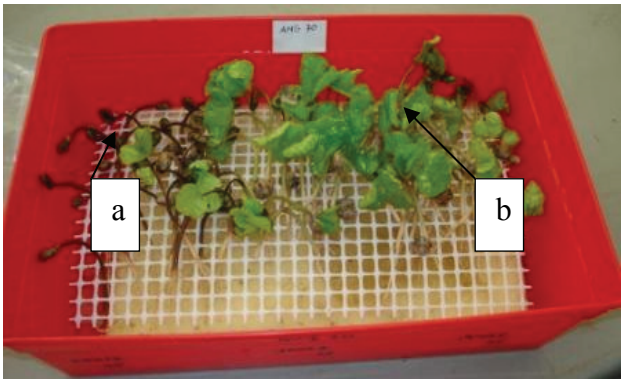


Figure 6. Resistance screening tested in *Coffea* sp. hypocotils inoculated with *C. kahawae* from various origins a: susceptible reaction, b: resistant reaction.

CONCLUSIONS

The results of this research represent an important step to the development of new studies towards a genetic breeding programme in Angola aiming at durable disease resistance, helping to overcome a major limitation to the development of coffee cultivation in this country.

REFERENCES

Masaba D.M., Waller J.M. (1992). Coffee berry disease: The current status. *In Colletotrichum - Biology and Control.* Eds. J.A. Bailey & M.J. Jegger. CAB International. Wallingford, UK. pp. 237-249.

Muller R. A. (1980). Contribution à la Connaissance de la Phytomycocénose Constituée par *Coffea arabica* L., *Colletotrichum coffeanum* Noack (sensu Hindorf), *Hemileia vastatrix* B. et br., *Hemileia coffeicola* Maulanc et Roger. I.F.C.C. Bulletin 6. pp. 9-38.

van der Graaff, N.A. (1981). Selection of arabica coffee types resistant to coffee berry disease in Ethiopia. Mededelingen Landbouwhogeschool. Wageningen. Netherlands. 110 pp.

Variation of *Colletotrichum kahawae* Isolates from Diseased Cherries of Montane Rainforest Coffee in Ethiopia

A. ZERU[1*], F. ASSEFA[2], G. ADUGNA[1], H. HINDORF[3]

[1]Jimma Agricultural Research Centre, P.O.Box 192, Jimma/Ethiopia
[2]Biology Department, Addis Ababa University, P.O. Box 1176, Addis Ababa/Ethiopia
[3]INRES-Phytomedizin, University of Bonn/Germany. [*]E-mail: aregazeru@yahoo.com

SUMMARY

Diseased green coffee cherries were collected from the montane rainforest areas Bonga, Yayu, Berhane-Kontir and Harenna for isolation and characterization of *Colletotrichum kahawae* Waller and Bridge strains from wild populations of *Coffea arabica* L. in Ethiopia. Fifteen representative *C. kahawae* isolates from forest coffee areas and two isolates from Gera (CBD hot spot area) were studied based on their cultural, morphological and pathological characteristics. Isolates could mainly be described in three groups, based on their colony colour manifestation on the obverse side of potato dextrose agar (PDA) and malt extract agar (MEA), i.e. light gray, dark gray and gray/dim gray mycelia forms. The mean radial colony growth rate of *C. kahawae* isolates ranged between 0.6 and 5.5 mm/24 hrs and 1.2 and 6.1 mm/24 hrs on PDA and MEA, respectively. Conidia sizes varied in average of 14.10 ± 0.9 µm in length and 4.21 ± 0.3 µm in width. All isolates showed variable conidia shapes and the most frequent and abundant type from each isolate was cylindrical and round at both ends. Highly significant ($P < 0.01$) variation was observed among *C. kahawae* isolates in their sporulation capacity (after 10 days of growth), which varied between 25.93 x 10^4 and 253.22 x 10^4 conidia ml^{-1}. Pathogenicity tests of 12 isolates on seedlings of four *C. arabica* cultivars indicated that there existed a highly significant difference ($P < 0.01$) among cultivars, isolates, and cultivar x isolate interactions. Hence, the results confirmed the existence of variations both in aggressiveness of the pathogen population and in resistance level of the host. Eventhough, the resistance manifested by cultivars is predominantly horizontal or non-biotype specific, the significant cultivar-by-isolate interaction revealed the presence of some coexistence vertical resistance in Arabica coffee genotypes. This up-to-date information enables as to develop durable CBD resistant coffee varieties, which could be utilized for economical and sustainable coffee production in the country. The difference in virulence and aggressiveness implies that care should be taken while developing resistant varieties. Accordingly, aggressive isolates should be used for successful screening of resistant coffee germplasms before releasing any newly developed coffee cultivars.

INTRODUCTION

Coffee Berry Disease (CBD) known as anthracnose of green and ripe coffee cherries is induced by *Colletotrichum kahawae* Waller and Bridge (syn. *Colletotrichum coffeanum* Noack sensu Hindorf). *C. kahawae* taxonomically belongs to the order of Melanconiales of the Fungi Imperfecti (Hindorf, 1975; Agrios, 2004). The pathogen is one of three species of *Colletotrichum* being isolated from coffee cherries, leaves and branches: *C. kahawae*, *C. gloeosporioides* and *C. acutatum* (Hindorf, 1970; Waller et al., 1993; Biratu, 1995).

The overall national average severity for coffee berry disease in Ethiopia ranged from 24 to 30 % on susceptible landraces (Derso, 1997). But CBD can cause under certain conditions up

to 100 % yield losses. Many research works have been conducted concerning the CBD pathogen and its variability since its confirmation in Ethiopia by Mogk (1971). Based on the colony colour and ability to form saltations *C. kahawae* isolates from Hararge were subdivided into two strains forming 'dark mycelium' and 'grayish mycelium', which showed light grayish to white colour differing to the strain forming saltations (Biratu and Hulluka, 1989). Waller et al. (1993) found common morphological, biochemical and pathogenic characteristics for isolates of *C. kahawae* taken from its range of distribution in Africa.

According to van der Graaff (1981) differential interactions between host and pathogen populations were seldom found in Ethiopia, but minor positive effects were probably caused by gene-for-gene specificity. Biratu (1995) studied variation/similarities within *C. kahawae* isolates collected from Hararge, Illubabor, Kaffa and Sidamo areas and recorded a presence of variations in aggressiveness and absence of races within *C. kahawae* populations based on pathogenicity tests. According to Derso and Waller (2003) the appearance of physiologic races within *C. kahawae* isolates as individual entities are unlikely, it would be useful to look at a profile of several isolates from widely differing coffee types existing in the country, in a locality over time. Apart from the host, the variation in CBD severity could be associated with differences in virulence between *C. kahawae* isolates occurring in different coffee growing regions.

The variability within *C. kahawae* populations from different indigenous forest coffee areas of Ethiopia so far was not known. Therefore in the present study variations within representative *C. kahawae* isolates from different montane rainforest coffee areas were studied using cultural, morphological and pathological criteria. A better knowledge of the pathogen variability allows to develop durable resistant varieties.

MATERIALS AND METHODS

Study areas

Four regions of montane rainforests in Ethiopia were selected for the study: Harenna in the Bale Mountains of the southeast, and in the southwest Bonga in the Kaffa zone, Berhane-Kontir of the Bench Maji zone and Yayu in the Illubabor zone. In each forest region representative sites of 100 x 100 m were chosen for disease assessments and sample collections of diseased materials.

Specimen collection

Infected green coffee cherries were collected in indigenous montane rainforest coffee habitats of Ethiopia. In each forest coffee area 2-6 different CBD affected sites were assessed, and then from 4-5 coffee trees 15-20 green coffee cherries with active CBD lesions were sampled from the respective areas during July to August 2005. *C. kahawae* isolates were sub-cultured on PDA for 10-14 days in an incubator adjusted to 22-25 ^{o}C. Pure cultures from monoconidial isolates were preserved in sterile distilled water at 18-25 ^{o}C for later use of characterization and pathogenicity studies.

Cultural and morphological characterizations of *C. kahawae* isolates

Ranges of cultural variations in the *C. kahawae* population were examined using representative isolates of each forest coffee area including isolates from Gera (CBD hot-spot area) by culturing on potato dextrose agar (PDA) and malt extract agar (MEA), adjusted at pH 5.5 ± 0.1 and incubated at 25 °C in complete darkness. For all experiments the ingredients of

PDA were 200 g/l potato infusion, 20 g/l dextrose and 15 g/l agar, where as the ingredients of MEA were 30 g/l malt extract, 5 g/l peptone and 15 g/l agar.

The vigor of aerial mycelium growth was scored as dense, irregular or very scarce on the obverse side of 10 days old cultures on PDA and MEA. The colony colour from the above and reverse side was also determined on PDA and MEA media using a red-green-blue colour chart (An., 2005) of 10 days old cultures. The radial growth rate was measured in mm/24hrs and the tendency to form saltation was described from 4 cultures per isolate. The conidia sizes (length and width) were computed from 7 days old cultures on PDA and 75-150 conidia per isolate were taken and measured with an ocular micrometer fitted into 10x eyepiece and 40x objective of the microscope. Conidia shapes of representative *C. kahawae* isolates were described using the most frequent five conidial shapes used by Hindorf (1973) and Biratu (1995). The frequency of each shape was tallied from 150 conidia per isolate of 14 days old cultures on PDA.

Pathogenicity tests on detached cherries

The detached cherry test was employed to confirm the infectivity of 18 *Colletotrichum* isolates; which were isolated from infected cherries collected from montane rainforest coffee of Ethiopia: Harenna (4), Bonga (6), Berhane-Kontir (2) and Yayu (6). Completely expanded green coffee cherries were collected, surface sterilized in laundry bleach for 2 minutes and washed in distilled sterilized water. Twenty-five cherries were arranged in a plastic box lined on tissue paper for each isolate inoculation, a plastic net with hollows was used as a substrate to maintain a high level of humidity. The inoculum of each isolate was prepared from 10 days old cultures grown on PDA by washing with distilled sterilized water. Drops of 25 µl of the conidia suspension (2 x 10^6 conidia/ml) were placed at the center of each cherry. Then the boxes were hermetically closed after inoculation to maintain the high relative humidity needed for the infection process and symptom development. After 14 days numbers of infected, healthy and rotten cherries were recorded.

Pathogenicity tests on coffee seedlings

Representative isolates of *C. kahawae* from Harenna (H40, H41, H43), Bonga (B52, B53, B55), Berhane-Kontir (S60, S61), Yayu (Y70, Y73, Y75) and Gera (G81) were cultured on PDA and inoculated on seedlings of resistant (754, 741), moderately resistant (74110) and susceptible (370) cultivars to study the pathogenic variability within the population. In a growth room, seeds of each cultivar were sown (40 seeds/box) in a heat sterilized and moistened sandy soil in disinfected plastic boxes (25.5 cm x 15 cm x 6 cm = 2295 cm^3) arranged on benches and covered with chip-wood. Five weeks after sowing, hypocotyls of the seedlings emerged and were maintained in a growth room at 20-25 °C with 12 hrs alternate light until unfolding stage of the hypocotyls. During the unfolding stage, hypocotyls of seedlings were maintained at 20 ^oC, kept wet with sterilized water, and covered with plastic sheets for 48 hrs to obtain 100% relative humidity. 48 hrs later, the conidia suspension of each isolate was prepared from 10 days cultures with 2 x 10^6 conidia/ml concentration. Then batches of three plastic boxes from each coffee cultivar containing 25 seedlings/box were arranged on benches for each isolate and inoculated by dipping a fine camel hairbrush into the conidia suspension and brushing each seedling stem with the inoculum. The second re-inoculation was after 48 hrs in the same procedure and maintained wet for additional 48 hrs. The temperature was adjusted to 20-25 °C for three weeks. A randomize complete block design in three replication was employed.

The reaction of each seedling of coffee cultivars against isolates was assessed 15 and 21 days after inoculation using the symptom classifications (0-4 scale) of van der Graaff (1981). A disease index (DI) for each assessment was expressed as a percentage of the maximum possible infection by modifying the equation used by O'Sullivan and Kavanagh (1991):

$$DI = 100 (w + 2x + 3y + 4z) / 4(v + w+ x + y + z)$$

where as v = number of seedlings in class 0, w = number of seedlings in class 1, x = number of seedlings in class 2, y = number of seedlings in class 3, z = number of seedlings in class 4. The fractions were analyzed after angular transformation.

Data analysis

All data were analyzed following the respective statistical procedures and treatment means were compared using Duncan's Multiple Range Test, DMRT (Gomez and Gomez, 1984; Townsend, 2002). MSTAT-C and SAS microcomputer statistical software packages were employed to perform analysis of variance (ANOVA) and mean comparisons. Excel microcomputer statistical software was also employed to manipulate graphs.

RESULTS AND DISCUSSION

Cultural and morphological characteristics of *C. kahawae* isolates from forest coffee

The pathogenicity test of isolates on detached coffee cherries revealed two forms of *Colletotrichum* species: *C. kahawae* being pathogenic and *C. gloeosporioides*, which behaved most probably as saprophytic or sequential colonizer of dead tissues. Similar results were reported by Biratu and Hulluka (1989), Biratu (1995) and Derso and Waller (2003). As Hindorf et al. (1997) stated for correct identification and characterization one still needs to isolate the fungal population, detecting morphological features in vitro such as mycelium colour, growth rate, conidial production and testing the pathogenicity on hypocotyls or cherries. Accordingly, 17 isolates (15 from forest coffee areas and 2 from Gera) were used for detail quantitative and qualitative analysis of the CBD pathogen. Out of 17 *C. kahawae* isolates tested for their aerial mycelia growth 47.1% showed consistently dense aerial mycelia growth on both PDA and MEA media, where as 11.8 and 5.8% isolates revealed irregular and very scarce aerial mycelia growth on both PDA and MEA media, respectively. The rest 35.3% isolates showed inconsistent aerial mycelia growth.

Based on their colony colour the manifestation of isolates on the obverse side of PDA and MEA media could be grouped as light gray, dark gray and gray mycelia forms (Table 1). Isolates from Berhane-Kontir (S60, S61), Bonga (B52, B53), Yayu (Y70, Y72, Y73, Y75) and Gera (G80) produced the distinct light gray mycelium form. In this group *C. kahawae* isolates showed a white mycelia colour during the first 4-6 incubation days and then changed into light gray mycelia on both PDA and MEA media. However, isolates B52, Y70 and Y71 revealed dark olivegreen or dark olivebrown, when becoming older and others remained light gray. Similarly, Hindorf (1970) observed that *C. kahawae* isolates initially had a white mycelium and then changed after 4-6 days to gray and eventually to dark olive brown. Biratu (1995) also reported of light bluish gray coloured *C. kahawae* isolates from Kaffa and Illubabor on PDA. In the second group, isolates H43 (Harenna), B51 (Bonga) and Y74 (Yayu) showed dark gray colony colour in the first 15 days of the incubation period. In other studies similar colony colours of *C. kahawae* isolates were observed. Biratu and Hulluka (1989) found a grayish mycelium form (light grayish to white) of *C. kahawae* from Hararge.

In the third group, isolates from Harenna (H40, H41), Bonga (B50, B55) and Gera (G81) revealed initially a light gray colour and changed from gray after 5-7 days of incubation and then into dark olivegreen after 15 days. Similarly, Hindorf (1975) reported that the culture of *C. kahawae* forms a gray to blackish colony on malt extract agar. On the reverse side of culture plates, colonies of the first group revealed whitish yellow or light yellow colours in the first 4-6 days of growth and then changed into various combinations of colours. The second group manifested a light gray colour in the first 4-6 days and then changed into various colour combinations. The third group showed light orange and/or rosy brown colour and then changed into various colour combinations.

The colours of *C. kahawae* isolates were consistent at 12-30 °C. Forty one percent of isolates showed saltations, which differ in colour from the mother isolates on PDA. Sectors in all groups were lighter in colour and/or some isolate like Y75 showed an orange or light yellow colour, which indicated the existence of mutations. The result confirmed the observation of Biratu and Hulluka (1989), that sectors originated in any part of the colony of the mother isolates and the strains indicated unstable characteristics, i.e. some were revertible while others remained stable.

The radial colony growth of isolates was faster on MEA than on PDA (Fig.1). The mean colony diameters of *C. kahawae* isolates were 35.9, 57.7 and 71.7 mm on MEA and 30.9, 46.7 and 57.3 mm on PDA after 7, 10 and 15 days of incubation, respectively. The mean radial colony growth rate of *C. kahawae* isolates ranged between 0.6 and 5.5 mm/24 hrs on PDA and 1.2 and 6.1 mm/24 hrs on MEA (Table 2). A faster growth rate on MEA could be the presence of peptone (5 g/l) in the MEA substrate composition. Utilization of peptone by the isolates revealed, that *C. kahawae* isolates could release peptidase to degrade peptone. The mean radial colony growth rate of *C. kahawae* isolates varied on PDA and MEA, i.e. 4.4 ± 0.36 and 5.1 ± 0.35 mm/24 hrs, respectively. Similarly, Waller et al. (1993) recorded 4 mm/24 hrs for the growth rate of the CBD pathogen. Our results indicated a faster mean growth rate as compared to Hindorf (1970; 1973), i.e. 1.9 ± 0.5 mm/24hrs for the average mycelia growth rate of CBD isolates at 22^{o}C incubation on 2% Oxoid malt extract agar. However, our results were comparable with those of Biratu and Hulluka (1989). These authors recorded a growth rate of 6.5 and 6.7 mm/24 hrs on PDA (25 ± 1 °C) for dark mycelia and grayish mycelia isolates of the pathogen, respectively.

The sizes of *C. kahawae* conidia varied. The conidia length and width ranged from 12.7-15.5 µm and 3.6-4.8 µm, respectively, and the average size was 14.10 ± 0.9 x 4.21 ± 0.3 µm. Hindorf (1973) recorded 13.1 ± 0.6 µm x 3.8 ± 0.2 µm and 10.8-23.0 x 3.4-4.7 µm as average and range of the conidia length and width, respectively. Biratu and Hulluka (1989) reported of 15.3 x 3.5 µm as the average conidia size from isolates. Biratu (1995) also reported of variable mean conidia length (13.5 -19.3 µm) and width (2.9-5.2 µm) on PDA. The conidia shapes of *C. kahawae* isolates proved to be as well variable. The five types of conidia shapes were frequently observed in different proportion, when being produced in each isolate. But the conidia shape of type 1 (cylindrical and round at both ends) dominated in all isolates and accounted a proportion ranging between 49 and 88%.

Table 1. Colony colour of *C. kahawae* isolates on PDA and MEA observed after 10 days of growth.

Isolate[1]	Colony colour on media			
	PDA		MEA	
	TOP	REV	TOP	REV
H40	Gray	Dark olivegreen	Dim gray	Dark olivegreen
H41	Gray	Dark olivegreen	Dim gray	Dark olivegreen
H43	Dark gray	Dark olivegreen	Dark gray	Dark olivegreen
B50	Gray	Dim gray	Gray	Dim gray
B51	Dark gray	Dark olivegreen	Gray	Dark olivegreen
B52	Light gray	Dark olivegreen	Gray	Dark olivegreen
B53	Light gray	Dim gray	Light gray	Dim gray
B55	Gray	Rosy-brown	Dark gray	Dark olivegreen
S60	Light gray	Gray	Light gray	Dim gray
S61	Light gray	Dim gray	Light gray	Dim gray
Y70	Light gray	Dark olivegreen	Light gray	Dark olivegreen
Y71	Light gray	Dark olivegreen	Light gray	Dark olivegreen
Y73	Light gray	Dim gray	Light gray	Dim gray
Y74	Dark gray	Gray	Gray	Gray
Y75	Light gray	Rosy-brown	Light gray	Rosy-brown
G80	Light gray	Rosy-brown	Light gray	Rosy-brown
G81	Gray	Dark olivegreen	Gray	Dark olivegreen

[1]*C. kahawae isolates coded with 'H, B, S and G' were collected from Harenna (Bale), Bonga, Berhane-Kontir, Yayu and Gera, respectively.* ***TOP:*** *Colony colour from above;* ***REV:*** *Colony colour from the reverse side of the Petri-dish.*

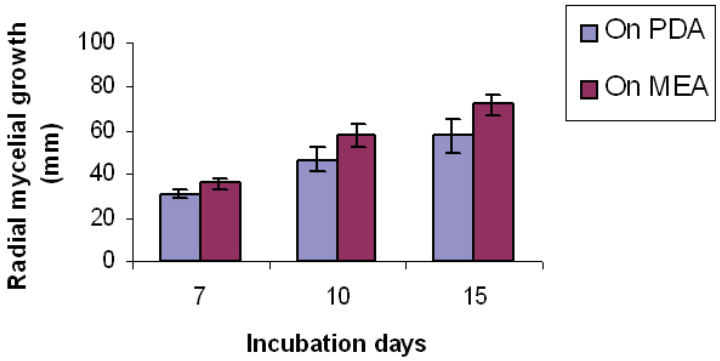


Figure 1. Radial mycelia growth of *C. kahawae* isolates on PDA and MEA after 7, 10 and 15 incubation days (error bars are standard deviations).

The conidial production of 10 days old cultures showed highly significant ($P < 0.01$) differences among isolates. The conidia production varied between 25.93 x 10^4 (Y70) and 253.22 x 10^4 conidia/ml (S60). The isolate S60 produced a high amount of conidia, followed by isolates B52, B53 and Y74 producing 148.22 x 10^4, 146.44 x 10^4 and 136.91 x 10^4 conidia/ml, respectively (Table 2). Isolate B52 with a highly aggressive reaction on all coffee cultivars produced an intermediate amount of conidia as compared to the isolate S60 (Table 2). Variations in conidia production of different cultures were observed confirming the findings of Biratu (1995).

Variation in the pathogenicity of *C. kahawae* isolates

Pathogenicity tests of 12 isolates on seedlings of four *C. arabica* cultivars indicated that there existed a high significant ($P < 0.01$) difference among cultivars, isolates and cultivar x isolate interactions. Seedlings of cultivars 741 and 754 exhibited resistant reactions to all isolates from montane forests and Gera, implying horizontal resistant reactions, where as cultivar 370 revealed highly susceptible reactions to all isolates (Table 3). Cultivar 74110 also showed susceptible reactions to nearly all isolates except isolate Y75, for which a low level of 21.0 % mean percent infection was recorded. The inheritance of resistance to coffee berry disease was studied by Mesfin and Belachew (1984). These authors suggested, that the CBD resistance in cultivars 741 and 754 was controlled by recessive three to five major genes of additive nature, where as the susceptibility was controlled by partial to complete dominant genes. According to Biratu (1995) cultivar 74110 had field resistance, but unusually susceptibility in hypocotyl reactions.

Table 2. Radial growth rate, aerial mycelia growth, sectoring and conidial production capacity of 17 C. *kahawae* isolates.

Isolate[1]	Radial growth rate (mm/24hr)		Sectoring	Aerial mycelia growth (vigor)[2]		Conidia production (x10,000 conidia/ml)
	PDA	MEA	PDA	PDA	MEA	PDA
H40	4.9 a-c	5.2 D	Absent	+	+	115.22 O
H41	4.6 b-d	5.2 D	Present	+	+	113.33 O
H43	4.7 b-d	6.0 AB	Absent	++	+	104.67 OP
B50	4.6 cd	5.9 A-C	Absent	++	+	50.89 S
B51	4.9 a-c	5.4 CD	Present	+	+	89.78 PQ
B52	4.8 b-d	5.7 A-D	Absent	+	+	148.22 N
B53	4.5 cd	6.1 AB	Present	+	+	146.44 N
B55	4.2 de	5.5 A-D	Absent	++	+	87.33 PQ
S60	5.5 a	6.0 A-C	Absent	+	+	253.22 M
S61	4.6 cd	5.4 B-D	Absent	++	++	73.78 QR
Y70	3.6 e	4.0 E	Present	+	++	25.93 T
Y71	4.8 b-d	5.3 D	Present	+	+	106.56 OP
Y73	5.3 ab	4.4 E	Absent	++	+	66.25 RS
Y74	5.5 a	6.1 A	Absent	++	++	136.91 N
Y75	0.6 f	1.2 F	Present	+++	+++	49.78 S
G80	3.6 e	4.4 E	Absent	++	+	73.44 QR
G81	4.4 cd	5.5 B-D	Present	+	+	76.89 QR

[1]*C. kahawae isolates coded with 'H, B, S and G' were collected from Harenna (Bale), Bonga, Sheko, Yayu and Gera, respectively. Means followed with the same letter(s) are not significantly different according to DMRT. LSD values (P = 0.05) for the radial growth rate on PDA, radial growth rate on MEA and the conidia production capacity comparisons are 0.29, 0.28 and 10.1, respectively.*

[2]*Aerial mycelia growth (vigor): += dense, ++= irregular (scarce), +++= very scarce*

Table 3. Pathogenicity of 12 *C. kahawae* isolates inoculated on seedlings of 4 *Coffea Arabica* cultivars 21 days after inoculation in growth room.

Isolate[1]	*Coffea arabica* cultivars[2]				Mean
	741	754	74110	370	
H40	14.0 gh	20.3 g	88.7 b-d	100 a	55.8 AB
H41	12.7 gh	17.8 gh	85.3 b-e	100 a	54.0 A-C
H43	14.2 gh	15.4 gh	78.3 d-f	98.0 a	51.5 CD
B52	20.2 g	17.9 gh	89.5 bc	100 a	56.9 A
B53	16.5 gh	13.8 gh	86.6 b-e	100 a	54.2 A-C
B55	17.7 gh	18.9 gh	77.8 d-f	98.3 a	53.2 B-D
S60	12.3 gh	13.7 gh	80.3 c-f	100 a	51.6 B-D
S61	10.7 gh	14.3 gh	76.9 ef	97.6 a	49.9 D
Y70	9.0 h	15.0 gh	79.0 d-f	100 a	50.8 CD
Y73	14.6 gh	18.5 gh	92.7 b	98.3 a	56.0 AB
Y75	16.0 gh	10.8 gh	21.0 g	70.3 f	29.5 E
G81	21.8 g	9.3 h	79.3 d-f	98.3 a	52.2 B-D
Mean	15.0 Z	15.5 Z	78.0 Y	96.7 X	

[1] C. kahawae isolates coded with 'H, B, S and G' were collected from Harenna (Bale), Bonga, Berhane-Kontir, Yayu and Gera, respectively. [2] Coffee cultivar 741 and 754 are CBD resistant and 74110 is intermediate resistant, where as 370 is a susceptible check. Means followed with the same letter(s) are not significantly different according to DMRT. LSD values (P = 0.05) for the cultivars, isolates and the interactions comparisons are 2.1, 3.7 and 7.3, respectively.

Significant differences of main effects, i.e. differences among host genotypes and/or isolates in the analysis of variance, indicated that resistance and pathogenicity are horizontal (van der Plank, 1984; Biratu, 1995). Their seedling inoculation tests confirmed the high aggressiveness in *C. kahawae* populations associated with lower latent periods and high mean percent infections of inoculated coffee hypocotyls. Eventhough, the resistance manifested by cultivars is predominantly horizontal or non-biotype specific, the significant difference of cultivars by isolate interactions revealed the presence of some coexistence vertical resistance in Arabica coffee genotypes with a small positive effect (van der Plank, 1984, van der Graaff, 1981). Isolates B52, Y73, H40, B53 and H41, followed by B55, G81 and S60, induced high percent of seedling infections on all Arabica coffee cultivars as compared to other isolates (Table 3). These isolates showed a significant high aggressiveness on cvs. 74110 and 370 and less aggressiveness on cvs. 741 and 754. Isolate Y75 was the weakest on cv. 74110 causing 21.0 % mean seedling infections, but this cultivar exhibited high susceptibility reactions to other isolates with mean percent infections ranging between 76.9 to 92.7% (Table 3). The isolate Y75 revealed with the lowest infection rate of 70.3% also less aggressive reactions on seedlings of coffee cv. 370 as compared to other isolates. Cvs. 741 and 754 showed significantly lower seedling infections, i.e. 15.0 and 15.5%, respectively.

CONCLUSION AND RECOMMENDATIONS

Fifteen representative *C. kahawae* isolates from forest coffee areas of Ethiopia and two isolates from the CBD hot spot area of Gera were studied based on their cultural, morphological and pathological criteria. No race difference was observed within *C. kahawae* isolates; however, certain cultural and morphological variations as well as significant variations in aggressiveness were detected among them. This information has assured to

develop durable CBD resistant coffee varieties, which could be utilized for economical and sustainable coffee production in the country. Although the likelihood of forming new races appeared to be rare, periodical checking of the pathogen consistency by collecting samples from all respective coffee producing areas of the country is paramount. The seedling inoculation test has confirmed high aggressiveness in the *C. kahawae* population associated with a high mean percent infection rate of inoculated hypocotyls of coffee seedlings. The difference in virulence and aggressiveness implies that care should be taken and for further development of resistant varieties aggressive isolates should be used for a successful screening of coffee germplasms before the release of new developed cultivars.

ACKNOWLEDGEMENTS

The work could be carried out as part of a joint project "Conservation of wild coffee in montane rainforests of Ethiopia (CoCE)" coordinated by the "Center for Development Research (ZEF)" of Bonn University and were financially supported by the German Ministry of Education and Research (BMBF). We kindly acknowledge the fruitful cooperation and assistance of the staff of the "Jimma Agricultural Research Center" of EIAR, Addis Ababa/Ethiopia.

REFERENCES

Anonymous (2005). http://HTM help central-com. Hexid-RGB colour chart-files. Accessed on 25/12/2005.

Agrios G.N. (2004). Plant pathology, 4th ed., Academic Press, Imprint of Elsevier, 635 pp.

Biratu T. and Hulluka M. (1989). *Colletotrichum* species associated with Coffee Berry Disease in Hararge. Eth. J. Agric. Sci. 11: 1-6.

Biratu T. (1995). Studies on *Colletotrichum* population of *Coffea arabica* L. in Ethiopia and evaluation of the reactions of coffee germplasms. PhD thesis, Bonn/Germany, 231 pp.

Derso E. (1997). Coffee diseases and their significance in Ethiopia. ASIC 17(I): 723-726.

Derso E. and Waller J.M. (2003). Variation among *Colletotrichum* isolates from diseased coffee berries in Ethiopia. Elsevier Science Crop Protection 22: 561-565.

Gomez. K. A. and Gomez, A. A. (1984). Statistical procedures for agricultural research. 2nd ed., John Wiley & Sons Inc., New York/USA, 680 pp.

Hindorf H. (1970). *Colletotrichum* spp. isolated from *Coffea arabica* in Kenya. Z. Pflanzenkrh. Pflanzenschutz 77: 328-331.

Hindorf, H. (1973). *Colletotrichum*-population on *Coffea arabica* L. in Kenya. II. Qualitative and quantitative differences in the *Colletotrichum*-population. Phytopath. Z. 77: 216-234.

Hindorf H. (1975). *Colletotrichum* occurring on *Coffea arabica*: A review. J. Coffee Res. 5: 43-56.

Hindorf H., Biratu T. and Omondi C. (1997). Correct identification of the pathogen *Colletotrichum kahawae* causing Coffee Berry Disease (CBD). ASIC 17: 599-603.

Mesfin A. and Belachew B. (1984). Resistance of the F1 to Coffee Berry Disease in six parent diallel crosses in coffee. In: Proc. 1st Workshop CBD, Addis Ababa/Ethiopia, pp.167-177.

Mogk M. (1971). Report on the coffee disease survey in Ethiopia. IAR Addis Ababa/Ethiopia.

O'Sullivan E. and Kavanagh J.A. (1991). Characteristics and pathogenicity of isolates of *Rhizoctonia* spp. associated with damping-off of sugar beets. Pl. Path. 40: 128-135.

Townsend J. (2002). Practical statistics for environmental and biological scientists. John Wiley & Sons Ltd., England, 276 pp.

Van der Graaff N.A. (1981). Selection for Arabica coffee types resistant to CBD in Ethiopia. PhD thesis, Wageningen/Netherlands, 110 pp.

Van der Plank J.E. (1984). Disease resistance in plants. 2nd ed., Ac. Press London/UK., 194 pp.

Waller J.M., Bridge P.D., Black R. and Hakiza H. (1993). Characterisation of the Coffee Berry Disease pathogen, *Colletotrichum kahawae* sp. nova. Mycol. Res. 97: 989-994.

Disease Resistance and Cup Quality in Arabica Coffee: the Persistent Myths in the Coffee Trade versus Scientific Evidence

H.A.M. VAN DER VOSSEN

Plant Breeding & Seed Consultant, 1606 CA 18 Venhuizen, the Netherlands.
E-mail: herbert.vandervossen@quicknet.nl

SUMMARY

Traditional cultivars of arabica coffee (*Coffea arabica*) are susceptible to coffee leaf rust (CLR, *Hemileia vastatrix*) and coffee berry disease (CBD, *Colletotrichum kahawae*). CLR is of worldwide importance, while CBD is still restricted to Africa, causing altogether to the coffee producing countries an estimated annual economic damage of some $ 2-2.5 billion in crop losses and costs of chemical control. Host resistances to both destructive diseases present in *C. canephora* have been successfully introgressed into *C. arabica*, after several decades of breeding and selection notably in Brasil, Colombia, India and for CBD resistance in East Africa. The CLR resistant cultivars Catimor, Sarchimor and their (hybrid) derivatives have since been grown on hundred thousands of hectares in Latin America and elsewhere, while CBD (and CLR) resistant cultivars, like Ruiru 11 of Kenya, are now increasingly being planted in East and South-Eastern Africa. They contribute to ecologically sustainable coffee production and to considerable socio-economic benefits for the coffee growers, by higher yields at much reduced production costs without necessarily affecting cup quality. Nevertheless, some representatives of the international coffee trade continue to denounce these modern cultivars for lack of flavour so typical of traditional varieties. However, in additon to the genetic background of a cultivar, there are many environmental factors influencing bean and cup quality characteristics, such as the agro-ecosystem (climate, altitude, soils and cultural practices) and post-harvest handling (primary processing, storage and shipping). This paper presents an overview of the considerable amount of scientific evidence accumulated over the years showing that, with all non-genetic factors at optimum level, these CLR and CBD resistant cultivars can be equal in quality to the best of traditional varieties in professional cup testing trials. The prejudices of the coffee trade appear, therefore, vastly exaggerated. On the other hand, coffee breeders should continue to apply maximum selection pressure for high quality and refrain from premature release of new disease resistant cultivars before exhaustive testing for cup quality.

INTRODUCTION

World production of arabica coffee (*Coffea arabica* L.) is still largely based on cultivars developed long ago by line selection within the *typica* and *bourbon* varieties, or in offspring of crosses between these two, for instance the Brazilian cultivar Mundo Novo. Semi-dwarf cultivars, such as Caturra and Villa Sarchi, originated as single-gene mutants (Krug & Carvalho, 1951). These traditional cultivars are often renown for their excellent cup quality, but most are very susceptible to the world-wide occurring coffee leaf rust (CLR), caused by *Hemileia vastatrix*, and coffee berry disease (CBD), caused by *Colletotrichum kahawae* and still restricted to Africa. The total economic damage in crop losses and costs of chemical control for these two diseases to world coffee production is estimated at $ 2-2.5 billion annually, not counting the potential environmental hazards of copper-based fungicides in particular.

Efforts to obtain host resistance to CLR, with first results reported in India around 1920, have had a long history of initial successes followed by disappointments because of repeated appearances of new virulent races of the rust fungus. However, some lines of the cultivar Catimor, which was developed from crosses between Caturra and Hibrido de Timor (HdT), have shown complete resistance to all physiological races of the CLR pathogen in most countries. These results were obtained by breeding plans normally applied to self-pollinating crops, including recombination crosses followed by backcrossing, inbreeding and pedigree selection. (Carvalho, 1988; Bettencourt and Rodrigues, 1988). The breeding programme in Kenya demonstrated the advantages of F1 hybrid cultivars also for arabica coffee, especially the simultaneous combination of compact plant type, high yield, good quality and different disease resistances (CBD and CLR) in one cultivar (Van der Vossen and Walyaro, 1981). Subsequently, breeding strategies aiming at F1 hybrid cultivars in arabica coffee have been adopted elsewhere, e.g. in Ethiopia (Bellachew, 1997), Tanzania (Teri et al., 2004) and in Central American countries (Bertrand et al., 1997, 2006).

The CLR resistant cultivars Catimor, Sarchimor and their (hybrid) derivatives have since been grown on hundred thousands of hectares in Latin America and elsewhere, while CBD (and CLR) resistant cultivars, like Ruiru 11 of Kenya, are now increasingly being planted in East and South-Eastern Africa. They contribute to ecologically sustainable coffee production and to considerable socio-economic benefits for the coffee growers, by higher yields at much reduced production costs without necessarily affecting cup quality. Nevertheless, some representatives of the international coffee trade continue to denounce these modern cultivars for lack of flavour so typical of traditional varieties (e.g. Illy, 2001). However, in additon to the genetic background of a cultivar, there are many environmental factors influencing bean and cup quality characteristics, such as the agro-ecosystem and post-harvest handling. This paper presents an overview of the considerable amount of scientific evidence accumulated over the years showing that, with all non-genetic factors at optimum level, these CLR and CBD resistant cultivars can be equal in quality to the best of traditional varieties according to professional cup tasting trials.

DEFINITION AND ASSESSMENT OF COFFEE QUALITY

Three main categories are distinguished in the coffee market (ITC, 2002): (1) mainstream washed and natural (dry- and semi dry-processed) arabica and robusta coffees of fair average quality (FAQ), which account for 85-90% of world coffee consumption, (2) high quality washed arabicas and robustas and (3) coffees of top quality and often in limited supply, usually single-origin washed arabicas, some superior washed robustas, but also a few natural arabicas (e.g. Ethiopian Harar,).

Bean size is determined mechanically using metal screens with round (normal bean) and slotted (pea berry) perforations varying in size, the presence of defective beans by counting and moisture content by moisture meters. Otherwise, coffee quality is entirely based on visual and sensory evaluation by expert coffee tasters. Green bean colour is a good indication of freshness, moisture content and homogeneity: a green-bluish colour of washed arabicas is a sign of high quality. Freshly roasted arabica coffee having a bright and even appearance with white and tight centre-cuts will usually produce a good quality beverage.

The beverage – generally an infusion of ground, freshly roasted beans (10 g) and hot water (150 ml) – is evaluated along the following major characteristcs (Wintgens, 2004):

- Fragrance: the smell of the dry roasted and ground coffee;
- Aroma: smell in the cup a few minutes after pouring hot water on the ground coffee;

- Acidity: a pleasing taste of the beverage, from low (sweet) to high (fruity/citrus);
- Body: mouth feel of the beverage
- Flavour: a combination of taste and aroma.

Flavour is the most important factor determining quality. Positive flavours are described in terms of winey, spicy and fragrant/floral; grassy, onion, woody and earthy are a few of many off-flavours (ITC, 2002). Low-quality natural arabica coffees often produce bitter and astringent beverages. Sometimes the aftertaste, which remains after the the mouth is cleared, is scored separately. Finally, the coffee is given a total or weighted average score to classify the quality of the sampled coffee lot. The scoring system applied in the ranking of coffee quality is either ascending (1 = poor) or descending (1 = best, e.g. in East Africa). In this review paper all quality data have been adjusted to ascending scores.

Taste and flavour requirements vary considerably with market segments. For instance, in northern Europe there is a general preference for acidity, light body and pleasant flavour of the cup, while in the south the coffee should taste sweet with a full body and strong flavour (Barel and Jacquet, 1994).

Green coffee beans consist mainly of endosperm with the following chemical composition: water (11-13%), cellulose and other polysaccharides (40-55%), sucrose and reducing sugars (5-9%), lipids (13-17%), proteins and free amino acids (11-16%), phenolic compounds including chlorogenic acids (10-15%), alkaloids including trigonelline (1-1.2%) and caffeine (0.7-3.5%), minerals (4%). Arabica coffees are generally higher in carbohydrate, sugar, lipid and lower in caffeine contents than robustas (Clifford, 1985). During roasting, the high temperature and pressure inside the beans trigger a large number of chemical reactions resulting in the dark colour and the 850 volatile and another 150 non-volatile compounds identified so far, many of which determine coffee aroma and flavour characteristics (Bonnländer et al., 2005). This complex situation has so far defied any analytical method of producing a quantitative chemical profile of green or roasted mild arabica coffees, which correlates well with the beverage quality as determined by cup tasting (Viani, 2000; Avelino et al., 2005; Bertrand et al., 2006). However, Farah et al. (2006) found a significant positive correlation between trigonelline and chlorogenic acid (mainly 3,4-dicaffeoylquinic acid) levels in green beans and cup tasting results for Brazilian natural coffees.

ENVIRONMENTAL FACTORS INFLUENCING COFFEE QUALITY

The agro-ecosystem

Arabica coffee originated from the highlands of south-western Ethiopia and its mild and pleasant beverage is best preserved under similar growing conditions. High altitudes are critical for the successful production of high-quality arabica coffees in equatorial regions (Illy and Viani., 2005). Lower temperatures, and their larger daily amplitudes, induce slower growth and more uniform ripening of the berries, that result in larger and denser beans, an increase of important precursors of aroma and flavour in the bean and superior beverage quality. Altitude has a significantly positive effect on acidity and aroma, while reducing bitterness (Avelino et al., 2005; Bertrand et al., 2006). Shade has a similar positive effect on coffee quality, particularly at medium altitudes, but also reduces yields (Guyot et al., 1996; Muschler, 2001; Decacy et al., 2003; Vaast et al., 2006). Shade did not improve cup quality at very high (> 1800 m) altitudes in Costa Rica (Avelino et al., 2007).

Rainfall requirements for arabica coffee production are at least 1200 mm per year with a maximum of 2500 mm. Coffee plants grow and yield better if exposed to alternate cycles of

wet and dry seasons, and moreover, a period of water deficit is important to synchronize flower bud differentiation. Areas with excess precipitation, especially during crop maturation, have a tendency to produce lower quality coffee due to irregular cherry ripening and poor conditions for drying the crop after harvesting. In years of excessively long dry seasons, shoot dieback and premature ripening of the berries will result in light beans producing a liquor with immature and astringent notes.

Coffee can be cultivated on a wide range of soil types, provided these are at least 2 m deep, free draining loams with a good water-holding capacity and a pH 5-6, fertile and contain at least 2% organic matter. High-quality, acidic arabica coffees are mostly produced on soils of volcanic origin. A balanced nutrient status of the coffee tree is essential for the production of high quality coffee. Nitrogen is important for vegetative growth and influences the caffeine content, but. excess N-fertilization may have a negative effect on cup quality. Potassium is a key nutrient during bean filling and influences total sugar and citric acid content. Phosphorus is required for root, shoot and fruit development. Excessive calcium and potassium produces a hard and bitter tasting liquor. Iron deficiency gives amber and magnesium deficiency brown and soft beans. Boron influences flowering and fruit set and consequently yields The effective control of pests and diseases is also essential for the production of quality coffee (Wintgens, 2004).

Harvesting and post-harvest handling

Inherently high quality coffee can easily be degraded by suboptimal harvesting, processing and storage practices. Only freshly harvested and fully ripe berries should be used in any of the three methods (washed, semi-dry and dry) of primary processing. This can be achieved by selective hand-picking or by mechanized techniques of separating the immature green from the ripe cherry before processing. Unripe coffee beans cause astringency, bitterness and a grassy off-flavour in the beverage. Delayed depulping and nipping of the beans due to faulty pulping machines, as well as prolonged fermentation can lead to onion flavour or still worse to “stinker” beans, which cause very unpleasant off-flavours. The fermented off-flavour is usually caused by under-fermentation. There are many other causes of off-flavours due to inadequate processing, drying and storage (Wintgens, 2004; Illy and Viani, 2005).

The method of mucelage removal during wet processing, either by fermentation or mechanically, does not very much influence coffee quality. Wet fermentation may somewhat improve flavour, as does soaking under water for 24 hours after mucelage removal and washing (Wintgens, 2004). Gonzales-Rios et al. (2007a & b) claim that the quality of green and roasted coffee, as measured by volatile aroma composition, was better after conventional fermentation than after mechanical mucelage removal. However, there were no data from sensory evaluation, which is the best means of determining quality of the coffee beverage. Besides, underwater soaking of the parchment after mechanical mucelage removal would probably have improved the quality of these coffees and so reduced the differences.

Beverage quality varies according to the processing methods applied. Washed arabicas from East Africa (Kenya, Tanzania, Ethiopia) and Latin America (Colombia, Guatemala, Costa Rica) are characterized by mild to pointed acidity, light body and intense aroma. Natural dry-processed arabicas from Brazil and Ethiopia have low acidity, less marked aroma, but much stronger body, which is sought after in espresso coffees (Illy and Viani, 2005).

THE QUALITY OF DISEASE RESISTANT CULTIVARS

Selection for bean size and cup quality has received much attention in arabica coffee breeding programmes – in Kenya, Tanzania and Colombia in particular – because the quality of new disease-resistant cultivars should be at least equal to that of the traditional cultivars in order to uphold the country's high reputation and special position in the world coffee market (Van der Vossen, 1985; 2001).

Kenya

The beverage quality of the CBD and CLR resistant cultivar Ruiru 11 has been challenged by the coffee trade since its first release by the CRF (Coffee Research Foundation) in Kenya in 1986. However, much evidence has been accumulated over the subsequent years, from national and international coffee tasting panels, showing that the beverage quality of Ruiru 11 does not deviate significantly from that of the best traditional *bourbon* – type cultivars like SL28 (Njoroge et al., 1989; Omondi, 2008). Interestingly, coffee lots of Ruiru 11 auctioned recently by a large coffee plantation company (E.Delbar, 2008, personal communication) got premium prices equal to those for SL28 or other traditional cultivars and sometimes even slighly (4-5%) higher: $ 3.8-4.8/ kg coffee of grade AA (ICO June 2008 average for Colombian Milds: $ 3.3).

A summary of the performance of Ruiru 11 against other cultivars, for yield, bean size and overall beverage quality, is presented in Table 1. The hybrid vigour is clearly reflected in higher productivity, while bean size and beverage quality compare well with SL28. Ruiru 11 also saves the coffee growers at least 30% in total variable costs of production, otherwise spent on chemical control of CBD and CLR (Karanja, 1993).

Table 1. Yield and quality of arabica cultivars in Kenya.

Cultivar	Pedigree	Clean coffee		Bean		Beverage
		(mean 1985-91)	PB	AA	AB	quality
		t/ha/year	%	%	%	(score 1 - 7)
SL28	Bourbon Drought Resistant II	2.0 - 2.5	11	39	42	5.0 - 6.5
K7	Kents	2.0 - 2.5	8	38	45	3.5 - 5.0
Catimor	F4 progenies ex Colombia	1.5 - 2.5	10	35	44	4.0 - 5.0
Ruiru 11	F1 hybrid	2.5 - 3.0	18	39	40	5.0 - 6.0
	(Catimor x selected clones of					
	Kenyan breeding programme)					

Note: Data from field trials at CRF, Ruiru; plant density 3,300 tr/ha; Sl28 and K7 sprayed with fungicides to control CBD & CLR; beverage quality: scores 1 = poor to 7 = excellent.

Tanzania

TaCRI (Tanzanian Coffee Research Institute) has been releasing clonal hybrid cultivars with host resistance to CBD and CLR since 2004. In addition to displaying hybrid vigour for yield, these new cultivars have in most cases a beverage quality, as assessed by professional tasters of the TCB (Tanzanian Coffee Board) and the private coffee sector, comparable to the best traditional cultivars like N39 and KP423 (Teri et al., 2004; TaCRI, 2005).

Colombia

In 1982, CENICAFE (National Coffee Research Centre) released the cultivar Colombia, which is made up of a number of Catimor lines selected for CLR resistance, yield, bean size and quality. The beverage quality was shown to be similar to the traditional *typica*, *bourbon* and Caturra varieties (Moreno et al., 1996). Continued selection and breeding has resulted in the new composite cultivar "Castillo". It is characterized by more durable CLR resistance and also by a level of beverage quality, that appears to meet the critical market for fine Colombian arabica coffees (Alvaredo et al., 2008).

Origin and nature of the Hibrido de Timor (HdT) variety

In the 1960s, about 40% of the coffee cultivated in Timor-Leste was robusta and 60% arabica coffee, which for 80% consisted of the CLR-resistant HdT variety (Krug and De Poerck, 1968). Initially planted on one coffee estate in the 1940s to replace the highly CLR-susceptible *typica* coffee, HdT was assumed to have arisen from a spontaneous cross between arabica and robusta coffee (Rodrigues et al., 1975). The original *typica* coffee introduced by the Portuguese was wiped out by the CLR. Present day arabica coffee in Timor-Leste is nearly all produced from the HdT variety grown at altitudes above 1000 m a.s.l., under forest shade, on some 50,000 ha of low-input smallholder farms yielding not more than 100-150 kg/ha of green coffee. The trees are of the arabica phenotype, variable in plant habit and leaf size, resistant to CLR, rather low yielding and the coffee has genuine arabica cup quality characteristics (Marsh, 2001). HdT is basically a *typica* arabica variety with some genomic material introgressed from robusta coffee at one time early in the 20th Century, which has made it highly CLR resistant However, the often heard assumption, that it is an interspecific hybrid and therefore should have many characteristics intermediate between arabica and robusta coffees including lower cup quality than a pure arabica, does not appear to hold. The fully washed arabica coffees from Timor-Leste, in particular those from the HdT variety grown at higher altitudes, are considered speciality arabicas comparable to some of the best mild arabica coffees (Table 2).

Table 2. Quality of Timor-Leste and other washed arabica coffees.

country: coffee area:	Timor-Leste Maubesse	Bali Kintamani	Kenya Hiriga	Tanzania Ngoro Ngoro	Costa Rica Tarrazu	Colombia Antioquia
Ground roasted coffee						
Fragrance (1 - 5)	3.0	3.5	4.0	3.6	3.8	3.5
Aroma (1 - 5)	3.0	3.4	4.0	3.7	4.1	3.8
Beverage						
Acidity (1 - 10)	8.0	7.5	9.4	8.9	9.1	8.4
Body (1 - 5)	4.0	4.3	3.2	3.6	3.5	3.9
Flavour (1 - 10)	8.4	8.4	9.4	8.7	8.8	8.9
Aftertaste (1 - 10)	8.5	8.4	9.2	8.6	8.8	8.8
Total Score	**34.9**	**35.5**	**39.2**	**37.1**	**37.6**	**37.3**

Scores: 1 = poor, 5 & 10 = exceptionally good.
Source: Sweet Maria's Coffee Cupping Reviews 2008 (www.sweetmarias.com/coffee. reviewarchive).

CONCLUSIONS

The above-mentioned high quality of Timor-Leste arabica coffees clearly refutes the repeated references in coffee publications to HdT as an interspecific hybrid having robusta-like

characteristics with negative consequences to the quality of HdT derivatives (Bertrand et al., 2003, 2005; Leroy et al., 2006).

Coffee traders and roasters often blame the "robusta blood" present in the new disease-resistant cultivars for the deteriorating beverage quality of traditionally fine arabica coffees, while sub-standard crop management, harvesting and primary processing, sometimes in consequence of low farmgate prices, are frequently the actual factors behind poorer quality.

Introgression of disease and pest resistances from related species is common breeding practice in many crops without necessarily resulting in permanent loss of quality. For instance, most modern tomato cultivars with excellent fruit qualities and taste have multiple disease and pest resistances derived from at least 5 different *Lycopersicon* species, but are never referred to as interspecific hybrids (Van der Vossen et al., 2004).

What matters is that breeders get rid of negative side effects (genetic linkage drag) after interspecific crosses by repeated backcrossing and rigorous selection to ensure that the new disease-resistant cultivars have not lost on quality and other important properties. Coffee breeders in Kenya, Tanzania and Colombia have obviously succeeded in this, but a cultivar like CR95 in Costa Rica (Leroy et al., 2006) shows that in other coffee producing countries this may not always have been the case.

The prejudices of the coffee trade appear, therefore, vastly exaggerated. On the other hand, coffee breeders should continue to apply maximum selection pressure for high quality and refrain from premature release of new disease resistant cultivars before exhaustive testing for cup quality.

REFERENCES

Alvaredo G.A., Puerta Q.G.I., González E.M., Montoya Restrepo C., Alarcón S.R. (2008). Atributos de calidad en taza de las variedades Castillo. Revista Cenicafé. In the press

Avelino J., Barboza B., Araya J.C., Fonseca C., Davrieux F., Guyot B., Cilas C. (2005). Effects of slope exposure, altitude and yield on coffee quality in two altitude *terroirs* of Costa Rica, Orosi and Santa María de Dota. Journal of the Science of Food and Agriculture 85: 1869-1876.

Avelino J., Barboza B., Davrieux F., Guyot, B. (2007). Shade effect on sensory and chemical characteristics of coffee from very high altitude plantations in Costa Rica. 2nd International Symposium on multi-strata agroforestry systems with perennial crops, 17-21 September 2007 at CATIE, Turrialba, Costa Rica. pp.5.

Barel M. and Jacquet M. (1994). La qualité du café: ses causes, son appréciation, son amélioration. Plantations, recherche, développement 1 (1): 5-13.

Bellachew, B. (1997). Arabica coffee breeding in Ethiopia: a review. Proceedings of the 16th ASIC International Coffee Conference, Nairobi,Kenya, ASIC, Paris, France, pp. 406-414.

Bonnländer B., Eggers R., Engelhardt U.H., Maier H.G. (2005). Roasting. In: A. Illy and R. Viani (eds). Espresso Coffee. The science of quality, 2nd edition. Elsevier Academic Press, Amsterdam, London, etc. pp. 179-214.

Bertrand B., Aguilar G., Santacreo R., Anthony F., Etienne H., Eskes A.B., Charrier A. (1997) Comportement d'hybrides F1 de *Coffea arabica* pour la vigueur, la production et

la fertilité en Amérique Centrale. Proceedings 17th International Scientific Colloquium on Coffee, Nairobi, Kenya. ASIC, Paris, France, pp. 415 - 423.

Bertrand B., Guyot B., Anthony F., Lashermes Ph. (2003). Impact of the *Coffea canephora* gene introgression on beverage quality of *C. arabica*. Theoretical and Applied Genetics 107: 387-394.

Bertrand B., Etienne H., Lashermes Ph., Guyot B., Davrieux F. (2005). Can near-infrared reflectance of green coffee be used to detect introgression in *Coffea arabica* cultivars? Journal of the Science of Food and Agriculture 85: 955-962.

Bertrand B., Vaast Ph., Alpizar E., Etienne H., Davrieux F., Charmetant P. (2006) Comparison of bean biochemical composition and beverage quality of Arabica hybrids involving Sudanese-Ethiopian origins with traditional varieties at various elevations in Central America. Tree Physiology 26: 1239-1248.

Bettencourt A.J., Rodrigues C.J. (1988). Principles and practice of coffee plant breeding for resistance to rust and other diseases. In: R.J.Clarke & R. Macrae (eds). Coffee Volume 4 – Agronomy. Elsevier Applied Science, London & New York. pp. 199-234.

Carvalho A. (1988). Principles and practice of coffee plant breeding for productivity and quality factors: *Coffea arabica*. In: R.J.Clarke & R. Macrae (eds). Coffee Volume 4 – Agronomy. Elsevier Applied Science, London & New York, pp. 129-165.

Clifford M.N. (1985). Chemical and physical aspects of green coffee and coffee products. In: M.N.Clifford & K.C.Willson (eds). Coffee: botany, biochemistry and production of beans and beverage. Croom Helm, London, New York & Sydney. pp. 305 – 374.

Decazy F., Avelino J., Guyot B., Perriot J.J., Pineda C., Cilas C. (2003). Quality of different Honduran coffees in relation to several environments. Journal of Food Science 68 (7): 2356-2361.

Farah A., Monteiro M.C., Calado V., Franca A.S., Trugo L.C. (2006). Correlation between cup quality and chemical attributes of Brazilian coffee. Food Chemistry 98: 373-380.

Gonzales-Rios O., Suarez-Quiroz M.L., Boulanger R., Barel M., Guyot B., Guiraud J-P., Schorr-Galindo S. (2007). Impact of "ecological" post-harvest processing on the volatile fraction of coffee beans: I Green coffee. Journal of Food Composition and Analysis 20: 289-296

Gonzales-Rios O., Suarez-Quiroz M.L., Boulanger R., Barel M., Guyot B., Guiraud J-P., Schorr-Galindo S. (2007). Impact of "ecological" post-harvest processing on the volatile fraction of coffee beans: II Roasted coffee. Journal of Food Composition and Analysis 20: 297-307

Guyot B., Gueule D., Manez J.C., Perriot J.J., Giron J., Villain L. (1996). Influence de l'altitude et de l'ombrage sur la qualité des cafés Arabica. Plantations, recherche, développement 3 (4): 272-280.

Illy E. (2001). The factors of quality in green coffee beans. The First Asian Regional Round-table on Sustainable, Organic and Speciality Coffee Production, Processing and Marketing, Chiang Mai 26-28 February 2001, Thailand. www.fao.org/DOCREP/003/X6938E/X6938e05

Illy A. and Viani R. eds (2005). Espresso Coffee: the science of quality, 2nd edition. Elsevier Academic Press, Amsterdam, London, New York. 398 pp.

ITC (2002). Coffee: an exporter's guide. ITC product and market development, UNCTAD/WTO, Geneva, Switzerland, 310 pp.

Karanja A.M. (1993). Ruiru 11, adoption and performance in the estate sector in Kanya. *Kenya Coffee* 58 (679): 1533-1543.

Krug C.A. and Carvalho A. (1951). The genetics of *Coffea.* Advances of Genetics 4: 127-158.

Krug C.A. and De Poerck R.A. (1968). Portuguese Timor, pp. 408-410. In: World Coffee Survey. FAO, Rome, Agricultural Studies no.76. 476 pp.

Leroy T., Ribeyre F., Bertrand B., Charmetant P., Dufour M., Montagnon C., Maraccini P., Pot D. (2006). Genetics of coffee quality. Brazilian Journal of Plant Physiology 18 (1): 229-242.

Marsh A. (2001). The Specialty Coffee Industry of East Timor. The First Asian Regional Round-table on Sustainable, Organic and Speciality Coffee Production, Processing and Marketing, Chiang Mai 26-28 February 2001, Thailand. www.fao.org/DOCREP/003/ X6938E/X6938e05

Moreno G., Moreno E., Cadena G. (1995). Bean characteristics and cup quality of the Colombia variety (*Coffea arabica*) as judged by international tasting panels. Proceedings of the 16th International Scientific Coffee Conference, Kyoto, Japan. ASIC, Paris, France, pp. 574-578.

Muschler R.G. (2001). Shade improves coffee quality in a sub-optimal coffee-zone of Costa Rica. Agroforestry Systems 85: 131-139.

Njoroge S.M., Morales A.F., Kari P.E., Owuor J.B.O. (1990). Comparative evaluation of the flavour qualities of Ruiru 11 and SL28 cultivars of Kenya arabica coffee. Kenya Coffee, **55** (643), 843-849.

Omondi C.O. (2008). Coffee quality assessment: the case of two Kenyan cultivars, Ruiru 11 and SL28. 22nd ASIC International Coffee Conference, Campinas, Brasil.

Rodrigues C.J.Jr., Bettencourt A.J.. Rijo L. (1975). Races of the pathogen and resistance to coffee rust. Annual Review of Phytopathol;ogy 13: 49-70.

TaCRI (2005). Tanzania Coffee Research Institute Annual Report 2005. Lyamungu, Moshi, Tanzania, 30 pp.

Teri J.M., Kilambo D., Mtenga D., Nyange N.E., Nzallawahe T.S., Chipungahelo G.S., Kipokola T.P., Kullaya I.K. (2004). Improved arabica varieties for the benefit of Tanzanian coffee growers. Proceedings of the 20th ASIC International Coffee Conference, Bangalore, India. ASIC, Paris, France. pp. 1187-1191.

Vaast Ph., Bertrand B., Perriot J.-J., Guyot B., Génard, M. (2006). Fruit thinning and shade improve bean characteristics and beverage quality of coffee (*Coffea arabica* L) under optimal conditions. Journal of the Science of Food and Agriculture 86: 197-204.

Van der Vossen H.A.M. and Walyaro D.J. (1981). The coffee breeding programme in Kenya: a review of progress made since 1971 and plan of action for the coming years. Kenya Coffee 46: 113-130.

Van der Vossen H.A.M. (1985). Coffee selection and breeding. In: M.N. Clifford & K.C. Willson (eds). Coffee: botany, biochemistry and production of beans and beverage. Croom Helm, London, New York & Sydney. pp. 48-96.

Van der Vossen H.A.M. (2001). Coffee breeding practices. In: R.J. Clarke & O.G.Vitzthum (eds). Coffee: recent developments. Blackwell Science Ltd, Oxford, UK. pp. 184-20.

Van der Vossen H.A.M., Nono-Womdin R., Messiaen C.-M. (2004). *Lycopersicon esculentum*. In: G.J.H.Grubben & O.A.Denton (eds). Plant Resources of Tropical Africa

2. Vegetables. PROTA Foundation, Wageningen / Backhuys Publishers, Leiden, Netherlands. pp. 373-379.

Viani R. (2000). Global perspectives in coffee quality improvement. Proceedings of the International Scientific Symposium on Coffee, 4 December 2000, Bangalore, CCRI – CBI, India, pp. 120-127.

Wintgens J.N., ed. (2004). Coffee: growing, processing, sustainable production. A guidebook for growers, processors, traders and researchers. Wiley–VCH Verlag GmbH & Co KGaH, Weinheim, Germany. 976 pp.

Coffees of Brazil Network: Participative Building of Knowledge within the Agroindustrial Coffee System

S.P. PEREIRA[1], C.M.G. DE AGUIAR[2], P.H. LEME[3]

[1]Instituto Agronômico de Campinas - IAC, Campinas-SP, Brazil
[2]EMBRAPA Café, Brasília-DF, Brazil
[3]P&A Marketing Internacional, Espírito Santo do Pinhal-SP, Brazil

SUMMARY

The speed of communication in the 21st century requires that institutions constantly modernize their relationship with their target audience, since any investments in science and technology will only yield tangible results if and when they actually reach the fields. In response to the new challenges, the Brazilian Consortium for Coffee Research and Development CBP&D/Café in partnership with the private company P&A Marketing International innovated one more time by participating in the Pearibus virtual social network platform: a collaboration, knowledge and business network known as "Rede Cafés do Brasil" (Coffees of Brazil Network). Peabirus (www.peabirus.com.br) is a social media platform designed to produce added value gains for production chains of most varied sectors that have among their members agents with common interests. The platform enables the participating virtual networks to organize themselves and articulate among each other in a way as to allow strategic alignment of the players involved. The networks, the communities and their respective members form a new relationship environment which, through collaboration, knowledge and business understanding, promotes continued innovation and economic development. What differentiates this virtual network is that members participation is guided and moderated. The concept of governance is also applied, with a Managing Group whose mission consists of facilitating the relationships building process, assessing and helping to keep focus on achieving the goals of the network. All actions undertaken within the framework of the Peabirus platform, Coffees of Brazil network, and Coffee Field Management and Coffee Marketing communities – object of the present study – involve the "social media" concept. The objective of this study is to evaluate the use of the social media Peabirus platform by the user-members of the Coffees of Brazil Network and /or participants of the Coffee Field Management and Coffee Marketing communities in finding technical and scientific information related to themes and topics deemed relevant to the coffee-producing and trading sector. An exploratory research approach was used to conduct this study. The active moderating approach can be regarded as key to the success of each of the communities that are part of the network. Relevant issues to the coffee sector reached an exponential number of readers. This turns Coffees of Brazil Network into a powerful tool to promote sustained and comprehensive interaction between major players in coffee agribusiness.

INTRODUCTION

The speed of communication in the 21st century requires that institutions constantly modernize their relationship with their target audience, since any investments in science and technology will only yield tangible results if and when they actually reach the fields. In response to these new challenges, the Brazilian Consortium for Coffee Research and Development (CBP&D/Café – Consórcio Brasileiro de Pesquisa e Desenvolvimento do

Café), in partnership with the private company P&A Marketing International, innovated one more time by participating in the Pearibus virtual social network platform: a collaboration, knowledge and business network known as "Rede Cafés do Brasil" (Coffees of Brazil Network).

Peabirus (www.peabirus.com.br) is a social media platform designed to produce added value gains for production chains of most varied sectors that have among their members agents with common interests. The platform enables the participating virtual networks to organize themselves and articulate among each other in a way as to allow strategic alignment of the players involved. The use of these integrated communication channels makes it possible to attain collective goals in a more systematic and efficient way as compared to the practical results obtained through traditional channels and means of interaction among players within a production sector.

The virtual networks are organized and set up as communities within the Peabirus framework, which places at disposal of the participants a series of tools that will allow them to gain professional and institutional visibility, which, in turn, will enable them to expand their possibilities for both personal development and that of the collective organizations they are part of. The networks, the communities and their respective members form a new relationship environment which, through collaboration, knowledge and business understanding, promotes continued innovation and economic development.

According to Silva (2007), with the emergence of the so-called digital economy, dynamic cooperative networks interlinking different types of social and economic agents have been considered as the most appropriate organizational format to promote intensive learning and the generation, communication and transfer of knowledge and innovation. Such forms of interaction not only interconnect different units within a company, but are also increasingly used to articulate different organizations and other agents towards the common goals of improving research, production and commercialization processes, enhancing competitiveness and exploring new market opportunities.

Within this context, this study allows to reflect on Paulo Freire's concepts of education and consciousness-raising through dialogue, and the model proposed by Juan Enrique Diaz Bordenave – both of which are basic reference sources in the field of rural communication – and rethink and analyze within a virtual environment the passiveness or non-passiveness of the contemporary reader with regard to the new communication models. Bordenave defined it as follows: "*Rural communication encompasses the whole formed by information flows, dialogue and reciprocal influences between and among the components that make up the rural sector and between the rural sector and the other sectors of the country that are affected by the workings and development of agriculture, or that have an interest in the improvement of rural life*" (Bordenave, 1983).

What differentiates this virtual network is that members participation is guided and moderated. The concept of governance is also applied, with a Managing Group whose mission consists of facilitating the relationships building process, assessing and helping to keep focus on achieving the goals of the network. All actions undertaken within the framework of the Peabirus platform, Coffees of Brazil network, and Coffee Field Management and Coffee Marketing communities – object of the present study – involve the "social media" concept, or in other words, the concept within which the user shifts from being a mere reader of information made available by third parties to assume a more constructive role. Thus, the user

takes an active part in elaborating the content of information made available via the internet, taking on the role of an active player with regard to his actions within the network.

Peabirus represents a revolution in the communications among agents of diverse cultural and professional backgrounds that make up the different segments of the coffee production chain by providing a tool for dynamic information exchange aimed at encouraging the incorporation of technologies generated by research and structuring the coffee production chain within the modern context of the information society.

The objective of this study is to evaluate the use of the social media Peabirus platform by the user-members of the Coffees of Brazil Network and /or participants of the Coffee Field Management and Coffee Marketing communities in finding technical and scientific information related to themes and topics deemed relevant to the coffee-producing and trading sector.

MATERIAL AND METHODS

An exploratory research approach was used to conduct this study. The choice of this method was based on Gil (1999), who states that the primary objective of exploratory research is to "provide a general view, of approximative nature, of a certain fact". According to Malhotra (2001), the main objective of this kind of research is to enable a researcher to understand a problem or subject matter that he is to study. Exploratory research is conducted when a problem needs to be defined with greater precision and in cases where relevant courses of action need to be identified or additional data need to be obtained in order to enable the development of the most appropriate research design and approach. As its name suggests, exploratory research aims to explore a problem or situation to provide criteria and insight or understanding.

As part of exploratory research, a wide variety of diversified and versatile methods may be employed. According to Vieira (2002), the methods most commonly used include: secondary research (such as reviewing available literature and/or data, etc.), informal discussions with consumers, employees, management or competitors, in-depth interviews, case studies and informal observation.

Being an exploratory study, an analysis was performed of the overall performance of the communities that make up the Coffees of Brazil Network over the period 01/09/2007 to 01/09/2008. The data and information analyzed were obtained by document research, traffic records of the communities using "Google Analytics" and the content made available on Peabirus.

THE COFFEES OF BRAZIL NETWORK WITHIN THE FRAMEWORK OF THE PEABIRUS PLATFORM

The Peabirus network is a social media tool that, based on concepts of collaboration, knowledge and business, utilizes the infrastructure of the internet to bring people together in communities with common interests in their search for innovation and increased competitiveness. The Coffees of Brazil Network is one of 25 sector-specific or thematic networks that currently form the backbone of the Peabirus platform (08/09/2008). At present, the Coffees of Brazil Network has over 2500 registered users, with more than 50 communities directly involved in coffee production and trade. Some of these communities are thematic, while other involve around specific institutions or companies. These communities were

created and developed to promote an environment in which researchers, field technologists, consultants, farmers and other professionals in the field of coffee growing and trading can share information, build on the demands of research and discuss the viability of applying research results in the field.

According to Silva (2007), the method used to build the Coffees of Brazil Network, and other networks embedded in Peabirus, is basically founded on interconnection of a number of key persons who act as cornerstones of the relationship-building process that underlies the network. These persons are grouped according to the role they play within the structure of the network, as depicted in the figure below:

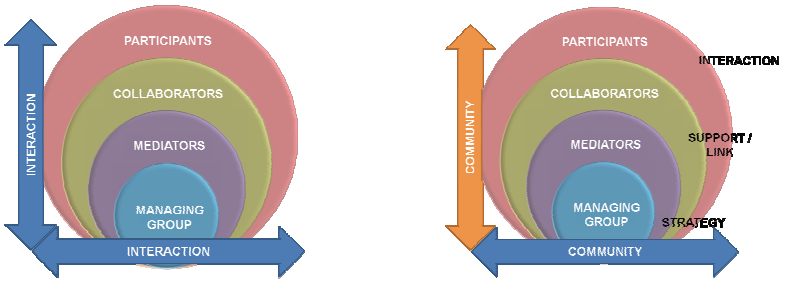


Figure 1. Network interconnections in Peabirus structure. Source: Silva (2007), Radiumsystems (2006) and authors.

The Managing Group (or Governance) is responsible for defining the contents, functions and plans of action, in addition to being in charge of monitoring and assessing the progress and functioning of the network. The role of the moderators is to encourage and support communication between the users of the network. They may or may not appoint individuals from their communities to act as collaborators and help the moderators in executing projects of the network (Silva, 2007).

The network members may join forces to create solutions specific to the development of the segment as a whole. The needs and demands are detected in a segmented way for each field of the sector involved, however, once they are exposed within the network they start interacting and complementing themselves, either in accordance with individual action undertaken by members of the network or as a result of strategic planning guidelines defined by the Managing Group. Interaction between the members within the virtual environment promotes rearticulation of the business chain of command from top to bottom with participation of all stakeholders.

In each of the communities it is possible to post topics within a discussion forum. These topics are related to the community and its interests and may take the form of a text message, news clippings, articles or simply questionings that may, depending on the degree of interest and encouragement of the members, generate a debate and an exchange of experiences within a communication and collaboration network in an attempt to serve a common interest: the enhancement of the competitiveness of the coffee producing sector. Other internet resources and applications may also be inserted in the space reserved to each community on the Network's website to promote strong interaction with other sites and social networks. Such resources and applications include downloadable videos, images, presentations, video conferences, RSS news feeds, music and audio player files, etc.

THE PROCESS OF CREATING COLLABORATIVE KNOWLEDGE WITHIN THE COFFEE FIELD MANAGEMENT COMMUNITY

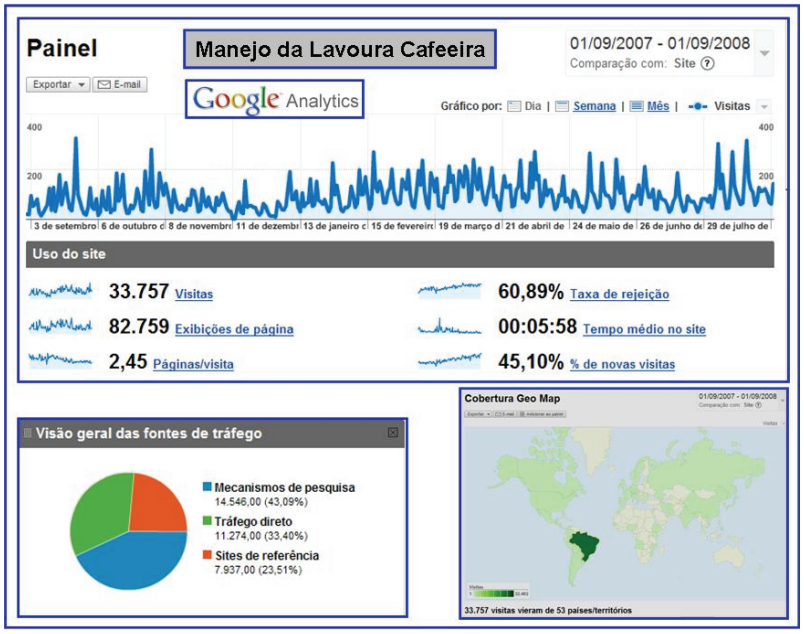
Among the communities that make up the Coffees of Brazil Network associated to CBP&D/Café, the major focus of this study will be on the Coffee Field Management Community which currently (08/09/2008) – two years after its implementation – has more than 800 members. Some issues or themes have gained increasing prominence within this community, have become a reading reference and serve as an encouragement for new postings. Furthermore, these themes draw a lot of attention from both the traditional mass media and the specialized press, thereby amplifying the reach and impact of the knowledge discussed. With these approaches, the Coffee Field Management Community consolidates itself as the virtual meeting place for the integration of agribusiness agents and discussion forum of themes and issues relevant to the success of the activity.

The present study will deal with the exploratory study of one of the 920 postings on the discussion forum of the Coffee Field Management community. This posting was entitled: "Frustration caused by unseasonable flowering of coffee trees in 2006".

The theme was posted by an agronomic engineer, also a consultant in the field of coffee growing, and says: "... frustration caused by unseasonable flowering of coffee trees in important coffee growing regions. Well-prepared fields, with high or at least average yield expectations, exhibiting poor and irregular flowering with little or poor flower bud differentiation. The main question is about the real causes and whether there is enough time or conditions for recovery in future flowerings and the impact and consequences of this phenomenon on harvest volumes. In a situation of extreme imbalance between production costs and prices, unfavorable crop prospects at this point cause great apprehension and difficulties and certainly will require more attention from all parties involved."

After the message had been posted, it was the task of the moderator of the Coffee Field Management community to send a message to all the members of the community inviting them to take part in the discussion. The importance of the issue raised, along with the encouragement to post reactions, produced effective results in terms of member participation. The encouragement, the response and the statements posted by the community's members resulted in the collective building of knowledge.

GENERAL VIEW OF THE COFFEE FIELD MANAGEMENT COMMUNITY THROUGH GOOGLE ANALYTICS



From September 2007 onwards, the "Google Analytics" tool was used to monitor the traffic statistics of each community. For the purpose of presenting some results, a 1-year period was analyzed (September 1st, 2007 to September 1st , 2008).
The statistics of the Coffee Field Management community over this time period (Figure 3) show that community was visited on 82.759 occasions by 33.757 people, clocking in an average of 2,45 pages per visit. With regard to the traffic sources, search engines accounted for 43,09% of the traffic to the community, direct traffic for 33,40% and referring sites for 23,51%. People from more than fifty-three (53) countries visited the community at least once in this period.

RESULTS AND DISCUSSION

During the period the theme was open for discussion, 30 postings concerning the proposed issue and more than 1000 visits were received from members of the community. Analysis of the topic shows the participation of a heterogeneous group of people, which included consultants, agronomic engineers, researchers, teachers, coffee farmers and market analysts. In the three situations observed, participants reported field experiences from the main coffee-producing regions, providing an up-to-date picture of the dynamics of the Brazilian coffee-growing sector. The relevance of the proposed themes and the subsequently posted statements encouraged an increasing number of community users to participate.

The topics discussed across the community allowed to map, locate and measure the extent of the problems discussed, once the participating members resided and worked in different coffee-producing regions.

Figure 2. Multiplication of the information sent in by members of the Coffee Field Management Community in response to the topic "Frustration caused by unseasonable flowering of coffee trees in 2006" on the main portals with a reputation of being a good source of news and information on coffee growing and the science of coffee.

The three topics analyzed were highlighted not only by the traditional media, but also by the specialized press and mass media, with significant multiplication and dissemination of the technical and scientific knowledge involved, building public awareness of the difficult situation coffee farmers are facing. A good example of this is the topic "Frustration caused by unseasonable flowering", which was originally published on the website of Embrapa Café and CBP&D/Café. Following this, a link to the report was placed on websites that specialize in agricultural readership materials, such as: the Brazilian Ministry of Agriculture, Embrapa, ABIC, Agrosoft, Cafepoint, Agrolink, Agroagenda, Página Rural, Revista Cafeicultura, Zoonews, CNC-CAFÉ, and other non-listed sites. It was also received coverage from important printed publications, including Folha Agrosul, Revista Agronegócios and periodic publications of the main coffee cooperatives.

The articles and reports published on the website of EMBRAPA Café presented technical-scientific opinions and comments from the community regarding information demands. The specialized media, particularly the internet-based media, provides the general public with reliable and well-qualified information. When such information reaches the fields, farmers and field technologists know how to use this information to advantage when making decisions and taking initiatives aimed at increasing competitiveness.

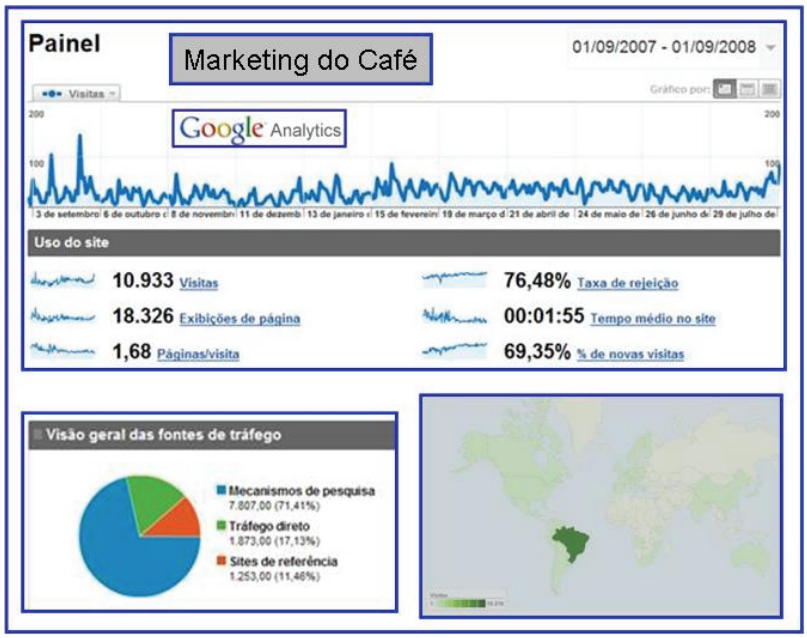
PROCESS OF BUILDING COLLABORATIVE KNOWLEDGE WITHIN COFFEE MARKETING COMMUNITY

Another community within the Coffees of Brazil Network that attained relative success among the people that access Peabirus is the Coffee Marketing community. The role of this community is to bring together coffee agribusiness players who wish to debate issues concerning the marketing of coffee, from seed to cup. Integration may occur either within the areas dedicated to the publication of contents, or directly among the participants themselves. In general, the objective is to promote interaction between the members on subject matters

directly related to the marketing of coffee, in a way so as to generate both knowledge and business.

Since its creation on 16/05/2006, a consultancy company that specializes in coffee growing, trading and distribution has performed the tasks associated with what could be described as a professional moderator of the community's website. In this study, we will analyze some of the more than 450 most visited topics of the marketing community and those that got the greatest participation from members.

GENERAL VIEW OF THE COFFEE MARKETING COMMUNITY THROUGH GOOGLE ANALYTICS



This study focuses on the period between September 1st, 2007 and September 1st, 2008. Analysis results of the traffic data relative to the Coffee Marketing community depicted in Figure 4 show that the community's website was visited 18.326 times by 10.933 people, with an average number of 1,68 pages per visit. The main traffic sources were search engines (Google, Yahoo and others), which were responsible for 71,41% of the flow of visitors to the community's website. Direct traffic – people that accessed the site via Pearibus or who typed the site URL directly into their browser - accounted for 17,13%, while referring sites accounted for 11,46% of total site traffic. The community received visitors from 44 different countries, with Brazil being the main geographic origin of traffic, followed by Portugal and the USA. Today (08/09/2008), the community has more than 950 members.

RESULTS AND DISCUSSION

There is a strong association between the interests of the end consumer and the most viewed topics on the Coffee Marketing community's website within the Coffees of Brazil network, notably the topics containing information on coffee shops. Of these topics, two are compilations of information and news clippings about coffee shops, the main subject of the first being Brazilian coffee shops, while the latter topic primarily focuses Starbuck's. Together, these two topics accounted for more than 4000 viewings. Other popular topics are related to specific online articles, with keywords that draw the attention of users that arrived at the community's site by way of electronic search engines.

Some of the topics visited by users promoted interaction with the public and attained considerable success as a result of a dynamic proposal from the moderator to stimulate user participation. The moderator introduced topics using simple and polemic questions, following a well-defined pattern, such as for example: "What kind of music do you prefer to hear in a coffee shop? (9 comments); Certified coffee or brand coffee? Where is the quality? (5 comments).

In addition to formulating a polemic question, the moderator also used the strategy of sending a message to the community users. This way, a new access channel to the proposed debate content was created for online users.

Valuable and intense interaction was generated as part of the discussions within the topic: "QUESTIONING: Production of special coffees (organic, gourmet, fair trade)", with more than 1000 views and 28 user comments. The theme had been proposed by a user who questioned the economic viability of producing specialty coffees. The most prominent result of the discussions around the topic was that the participants, even without any previous intention, ended up reviewing and commenting on the major issues involved in the production of specialty coffees, i.e. quality, quality standards, sustainability (environmental, social and economic), certifications, commercialization alternatives, and consumer perception of special coffees. The community's moderator drew up a summary of the subject matters addressed and posted a new topic: "QUESTIONING SUMMARY: Production of special coffees", which on its turn also attracted debate and interaction, with 700 visits and 8 comments.

CONCLUDING REMARKS

The Coffees of Brazil Network is consolidating itself as a channel of communication and collaboration within the Brazilian agri-industrial sector. The active moderating approach can be regarded as key to the success of each of the communities that are part of the network. It is the task of the moderator to assemble relevant and pertinent information materials, provide help to users and promote the debate. In other words, the role of the moderator is to act as an efficient agent in charge of channeling communication across the virtual and real worlds. The results produced by analysis of the two communities – Coffee Field Management and Coffee Marketing – of the Coffees of Brazil Networks substantiates the importance of the moderator in effective member participation and, consequently, to the building of collaborative knowledge.

On the other hand, the Peabirus platform proved to be an excellent tool for positioning the coffee sector within a reality increasingly influenced by the internet, serving as an efficient communication channel for its participants, who get the opportunity to democratically express their opinions and establish contact with other agents. Hence it is possible to conclude that the new social media tools can be successfully used to promote interactions among geographically dispersed agents and contribute to creating collective knowledge.

The experience of success of the Coffees of Brazil Network in bringing together agents and players of the agri-business sector in a virtual environment inspired the International Coffee Organization (ICO) to hire the companies P&A Marketing International and RadiumSystems to set up and coordinate a moderated network – the CoffeeClub Network (www.coffeeclubnetwork. com) – with the objective of promoting the integration of the coffee agri-business community on a worldwide level.

Information on issues and themes of relevance to the coffee sector reach an exponential number of readers. This feature turns the Coffees of Brazil network into a powerful tool of integration for everyone involved in the Brazilian coffee agri-business sector. The themes receive nationwide media coverage and contribute to the collective building and dissemination of knowledge, which is the ultimate goal of the "Coffees of Brazil Network" and CBP&D/Café.

BIBLIOGRAPHY

Bordenave J.D. O que é comunicação rural. São Paulo: Brasiliense, 1983.

Freire P. Extensão ou comunicação?. Tradução de Rosisca Darcy de Oliveira. Rio de Janeiro: Paz e Terra, 1997. 93 p.

Gil A.C. Métodos e técnicas de pesquisa social. 5. ed. São Paulo: Atlas, 1999.

Malhotra N. Pesquisa de marketing: uma orientação aplicada. 3.ed. Porto Alegre: Bookman, 2001. 719p.

Silva H.D. Rede de Colaboração, Conhecimento e Negócios dos Cafés do Brasil – Um novo modelo de comunicação e transferência de tecnologia para o CBP&D/Café. 5° Simpósio de Pesquisa dos Cafés do Brasil. Lavras, MG.

Vieira M.F.V., Zouain D.M. (Org.). 2. ed. Pesquisa qualitativa em administração. Rio de Janeiro: FGV, 2006.

Coffee Leaf Rust (*Hemileia vastatrix*) in Wild Forest Coffee Accessions and Released Varieties in Southwestern Ethiopia

C. JEFUKA[1*], G. ADUGNA[1], H. HINDORF[2], C.H. FININSA[3]

[1]Jimma Agricultural Research Centre, P.O.Box 192 Jimma/Ethiopia.
E-mail: challa1996@yahoo.co.uk
[2]INRES, University of Bonn/Germany
[3]Haramaya University, P.O.Box 138 Dire Dawa, Ethiopia

SUMMARY

Coffee leaf rust caused by *Hemileia vastatrix* Be. and Br is one of the third most important diseases of *Coffea arabica* L. in Ethiopia. Development of coffee leaf rust epidemics was monitored on wild forest coffee accessions collected from southwest Ethiopia and released varieties (Catimor J19 (CIFC 7362/17), Catimor J21(7363/46), Geisha and 7454) planted at Jimma and Tepi Agricultural Research Centres, respectively unnder field condition. Rust incidence (percent rusted leaves) and pustule per leaf were recorded at every month interval. Area under disease progress curve (AUDPC) and rate (r) of rust progression was estimated from rust incidence and expressed as proportion day^{-1} and logit day^{-1}, respectively. A highly significant differences were found among forest coffee accessions with respect to initial and final incidence, rate of disease progression and AUDPC values ($P<0.01$). Each released varieties significantly varied and the introduced Catimor lines accumulated higher amount of leaf rust than indeginious varieties at Tepi. These variable response provide opportunity to develop resistant varieties among enormous forest coffee genetic resources and require strategic conservation of their original area for future utilization in coffee improvement program. The susceptibility of Catimor lines might have indicated the appearnce of virulent race which may impede the current promotion of these lines for large scale production.

INTRODUCTION

Coffee (*Coffea arabica* L.) remains as the backbone of the Ethiopian economy, contributing 41 per cent of total foreign exchange earnings (IMF, 2006). Currently, it is produced on about 600,000 hectares of land yielding an estimated annual production of 350,000 tons of clean coffee (Alemayehu, 2008). However, the crop is prone to several diseases among which coffee berry disease (CBD), coffee wilt disease (CWD) and coffee leaf rust (CLR) caused by C*olletotrichum kahawae*, *Gibberella xylarioides* and *Hemileia vastatrix,* respectively, are the three major fungal diseases in Ethiopia.

Coffee leaf rust caused by *Hemileia vastatrix* Be & Br. is one of the most important diseases of *C. arabica* in the world (Kushalappa and Eskes, 1989). The disease may cause yield losses varying between 10 to 40% (Silva *et al.,* 2006*)* in different countries. In Ethiopia, leaf rust has been considered as minor diseases of coffee since it had never reached epidemic proportion as in other countries. Currently, CLR is widely distributed all over coffee growing regions of the country with varying intensities. The average national infected trees were estimated to about 36.3% in 1990 (Meseret, 1991). Eshetu *et al.* (2000) reported as high as 27% CLR severity in Hararghe region (Eastern Ethiopia). The disease incidence has been increaseing from time to time due to change in coffee production system. Therefore management intervention such as development and use of resistant varieties is essential before

the disease cause severe crop loss. In escalating price of fungicides and growing demand for organic coffee, the development and use of resistant varieties play vital role for sustainable supply of fine qulity coffee to the world market. In this line, development of coffee leaf rust epidemics was monitored on wild forest coffee accessions collected from southwest Ethiopia and four released varieties to assess their field susceptibility under natural infection.

MATERIALS AND METODS

The epidemics of coffee leaf rust was monitored on 45 forest coffee accssions collected from Yayu, Bonga and Berhane-Kontir forests and established at Jimma Agricultural Research Center (JARC) experimental field. Further experiment was also undetaken in lowland altitude belts at Tepi Agricultural Research Center (TARC) on four released C. *arabica* varieties namely Catimor J19 (CIFC 7362/17), Catimor J21 (CIFC 7363/46), Geisha and '7454'. Both the collections and released varieties were planted at 2m X 2m meter spacing between plants (2500 trees/ha). Uniform shade was provided by planting *Sesbania sesban* (Legume tree) at 4-meter interval between each coffee row and at the border of the field.

Three to six coffee plants were randomly selected and a pair of opposite branches from each (upper, middle and lower) canopy layers was used to determine rust incidence and SLD at every month interval (Brown *et al*., 1995). The rate (r) parameter was estimated as a slope of regression line after the logistic (logit x = ln (x/1-x) (Vander Plank, 1963) transformation of the disease incidence. The area under disease progress curve (AUDPC) was estimated using trapezoidal integration method (Campbell and Madden, 1990) based on monthly rust incidence. Because disease incidence was expressed in proportion and time in days, AUDPC is expressed here as proportion day^{-1}. Coffee tree and canopy was considered as replication and blocks, respectively, to analyze incidence, rate, and AUDPC of each location usig SAS statistical package (SAS version 8.1). Incidence was logarithmically transformed due to heterogeneity of variance.

RESULTS AND DISCUSSION

Forest coffee accessions showed significant differences with respect to initial and final incidence, AUDPC and rate of disease progression which varied from 7.6-73, 0-56, 23-112.5 and -0.056-0.003 logit day^{-1}, respectively. Coffee accessions P36, P37, P39 and P310, obtained from Berihane-Kontir forest had significantly lower respective AUDPC values of 28, 23.3, 30.9 and 31.6 proportion day^{-1}. Comparable AUDPC values of 31.8, 24.8 and 25.9 corresponding to P26, P29 and P215 accessions were found from Bonga but highest value of 112.5 proportion day^{-1} was observed on accession P46 from Yayu wild forest area (Table 1). Significant difference ($P < 0.05$) were exhibited among released coffee varieties in initial, final incidence, rate and AUDPC that ranged from 0, 1.9 and 0.009 to 25.2, 53.6 and 0.048 logit day^{-1} and 14.9-132.5 proportion day^{-1} respectively. Catimor J19 (132.5) and Geisha (14.9) had the highest and lowest AUDPC values. Canopy position significantly influenced rust incidence and the lower canopy accumulated high rust. Although precise time varied between locations and within forest coffee accessions and varieties, only single rust peak epidemics was attained in August to September and started declining in October at both locations. For instance Geisha and '7454' attained highest vales in July and October, respectively (Figure 1)

Table 1. Area under rust progress curve (AUDPC) on three forest coffee accessions at Jimma Research Center.

BONGA		BERHANE-KONTIR		YAYU	
Accession	AUDPC	Accession	AUDPC	Accession	AUDPC
P21	79.0	P31	36.2	P41	68.7
P22	60.6	P32	57.2	P42	75.3
P23	83.1	P33	51.8	P43	90.3
P24	52.1	P34	53.6	P44	69.0
P25	67.1	P35	42.1	P45	56.7
P26	31.8	P36	28.1	P46	79.8
P27	36.2	P37	23.3	P47	78.5
P28	38.6	P38	36.3	P48	94.0
P29	24.8	P39	30.9	P49	85.1
P210	53.3	P310	31.6	P410	112.5
P211	40.9	P311	52.9	P411	76.3
P212	78.9	P312	44.0	P412	66.6
P213	57.4	P313	54.8	P413	65.8
P214	79.0	P314	40.8	P414	82.3
P215	25.8	P315	46.4	P415	82.9
Mean					58.3
LSD(0.05)					12.92
CV (%)					14.0

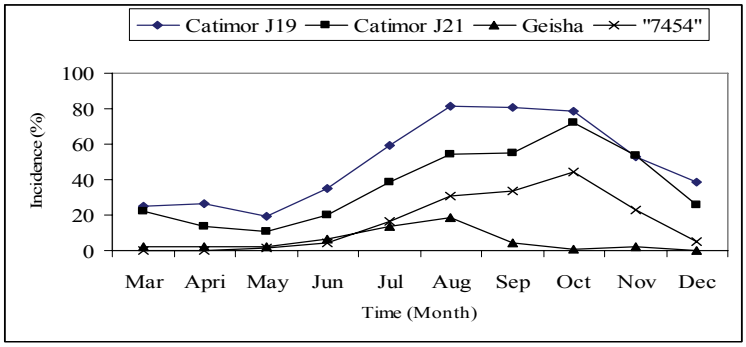


Figure 1. CLR progress curve on four varieties at Tepi Research Centre.

Higher rust incorporated into the lower coffee canopy is due to modification of microclimate through over shading effects of coffee branches which created conducive environment for rust development through lowering temperature, increased humidity and extended duration of leaf wetness favouring germination, penetration processes and subsequent development. According to Muthappa et al. (1989), heavy foliage density provides a favourable microclimate for the development of leaf rust within the leaf canopy.

The peak epidemic at Jimma appeared after receiving a total of 205.6, 210.4 and 250.2 mm of rainfall distributed over 23, 21 and 25 rainy days had occurred in July, August and September, respectively with a mean minimum and maximum monthly temperature of greater than 15 °C and less than 25 °C, respectively. These circumstances created ideal condition for spore dispersal within the crop canopy facilitating germination and infection processes for successive rapid development of CLR. The occurrence of severest rust incidence was reported to occur during well distributed rainy seasons and months in which the temperatures range from 19 °C to 24 °C (Sung, 1987). In October to December, removal of infected leaves and

occurrence of unfavourable temperature and rainfall caused rapid deceleration of the epidemics. Brown et al. (1995) reported the existence of significant negative correlation between number of days in a month on which the minimum temperature below 15 ^{0}C over the main period of epidemic development and severity of CLR. The peak epidemic was also related to ripening stage of the crop just before onset of the dry season. Coffee leaf rust susceptibility was reported to increases as the crop is ripening and its incidence was high when trees had high berries (Avelino et al., 2004). It was noted that coffee plants of the same genotypes can be receptive and immune when in the fructification and vegetative stage, respectively (Muller et al., 2004). Therefore the combined effects of weather condition, residual inoculum and fruit load may determine epidemics of CLR and observed peak time could be considered during field screening program to evaluate performance of coffee progenies.

The variable response of forest coffee accessions to rust at same ecological niche provide an opportunity to develop resistant varieties among enormous forest coffee genetic resources. Indeed, the substantial variability persisted in the forest coffee urge strategic conservation to rescue this gene pool from destruction by current expansion of farm lands, and to allow its natural co-evolutionary processes for future utilization in coffee improvement program. The susceptibility of introduced Catimor lines might have indicated the appearance of virulent race of the pathogen. This may impede current promotion of these lines for large scale production in rust hotspot localities and it necessitate alternative search for durable resistance.

REFERENCES

Alemayehu T., Esayas K. and Kassu K., 2008. Coffee development and marketing improvement plan in Ethiopia. *In*: Coffee diversity and knowledge; Girma, A., Bayetta, B., Tesfaye. S., Endale, T. and Taye, K. (eds). EIAR, Addis Ababa, Ethiopia. Pp 375-381.

Avelino J., Willocquet L. and Savary S., 2004. Effects of crop management pattern on coffee rust epidemics. Plant Pathology; 53:541–547.

Eshetu D., Teame G. and Girma A., 2000. Significance of minor coffee diseases of *Coffea arabica L.* in Ethiopia; a review. *In:* Proceedings of the workshop on control of coffee berry disease in Ethiopia, 13-15 August 1999, Addis Ababa, Ethiopia. Pp 58-64.

Campbell C.L. and Madden L.V. 1990. Introduction to plant disease epidemiology. John Wiley & Sons, New York. Pp 532.

Brown J.S., Kenny M.K., Whan J.H and Marrian P.R. 1995. The effect of temperature on the development of epidemics of coffee leaf rust in Papua New Guinea. Crop Protection, 14 (8): 671-676.

IMF, 2006. The Federal Democratic Republic of Ethiopia: Selected Issues and Statistical Appendix. International Monetary Fund Country Report 6/122. Washington.

Meseret W.1991. Epidemiology and resistance of coffee leaf rust in Ethiopia. Ministry of Coffee and Tea Development. Addis Ababa, Ethiopia.

Muller R.A., Berry D., Avelino J. and Biesse D. 2004. Coffee disease. In: Coffee growing, processing, sustainable production: A guide book for growers, processors and producers (Wintgens, J.ed.). WILEY-VcH Verlag GmbH & Co. kGaA. Weinheim.

SAS StatisticalAnalysis System Software Release 8.2. SAS Institute Inc., Cary, NC, USA.

Silva M.C., Varzea V., Guerra-Guimaraes L., Azinheira H.G., Fernandez D, Petitot A.S., Bertrand B., Lashermes P. and Nicole M. 2006. Coffee resistance to main diseases: leaf rust and coffee berry disease. Brazilian journal of plant physiology, 18 (1) 119-147.

Sung P.Q. 1987. Results of investigation on CLR in Vietnam. In: 12th International Conference on Coffee Science, 4 June - 3 July 1987, Montreux, France. Pp 648-656.

Vandrplank J.E. 1963. Plant disease: epidemiology and control. Academic press, New York. Pp 334.

Effects of Climate Changes on Coffee Diseases in Minas Gerais State, Brazil

S.M. CHALFOUN[1], E.P. XAVIER[2], M.C. PEREIRA[3]

[1]Resercher of Agricultural and Livestock Institution of Minas Gerais State-Lavras, MG- Brazil. E-mail: chalfoun@ufla.br
[2]Agriculture Technician of Agricultural and Livestock Institution of Minas Gerais State-Lavras, MG- Brazil
[3]Resercher of Agricultural and Livestock Institution of Minas Gerais State-Lavras, MG- Brazil

SUMMARY

Minas Gerais State is the major Brazilian coffee producer, responsible for more than a half of the total national production, so any event that may affect the coffee yield has national and international repercussion. The effect of climate on diseases occurrence and its severity is well known. However, some climate conditions have changed in the last years, causing impact in the behavior of the main coffee diseases, capable of leading the control strategies to a different direction. Historical series of data about coffee leaf rust (*Hemileia vastatrix* Berk. & Br.), considered the most important disease affecting coffee, were used to study the effect of the climate changes on the disease behavior. The results confirm that the chemical control based on calendar must definitively be replaced by a control based on disease monitoring measures, taking into account that that climate conditions can change through the years.

INTRODUCTION

Minas Gerais State is the major Brazilian coffee producer, responsible for more than a half of the total national production, so any event that may affect the coffee yield has national and international repercussion. The effect of climate on diseases occurrence and their severity is well known (Vale & Zambolim, 1997; Kimati et al. 1997). However, some climate conditions have changed in the last years, causing impact on the behavior of the main diseases, capable of leading the control strategies to a different direction (Vale et al., 2004).

EXPERIMENTAL APPROACH

Historical series of data about coffee leaf rust (*Hemileia vastatrix* Berk. & Br.), considered the most important disease affecting coffee (Becker-Raterink et al., 1991) were used to illustrate the effect of the climate changes on the disease behavior (Tables 1 and 2 and Figures 1and 2). We can see that the data about the disease progress under normal climate conditions in São Sebastião do Paraíso, Minas Gerais State, an important and representative coffee region, in the years of 1991, 1992, 1998, and 1999 (Figure 1), is very different from the data about the disease progress under different condition in the years of 1994, 1995, 2000 and 2003 (Figure 2).

RESULTS

Comparing the two patterns of coffee leaf rust disease progress curves with the data about rain, it was verified that in the years with a normal coffee leaf progress curve, the rain pattern

had a good distribution during the year, with the beginning of the rainy season on September. On the other hand, in the years with changed coffee leaf rust curve progress, the rain pattern had a worse distribution, with a delayed beginning of the rainy season to December (Figure 2).

The temperature, another factor responsible for changes on coffee leaf rust disease progress curve, was higher in the years of changed coffee leaf progress curve (Figure 2), mainly in the beginning of the summer, during the months of December, January and February. It is important to remember that during this period the maximum temperature remained toward 30 °C that is higher than the ideal temperature to the coffee leaf rust disease development, that is near 23 °C.

So, worse rain distribution pattern during the year and higher temperatures, mainly in the beginning of the summer season, were the main factor responsible for the delay of the phase of intense activity of the pathogen, making a change on the inflection point the disease curve progress to some months later, when it's compared with the normal curve.

The present case study explains the failure of chemical control strategies adopted by some coffee planters, based in the calendar method, so this method must be avoided. The chemical control strategies must be based on the disease monitoring measures once the climate conditions can change through the years.

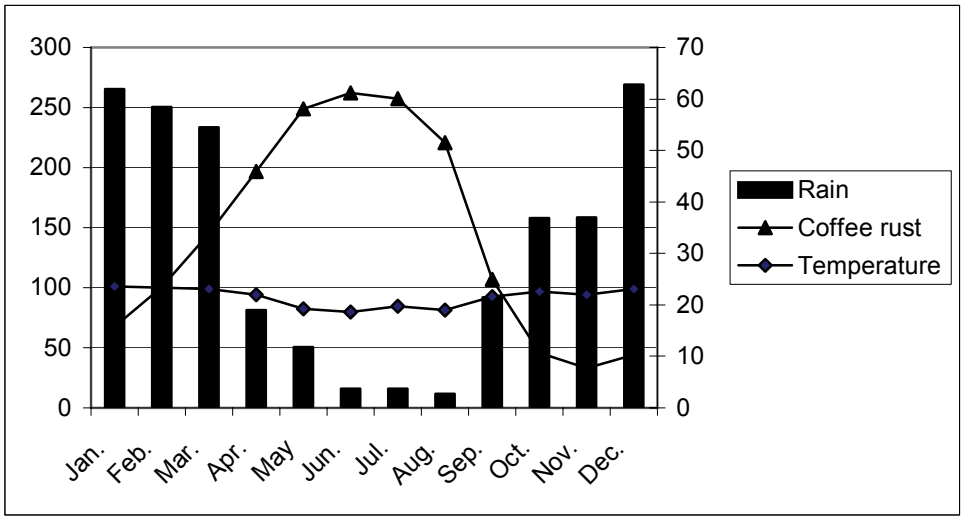


Figure 1. Normal coffee leaf rust progress curve and climate. Average data of 1991, 1992, 1998 and 1999. São Sebastião do Paraíso, Minas Grais State, Brazil.

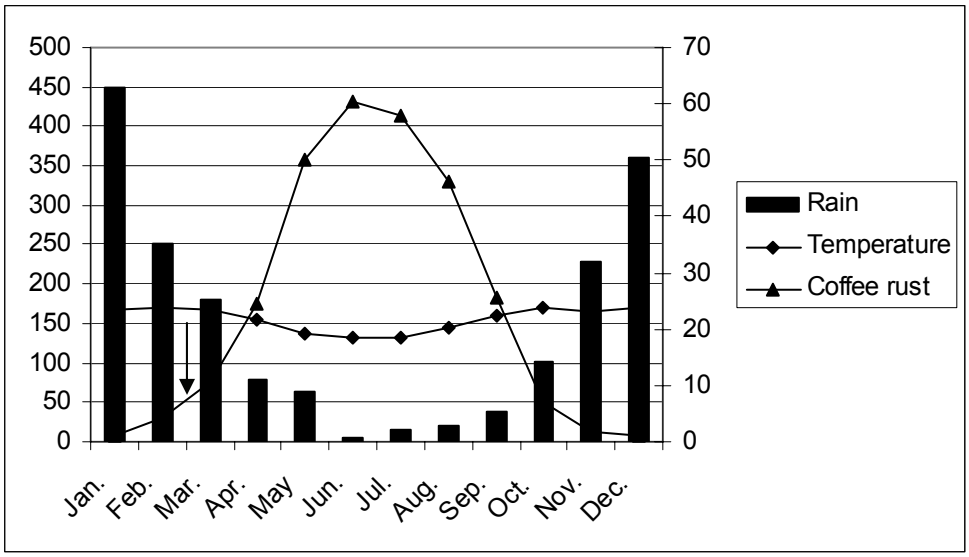


Figure 2. Changed coffee leaf rust progress curve and climate. Average data of 1994, 1995, 2000 and 2003. São Sebastião do Paraíso, Minas Grais State, Brazil. (↓) inflection area.

ACKNOWLEDGEMENTS

The authors are grateful to EPAMIG's Research Experimental Farm of São Sebastião do Paraíso, Minas Gerais State, Brazil.

REFERENCES

Bercker-Raterink S., Moraes W.B.C., Quijano-Rico M. La Roya Del Cafeto. Conocimiento y control. Deutsche Gesellschaft für Technische Zusammennarbeit (GTZ) GmbH. Eschborn, 1991, 281p.

Kimati H., Amorim L., Bergamin Filho A., Camargo L.E.A. and Rezende J.A.M. Manual de Fitopatologia, 3ª edição. Ed. Agronômica Ceres Ltda. São Paulo, 1997. v.2, 774 p.

Vale F.X.R and Zambolim L. Controle de doenças de plantas. 1ª edição. Ed. Universidade Federal de Viçosa. 1997 v1. 554p.

Vale F.X.R., Jesus Júnior W.C. and Zambolim L. Epidemiologia aplicada ao manejo de doença de plantas. 1ª edição. Ed. Perffil Universidade Federal de Viçosa. 2004. 531p.

Leaf Rust Resistance Heterosis and Gene Accumulation in Arabica Coffee Hybrids

L.C. FAZUOLI, M.T. BRAGHINI, J.C. MISTRO, M.R. PETEK, M.B. SILVAROLLA

Instituto Agronômico de Campinas, São Paulo, Brasil. Parcialmente financiado pelo CBP&D/Café e CNPq

SUMMARY

Several F_1 hybrids were obtained using the traditional coffee breeding methods, under homozygote conditions, to incorporate into a single genotype the major part of the SH_1 to SH_9 genes and smaller gene effects or quantitative genes related to the rust resistance, plant rusticity and vigor, and other agronomic characteristics. In this way, two hybrids were obtained from Obatã IAC 1669-20 x Icatu Vermelho IAC 4045 crossing; and 13 hybrids from Tupi IAC 1669-33 x Icatu Vermelho IAC 4045 crossing, totalizing 790 plants evaluated for high leaf rust resistance, associated to high yield, plant vigor, rusticity and other agronomic characteristics. The H 15483 hybrid derived from Obatã x Icatu and the H 15488, H 15490, H 15491, H 15493 and H 15495 hybrids derived from Tupi x Icatu crossing presented higher yields, higher plant vigor, higher rust resistance and higher heterosis values.

INTRODUCTION

The development of cloning techniques using biotechnology has turned possible to discern about using the heterosis (for high productivity and high plant vigor) existent in Arabica coffee hybrids, for commercial purposes, by means of hybrid cultivar cloning. Besides, this technique allows to add and combine genes of resistance to pests and diseases much faster than it would be in a long-range traditional coffee breeding planning. The objective of this work was to obtain several F_1 hybrids (Obatã IAC 1669-20 x Icatu IAC 4045 and Tupi IAC 1669-33 x Icatu IAC 4045), with high level of leaf rust resistance, associated to high yield, plant vigor, rusticity and other agronomic characteristics.

MATERIALS AND METHODS

The Coffee Breeding Program at Instituto Agronômico, State of São Paulo, Brazil, has developed several coffee cultivars resistant to the leaf rust (*Hemileia vastatrix*). Some of these, are the 'Obatã IAC 1669-20' and 'Tupi IAC 1669-33', which are short-tree cultivars, highly productive, immune to leaf-rust, but highly nutrient demanding and with low plant vigor. Another one, is the 'Icatu Vermelho' originated from *Coffea canephora*, which is a tall-tree cultivar, highly vigorous and productive, tolerant to high air temperatures and moderately susceptible to the main rust races prevalent in the Brazilian coffee cropping. Several F_1 hybrids were obtained, facing all technical difficulties of the traditional coffee breeding methods, under homozygote conditions, to incorporate into a single genotype the major part of the SH_1 to SH_9 genes and smaller gene effects or quantitative genes related to the rust resistance, plant rusticity and vigor, and other agronomic characteristics. In this way, two hybrids were obtained from Obatã IAC 1669-20 x Icatu Vermelho IAC 4045 crossing; and 13 hybrids from Tupi IAC 1669-33 x Icatu Vermelho IAC 4045 crossing, totalizing 790 plants evaluated.

The field experiment was carried out at Campinas, State of São Paulo, Brazil, in January/2002, in a nutritionally poor soil. The four first coffee grain yields during the periods of 2004 to 2007 were evaluated. Heterosis was calculated as percent of the standard cultivar for the two types of hybrids, using the formula:

$$H(\%) = \left(\frac{F_1}{Culti\,\mathrm{var}_{padrrão}} - 1 \right) * 100$$

RESULTS AND DISCUSSION

The data on the average yearly yield per plant (kg of coffee berry) during 2004-2007 of the F_1 hybrids obtained from the Obatã IAC 1669-20 x Icatu Vermelho IAC 4045 crossing, as well as the yield variation ranges and the heterosis values (%) in relation to the IAC 1669-20 standard cultivar are presented in Table 1. The coffee berry yield variation range was 1.75 to 10.52 kg per plant, and heterosis for the F_1 hybrid H15483 was 57.69%, which is considered a good value for heterosis. Therefore, the most productive F_1 coffee plants from this hybrid, among the 65 plants analyzed, can be multiplied by vegetative reproduction.

The F_1 hybrids obtained from the Tupi IAC 1669-33 x Icatu Vermelho IAC 4045 crossing and the respective average yearly yields (kg of coffee berry per plant, yield variation ranges and the heterosis values (%) in relation to the Tupi IAC 1669-33 standard cultivar, are presented in Table 2. All F1 hybrids among coffee plants from the Tupi IAC 1669-33 x Icatu Vermelho IAC 4045 crossing resulted in positive heterosis varying from 32.50 to 89.37%. Similar or even higher heterosis values were obtained for *Coffea arabica* F_1 hybrids by Araujo Netto and Pereira (1980), Araujo Netto *et al*. (1982), and Fontes (2001).

The five F_1 hybrids presenting high heterosis were IAC H15488, IAC H15495, IAC H15490, IAC H15491 and IAC H15493, with heterosis values of 89.37%, 65.94%,62.81%, 59.38% and 53.75%, respectively.

Large average yield variation ranges for these F_1 hybrids were observed that might be explained by the 'Icatu' heterozygosis. This variation might allow selecting F1 hybrids with yearly average yields of 8.00-9.25 kg of coffee berry per plant, which is well above the standard cultivars' productivity. These elite F_1 hybrids might also be multiplied by vegetative reproduction. Such coffee F_1 hybrids showed not only higher productivities, but also higher vigor, rusticity and rust resistance compared to the standard cultivars (Tupi IAC 1669-33 and Obatã IAC 1669-20). Besides, they are also auto-fertile plants.

It is important to point out that Icatu Vermelho cv. has presented high adaptability to the warm regions of the State of São Paulo, probably because of its origin from *C. canephora* that is a more heat tolerant species.

Such high heterosis for productivity and plant vigor hybrids shall be multiplied via vegetative reproduction (stalk or tissue culture) and destined to commercial cropping. Therefore, they might be a new option for Arabica coffee cropping in the State of São Paulo in regions of nutrient poor soils and high average yearly air temperatures.

CONCLUSIONS

The evaluated F_1 hybrids presented high productivity, short trees, rust resistance, rusticity and high heterosis values (varying from 29.33 to 89.37%) for coffee yields.

The H 15483 hybrid derived from Obatã x Icatu and the H 15488, H 15490, H 15491, H 15493 and H 15495 hybrids derived from Tupi x Icatu crossing presented higher yields, higher plant vigor, higher rust resistance and higher heterosis values.

Table 1. Yields and average yearly yield variation ranges (kg of coffee berry per plant), referent to four harvests (2004-2007) of F_1 hybrids obtained from Obatã IAC 1669-20 x Icatu IAC 4045 cultivar crossing and the respective heterosis values (%), in relation to the Obatã IAC 1669-20 standard cultivar.

F_1 Hybrid	Number of plants	Fruit yield	Heterosis	Amplitude
		kg	%	kg
IAC H 15483	65	6,56	57,69	1,75 - 10,52
IAC H 15497	32	5,38	29,33	1,95 - 8,78
Obatã IAC 1669-20	27	4,16	-	0,73 - 7,33

Table 2. Yields and average yearly yield variation ranges (kg of coffee berry per plant), referent to four harvests (2004-2007) of F_1 hybrids obtained from Obatã IAC 1669-20 x Icatu IAC 4045 cultivar crossing and the respective heterosis values (%), in relation to the Tupi IAC 1669-33standard cultivar.

F_1 Hybrid	Number of plants	Fruit yield	Heterosis	Amplitude
		kg	%	kg
IAC H 15484	105	4,41	37,81	1,48 - 6,60
IAC H 15485	100	4,69	46,56	1,20 - 7,25
IAC H 15486	34	4,46	39,38	1,75 - 7,73
IAC H 15487	103	4,46	39,38	1,98 - 7,58
IAC H 15488	36	6,06	89,37	1,75 - 9,25
IAC H 15489	67	4,37	36,56	1,25 - 6,65
IAC H 15490	69	5,21	62,81	0,83 - 8,03
IAC H 15491	69	5,10	59,38	1,05 - 7,88
IAC H 15492	57	4,61	44,06	2,03 - 6,98
IAC H 15493	20	4,92	53,75	3,40 - 6,45
IAC H 15494	9	4,24	32,50	2,00 - 6,85
IAC H 15495	9	5,31	65,94	4,43 - 8,00
IAC H 15496	15	4,78	49,38	2,88 - 7,88
Tupi IAC 1669-33	27	3,20	-	0,35 - 5,05

REFERENCES

Araújo Netto K., Paulino A.J., Pereira J.B.D. Estado de híbridos de *Coffea arábica* - Catimor versus Catuaí, Catindu versus Catuaí e outros In: Colóquio Da Association Scientifique Internationale Du Café 10°, Salvador 1982 Resumos, Salvador, ASIC, 1982, p73

Araújo Netto K.E., Pereira J.B.D. Vigor híbrido em cruzamento de *Coffea arabica* In: Congresso Brasileiro De Pesquisas Cafeeiras, 8, Campos do Jordão, 1980, p14-16.

Fontes J.R.M. Heterose, Capacidade Combinatória e Divergência Genética estimada por análise de marcadores RAPD em cruzamento entre cafeeiros Catuaí (*Coffee arabica L.*) e Híbrido de Timor. Tese de Doutorado Viçosa - UFV, 2001,122p.

Simultaneous Control of Pests and Diseases in Coffee Plants

P. REBELLES REIS[1,2], J.C. DE SOUZA[1,2]

[1]EPAMIG-CTSM/EcoCentro, P.O. Box 176, Zip Code 37200-000, Lavras, MG, Brazil
[2] CNPq and Fapemig research, respectively

SUMMARY

Pests and diseases can cause heavy losses on coffee cropping. The leaf miner, *Leucoptera coffeella* (Lepidoptera: Lyonetiidae), is presently the main pest of coffee plants, mainly on high temperature and dry regions. Nymphs of cicadas are important pests of coffee crops and can also cause heavy losses; *Quesada gigas* (Hemiptera: Cicadidae) is the largest in size and the most harmful species. The coffee rust (*Hemileia vastatrix* Berk. et Br.), main disease of coffee, can also cause heavy defoliation, leading to the complete weakening of the plant, resulting in losses of up to 80% in production. Essays conducted by EPAMIG in "Minas Gerais" State regions demonstrated that thiamethoxam 250 WG (insecticide) alone or in formulations with cyproconazole 300 WG (fungicide), both with good systemic properties, is highly efficient in controlling the coffee leaf miner (> 80%), mobile sucking cicada nymphs (> 90%) and mealybugs (Pseudococcidae) (> 90%) on roots when applied in drench on low plant stem, in soil along planting line or in irrigation water. On the other hand, cyproconazole is highly efficient in coffee rust disease control (> 90%). The drench method using a manual backpack sprayer with regulator, for small and medium coffee crops, or in tractor with spraying bar, for larger crops, in continuous spray applied in soil along planting line is an excellent option for the control of coffee rust disease and key pests of the crop (coffee leaf miner, cicadas and mealybugs) in one operation alone.

INTRODUCTION

The insecticide thiamethoxam 250 WG or the mix insecticide + cyproconazole 300 WG (fungicide) is highly systemic and is absorbed when applied in drench at low plant stem, in stripes on soil along coffee plant lines and on irrigation water (drip or LEPA). After absorption by the plants, they are incorporated to elaborated sap (phloem) or raw sap (xylem) and oriented toward roots and aerial part, respectively, where they act against pests.

COFFEE LEAF-MINER CONTROL

The coffee leaf miner, *Leucoptera coffeella* (Lepidoptera: Lyonetiidae), is presently the most important pest of coffee plantations, mainly on temperature regions on high elevations and water deficiency (Reis and Souza, 1986). The majority of lesions are found on the upper part of the plant (Reis et al., 1975). Resulting lesions are mostly found on leaves at top of the canopy, and chances are of complete defoliation of the plant and consequent significant reduction in production. Crops with intense defoliation may last up to two years to recuperate. In South of Minas Gerais, Brazil, in 1975, a reduction in production of about 52% was detected resulting from the attack of this pest (Reis et al., 1976). Over 80% in efficiency in controlling the leaf miner is found along the long control period when thiamethoxam is used (Table 1).

Table 1. Evolution of leaf miner infestation in percent of mined leaves (%ML) and percent of efficiency (%E) on each sampling, in treatments applied on 02/28/2002. Monte Carmelo, MG, Brazil (Souza et al., 2006°).

Treatments[1]	Percent of mined leaves and efficiency of treatments					
	05/02/2002		06/05/2002		07/09/2002	
	% ML	% E	% ML	% E	% ML	% E
1. Thiamethoxam 250 WG (drip)	3,0 d	87,6	8,2 d	88,5	17,9 d	79,0
2. Thiamethoxam 250 WG (drench)	0,4 e	98,4	5,2 d	92,7	20,5 d	70,1
3. Thiamethoxam 250 WG (line)	15,0 b	38,3	50,7 bc	28,7	58,2 c	32,3
4. Imidacloprid 700 WG (drip)	21,5 a	11,5	67,4 ab	5,2	83,6 ab	2,8
5. Imidacloprid 700 WG (drench)	9,0 c	63,0	46,1 c	35,2	66,5 bc	22,6
6. Thiamethoxam 10 GR (furrow)	18,6 ab	23,4	53,3 abc	25,0	74,9 abc	12,8
7. Aldicarb 150 GR (furrow)	20,1 ab	17,3	70,3 a	1,2	84,0 ab	2,2
8. Control	24,3 a	-	71,1 a	-	85,9 a	-
CV (%)	15,3		17,5		13,7	

[1]*Treatments: 1 = Actara 2000 g c.p. */ha, drip irrigation; 2 = Actara 2000 g c.p./ha, drench on low plant stem; 3 = Actara 2000 g c.p./ha, two lines on soil under canopy projection; 4 = Premier 1600 g c.p./ha, drip irrigation; 5 = Premier 1600 g c.p./ha, drench at low plant stem; 6 = Actara 50 kg c.p./ha, two furrows on soil under canopy projection; 7 = Temik 25 kg c.p./ha, two furrows on soil under canopy projection and 8 = Control with no application of pesticides. * c.p. = commercial product.*

COFFEE CICADAS CONTROL

Cicadas can cause severe loss to infested crops and the most harmful species is *Quesada gigas* (Hemiptera: Cicadidae), larger of all species. Nymphs attach to coffee plant roots sucking sap. Up to 540 nymphs per plant have been reported and 35 nymphs and up per plant causes heavy losses. Continuous sucking of sap induces weakening of the plants revealed by shortening of above ground parts, early leaf drop and up to 87% loss in production with possibility of complete loss of the entire crop. Thiamethoxam is highly effective in control of all cicada species with efficiency of over 93%, regardless the application technique used in December, a rainy period (Table 2).

Table 2. Mean of live cicada nymphs per infested coffee plant and percent of efficiency on December treatments. São Sebastião do Paraíso, MG, Brazil, 07/28/2003.

Treatments	Dosage kg c.p.*/ha	Methods of application	Live nymphs/ plant	Efficiency (%)
1. Thiamethoxam 10 GR	50,0	Two furrows on soil under canopy projection	2,12 c	97,7
2. Thiamethoxam 250 WG	2,0	Drench on low plant stem	3,88 c	95,8
3. Thiamethoxam 10 GR + thiamethoxam 10 GR + cyproconazole 20 GR	25,0 + 30,0	Two furrows on soil under canopy projection	6,38 c	93,0
4. Thiamethoxam 250 WG and thiamethoxam 300 WG + cyproconazole 300 WG	1,0 + 1,0	Drench on low plant stem	1,92 c	97,9
5. Imidacloprid 700 WG	1,7	Drench on low plant stem	14,38 b	84,3
6. Aldicarb 150 GR	25,0	Two furrows on soil under canopy projection	15,75 b	82,8
7. Control	-	-	91,62 a	-
CV (%)			20,80	

** c.p = commercial product*

COFFEE RUST DISEASE CONTROL

Coffee rust disease (*Hemileia vastatrix*), main disease of coffee plants, can cause heavy losses to production due to intense defoliation resulting to infestation, and can result in complete weakening of the plants. Losses in production can go over 90% with defoliation. Cyproconazole, active ingredient of Verdadero 600 WG brand (thiamethoxam + cyproconazole) applied on November-December in drench is highly effective against leaf rust with over 92% in efficiency (Table 3).

Table 3. Percent of leaves with rust in plots, mean percent of infection and percent of efficiency of November treatments. São Sebastião do Paraíso, MG, Brazil, 07/05/2004.

Treatments[1]	Replicates				Mean %	Efficiency %
	I	II	III	IV		
1.Thiamethoxam 300 WG + cyproconazole 300 WG	2,0	2,0	0,3	4,0	2,00 c	95,2
2.Thiamethoxam 300 WG + cyproconazole 300 WG e thiamethoxam 250 WG	2,0	2,0	4,0	6,0	3,50 bc	91,6
3.Imidacloprid 700 WG	24,0	52,0	22,0	40,0	34,50 a	16,9
4.Triadimenol + Disulfoton GR	4,0	18,0	8,0	12,0	10,50 b	74,7
5.Control	38,0	44,0	38,0	46,0	41,50 a	-
C.V. (%)					27,70	

[1]Treatments: 1 = Verdadero at 1 kg c.p./ha applied in November, in "drench"; 2 = Verdadero at 1 kg c.p./ha applied in November, in "drench" + Actara at 1 kg c.p./ha applied in February, in "drench"; 3 = Premier at 1,5 kg c.p./ha in November, in "drench"; 4 = Baysiston GR a 50 kg c.p./ha applied in November, in two furrows on soil under canopy projection and 5 = Control with no application of pesticides. * c.p = commercial product.*

VIGOR AND PRODUCTIVITY OF COFFEE PLANTS

The use of thiamethoxam in single or sequential applications induces high vigor and extensive foliage in coffee plants (increase in root system and leaf production), which result in increase of production on actual season and favor plants for the incoming seasons (Table 4), along with excellent efficiency on key-pests control.

Table 4. Coffee production related to coffee leaf miner control with thiamethoxam 250 WG by drip irrigation water on three times of application. Monte Carmelo, MG, Brazil (Souza et al., 2006).

Treatments[1]	Percent of mined leaves [2] on 06/08/2002	Production	
		Bags of 60 kg of milled coffee / ha[3] (harvest in July of 2003)	Index of increment
1. Control	97,8 c	23,0 b	100
2. Thiamethoxam in February	30,3 ab	57,7 a	251
3. Thiamethoxam in March	20,6 a	54,6 a	237
4. Thiamethoxam in April	37,0 b	52,0 a	226
CV (%)	19,8	13,8	

[1]Treatments: 1 = Control with no application of pesticides; 2, 3 and 4 = Actara (thiamethoxam) 250 WG at 2 kg c.p./ha on drip irrigation water (0.56 g c.p./plant) on February, March and April of 2002, respectively. [2] Means followed by the same letter in columns are not statistically different (Duncan 5%). [3] Means followed by the same letter in columns are not statistically different (Scott-Knott 5%). * c.p = commercial product.*

FINAL REMARKS

Coffee cropping reached high technical level in pest control with the introduction of the neonicotinoid insecticide thiamethoxam. The advantages of using of WG (water dispersible granules) formulation includes its easiness of application by drench at lower part of plant stem, a continuous stripe along planting line or on irrigation water (drip irrigation or LEPA). The use of this insecticide alone (Actara 250 WG) or mixed to the fungicide cyproconazole (Verdadero 600 WG) has demonstrated its effectiveness during long periods of control for the leaf miner, cicadas, mealybugs on roots and of the coffee rust, besides great vigor to the plants and consequently augmentation in crop productivity. Another advantage of liquid application is the use of extremely simple equipment, manual costal knapsack sprayer with dispenser shaft and regulator or tractor with spray bar, allowing faster and more precise applications. Thiamethoxam has brought coffee cropping to a new level for pest control of species which cause economic damage to the crop and excellent gains in productivity and quality, with good returns to the growers due to high plant vigor.

ACKNOWLEDGMENTS

To Syngenta Crop Protection Ltda. for the financial support and furnishing of products, and to CNPq and Fapemig for the scholarships.

REFERENCES

Reis P.R. and Souza J.C. de. Pragas do cafeeiro. In: Rena A.B., Malavolta E., Rocha M. and Yamada T. (Ed.) Cultura do cafeeiro; fatores que afetam a produtividade. Piracicaba: POTAFOS, 1986. p.338-378.

Reis P.R., Lima J.O.G. and Souza J.C. de. Flutuação populacional do "bicho-mineiro" das folhas do cafeeiro, *Perileucoptera coffeella* (Lepidoptera: Lyonetiidae), nas regiões cafeeiras do Estado de Minas Gerais e identificação de inimigos naturais. In: Congresso Brasileiro De Pesquisas Cafeeiras, 3, 1975, Curitiba. Rio de Janeiro: IBC, 1975. p.217-218.

Reis P.R., Souza J.C. De, Lima J.O.G. and Melo L.A.S. Controle químico do “bicho-mineiro” das folhas do cafeeiro, *Perileucoptera coffeella* (Lepidoptera: Lyonetiidae). In: Congresso Brasileiro De Pesquisas Cafeeiras, 4, 1976, Caxambu. [Resumos]... Rio de Janeiro: IBC, 1976. p.238-239.

Souza J.C. De, Reis P.R., Rigitano R.L. De O. and Ciociola Júnior A.I. Eficiência de thiamethoxam no controle do bicho-mineiro do cafeeiro. I – Influência da modalidade de aplicação. Coffee Science, Lavras, v.1, n.2, p.143-149, jul/dez. 2006a.

Souza J.C. De, Reis P.R., Rigitano R.L. De O. and Ciociola Júnior A.I. Eficiência de thiamethoxam no controle do bicho-mineiro do cafeeiro. II – Influência da época de aplicação via irrigação por gotejamento. Coffee Science, Lavras, v.1, n.2, p.150-155, jul/dez. 2006.

Evaluation of Natural Products for the Control of Coffee Leaf Rust, *Hemileia vastatrix* Berkeley & Brues

D. KUTYWAYO, N. MTETWA AND D.H. NZARAYEBANI

Coffee Research Institute, P.O. Box 61, Chipinge, Zimbabwe

SUMMARY

Coffee leaf rust is an important disease causing severe defoliation and decline in yield in many coffee producing countries. Current management practices rely on the use of expensive inorganic fungicides and host plant resistance. *Carica papaya* aqueous leaf extracts (100%, 50%, 33%, 20%, 16.7% and 12%) and fermented cow urine (0.1%, 0.5%, 1%, undiluted) were evaluated for their efficacy against coffee leaf rust under laboratory conditions. Undiluted *C. papaya* extract had the least spore germination of 16.5% followed by the 50% concentration while the weakest dilution (12%) had the highest spore germination percentage of 69.88%. The standard, copper oxychloride applied at 8 g/litre, had 23% spore germination. Fermented cow urine at concentrations of 0.1%, 0.5% and 100% was more effective than the conventional fungicide Copper oxychloride. *Carica papaya* and cow urine have potential for use as components of an integrated coffee leaf rust management programme.

INTRODUCTION

Coffee leaf rust is a major disease in many coffee producing countries. The disease devastated the coffee industry in Ceylon in the 1880s with average yields falling by over 50% (Ward, 1881). The disease caused similar devastations in Indonesia, India and South America though not as destructive as in Ceylon (Sreenivasa, 1989; Monaco, 1977; Castilo, 1989). Coffee leaf rust leads to premature leaf abscission, dieback of trees and reduction of yield (Waller, 1982; Cannel, 1973). It is mainly managed by chemical means, using inorganic fungicides such as triazoles and copper-based fungicides as well as host plant resistance (Waller, 1982). The use of inorganic fungicides is very expensive especially to smallholder resource-poor farmers and is also not environmentally friendly. Natural products such as papaya leaf extracts, cow urine and even human urine could have potential in management of plant diseases. This trial was conducted in order to evaluate natural products for their efficacy in the control of coffee leaf rust.

MATERIALS AND METHODS

Work was carried out in the laboratory, greenhouses and fields at the Coffee Research Institute, Chipinge, Zimbabwe. Aqueous leaf extracts of Carica papaya were prepared by pounding 1 kg of fresh leaves and mixing with 1 litre water. The papaya extract was diluted into concentrations of 50%, 33%, 20%, 16.7% and 12%. The dilutions were compared with the neat extract (100%) and the standard, copper oxychloride under laboratory conditions. Cow urine was collected from a dairy farm and fermented for 2 weeks in the laboratory. Different concentrations of fermented cow urine were tested in the laboratory (1ml/litre, 5ml/litre, 10 ml/litre and the neat (undiluted). These were compared with copper oxychloride. Laboratory treatments were administered by mixing the leaf extract or cow urine solutions with 9 ml of 2% water agar in sterile glass petridishes. Efficacy of the treatments was determined by evenly spreading 0.5 ml of freshly prepared coffee leaf rust spore suspension

over agar/treatment mixtures in petridishes. These were then incubated at 25 °C for +/- 18 hours in the dark. A control with no fungicide treatments was included for comparison and to calculate percent spore germination. After incubation, petri-dishes were flushed with 0.5 ml of 1% mercuric chloride aqueous solution to prevent further germination. Rust spore germination was recorded using microscopic examination. The results were expressed as percentage spore germination in terms of the germination recorded in the controls. The data on percent spore inhibition were transformed by using the arcsine square root transformation before analysis of variance using Genstat. Means were separated according to Duncan's multiple range test. A field trial for cow urine was set up in a randomized block design with 7 treatments replicated 3 times at Coffee Research Institute. Treatments were as per Table 2. Percentages were transformed and analyzed for variance. Data on percent leaf infection were collected from 4 central covas each with 5 marked primaries on a monthly basis.

Table 1. Cow urine treatments evaluated for efficacy against Coffee Leaf Rust under field conditions at Coffee Research Institute, Chipinge, Zimbabwe.

Treatment	Application rate
1. Cow urine	250 ml/litre of water
2. Cow urine	500 ml/litre
3. Cow urine	1000 ml/litre
4. Cow urine + spreader	1000 ml+spreader/litre
5. Cow urine - neat	Undiluted
6. Copper oxychloride 85% WP	10 g/litre of water
7. Control	Untreated

RESULTS

Aqueous papaya leaf extract applied as undiluted (neat) gave the lowest spore germination, which was significantly different from the standard copper oxychloride at 8ml/l under laboratory conditions (Table 2). There appeared to be a dose- dependent increase in efficacy of the aqueous papaya extracts against coffee leaf rust. This suggests that a lot of leaf materials will be required to achieve good efficacy under field conditions.

Table 2. Effect of aqueous papaya leaf extracts on coffee leaf rust spore germination under laboratory conditions at Coffee Research Institute, Chipinge, Zimbabwe.

	Assessment dates				
Treatment	1	2	3	4	Mean
50%	31.1c*	28.5d	27.8c	30.1cd	29.4a
33%	47.3b	42.9c	38.9c	40.0c	42.3b
20%	59.3ab	55.7b	51.0b	58.3b	56.1c
16.7%	60.2a	60.3b	52.3b	70.7a	60.9d
12%	66.6a	71.6b	69.6a	71.7a	69.9e
Copper oxychloride	19.7cd	23.5de	20.9de	28cd	23.0f
100% (neat)	11.6d	15.7e	15.2e	23.5d	16.5g
$LSD_{0.05}$	12.5	8.58	7.87	12.04	3.46
CV	16.6	11.30	11.20	14.70	4.60

**Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test.*

Undiluted fermented cow urine (neat) compared well with the standard (copper oxychloride) in reducing spore germination under laboratory conditions (Figure 1). There were no significant differences in efficacy against leaf rust between the other concentrations but there was a decrease in leaf rust spore germination with increase in concentration.

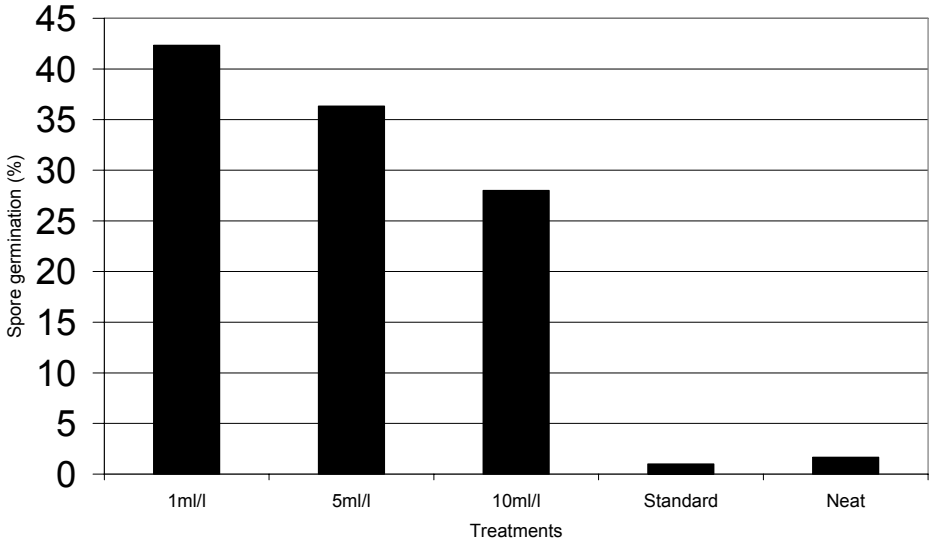


Figure 1. Effect of different concentrations of fermented cow urine on coffee leaf rust spore germination under laboratory conditions.

There were no significant differences between different concentrations of fermented cow urine and the standard (copper oxychloride) under field conditions. However, undiluted urine caused leaf burn in the field.

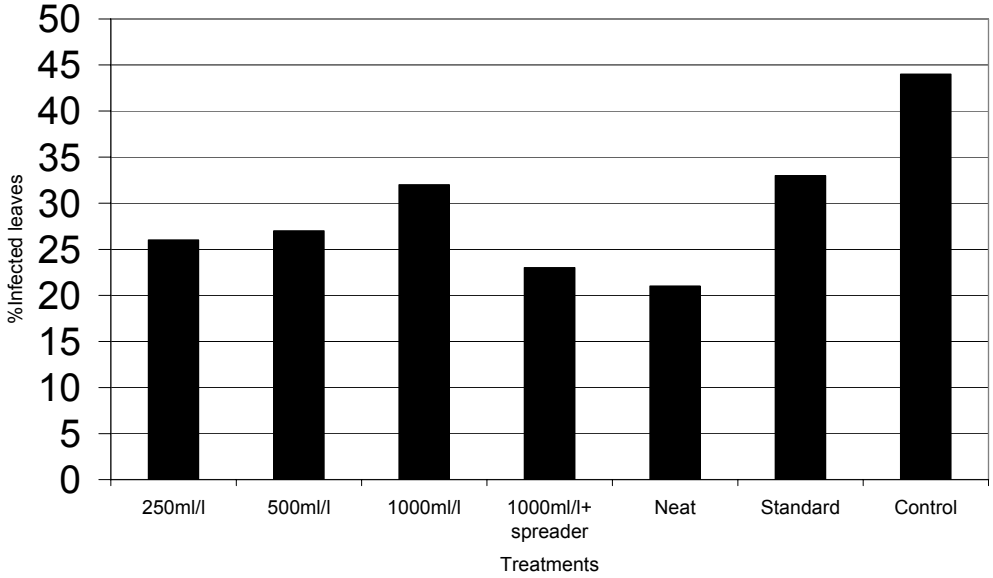


Figure 2. Effect of different concentrations of fermented cow urine on coffee leaf rust infection under field conditions at Coffee Research Institute, Chipinge, Zimbabwe.

CONCLUSION

Papaya aqueous leaf extracts showed potential in inhibiting coffee leaf rust spore germination under laboratory conditions whereas cow urine compared well with the standard under laboratory conditions but there were no significant differences in efficacy in the field. The

increase in efficacy with increase in concentration of both papaya leaf extract and fermented cow urine suggests that more materials are required to achieve good efficacy under field conditions.

More work still needs to be done to evaluate papaya leaf extracts and fermented cow urine under field conditions.

ACKNOWLEDGEMENTS

The Director, Agriculture Research for Development, Ministry of Agriculture, Zimbabwe is sincerely thanked for encouraging us to participate while the Technical Centre for Agricultural and Rural Cooperation (CTA) and the scientific committee of ASIC facilitated the funding to enable attendance at the conference.

REFERENCES

Cannel M.G.R. Effects of irrigation, mulch and N-fertilisers on yield components of Arabica coffee in Kenya. Exp. Agric. 1973. 9:225-232

Castilo-Z J. Breeding for rust resistance in India. In Kushalappa A.C. and Eskes A.B (eds). Coffee Rust Epidemiology, Resistance and Management CRC Press Inc., Florida 1989. pp. 307-316

Monaco L.C. Consequences of the introduction of coffee rust into Brazil. In Day, P.R. (ed). The Genetic basis of epidemics in Agriculture. Ann. NY Aca. Sci. 1977. 287:57-71

Sreenivasa M.S. Breeding for rust resistance in India. In Kushalappa A.C. and Eskes A.B (eds). Coffee Rust Epidemiology, Resistance and Management CRC Press Inc., Florida 1989. pp. 316-323

Waller J.M. Coffee rust epidemiology and control. Crop Protection 1982 1:(4):385-404

Ward H.M. From Mr Marshall Ward's report. Tropical Agriculturalist. 1881. 1:506-30

Evaluation of the Effect of the Fungicide Priorixtra (Azoxystrobin + Cyproconazole) on the Control of Leaf Rust and Cercospora of Irrigated Coffee Fields, Irrigated by the Net Sprinkler Irrigation System

A.L.T. FERNANDES[1], C.A.V. BECKER[2], R. DE OLIVEIRA SILVA[1], G. BECKER[1]

[1]University of Uberaba and FAZU, Uberaba, MG, Brazil
[2]Syngenta, São Paulo, SP, Brazil

SUMMARY

Leaf rust is the principal disease of coffee growing. It can be found in all fields cultivated in Brazil, and can lead to losses above 35% in regions that are favorable to the disease. Under conditions of prolonged drought, this can exceed 50% (ZAMBOLIM et al., 1999). According to Santinato and Fernandes (2002), it is caused by the fungus *Hemileia vastatrix*, and the principal damaged caused is premature leaf loss and dry branches, which, as a result, do not produce fruit the following year. If it is not controlled, with intense and successive attacks, it can lead to major losses in production and deterioration of fields, requiring producers to invest large sums of money to recover their plantations. According to Santinato and Fernandes (2002), the disease may be controlled genetically and chemically. Chemical control, according to Zambolim et al. (1999), may be done preventively, with contact fungicides, of which cupric fungicides are the most effective, or through the use of systemic fungicides applied to the foliage or the ground. The forms of soil application of these products may be for GR formulations with granulators and for WG formulations in liquid form using special equipment (application bars), or through the irrigation water (chemigation). Another method of application is through the irrigation system itself, using a technique known as fungigation and insectigation, used more often in central pivot irrigation system with localized sprinklers and drip. In order to evaluate the agronomic efficiency of the systemic fungicide Azoxystrobin + Cyproconazole in chemical control of leaf rust and cercospora leaf spot (*Hemileia vastatrix*), research was set up at the Research and Teaching Farm, at the University of Uberaba, Minas Gerais, Brazil, in December 2005. The research was conducted through June 2006, at a plantation of *Catuaí Vermelho* coffee irrigated by the net sprinkler system. The treatments were: 1) Azoxystrobin + Cyproconazole + Nimbus in 3 applications of 0.5 l/ha (Dec/05; Feb/06 and Mar/06); 2) Azoxystrobin + Cyproconazole + Nimbus in 2 applications, of which 0.75 l/ha in Dec/05 and 0.75 l/ha in mar/06; 3) Azoxystrobin + Cyproconazole + Nimbus in 2 applications, of which 1.0 l/ha was in Dec/05 and 0.5 l/ha in Mar/06; 4) Epoxiconazole + pyraclostrobin, in two applications, of which 1.5 l/ha in Dec/05 and 1.0 l/ha in Mar/06; 5) Control. All the treatments, as of March 2006, presented satisfactory control of leaf rust and cercospora, in spite of the high rate of infection in the control, with a 50% infection rate. Beginning in May 2006, the only treatment effective in the control of the disease was Azoxystrobin + Cyproconazole, applied 3 times (Treatment 1), a performance that remained satisfactory until June. In May and June, the control reached a high infection rate, 99 and 100%, respectively. The other treatments (2, 3 and 4) were all statistically inferior to Treatment 1. With regards to cercospora on the leaves, a similar superiority as that in Treatment 1 was seen, with only 1.5% infection in the leaves, compared to 15.3, 17, 8, 35.3 for treatments 2, 3 and 4, and a high control infection, reaching 88.5%. In

the evaluation of cercospora in the beans, the best treatment was the application of a product composed of Azoxystrobin + Cyproconazole in 3 times of 0.5 l/ha.

INTRODUCTION

Leaf rust is the principal disease of coffee tree growing. It can be found in all plantations grown in Brazil, with losses, in regions favorable to the disease, in excess of 35%, which can, under prolonged drought conditions, exceed 50% (ZAMBOLIM et al., 1999). If not controlled, with intense and successive attacks, it can lead to high losses in production and to decline of the plantations, requiring high investments from producers to replenish them. According to Santinato and Fernandes (2002), control of this disease can be genetic or chemical. Chemical control, according to Zambolim et al. (1999) can be done preventively, with contact fungicides, of which the cuprics are the most effective, or through the use of systemic fungicides through via leaves or soil. The forms of soil application of these products can be done for GR formulations with granulators for the WG formulations in liquid form, using backpack/manual equipment (localized application at the base of the plants) or mechanized with the "*beckini*" bar adapted to the PH or to atomizers (application as a continuous thin line along planting line). Another application method is through the irrigation system, using the technique known as fungigation (sprinkler application) and insectigation, which are more often used in the center pivot system with localized emitters and drip irrigation. Several active ingredients are being launched by manufacturers to better control the disease, preferably with products with lower toxicity rates. Within this context, with the aim of evaluating a new fungicide, PrioriXtra (azoxystrobin + cyproconazole), in the control of leaf rust and cercospora of the coffee tree, at three stages of application, this experiment was conducted at the Experimental Field Farm School of the University of Uberaba, in Uberaba – MG, in a Red Catuai plantation irrigated by the net sprinkler system.

MATERIAL AND METHODS

The experiment was conducted at the Experimental Field Farm School of the University of Uberaba, Uberaba – MG, on cultivated Red Catuai coffee trees – IAC 144; age: 7 years old; spacing: 4,0 x 0,5 m (5.000 plants per ha). Evaluations of leaf rust and cercospora control were performed between January and June 2006. The experimental delineation was random blocks, with 5 treatments and 6 repetitions, with plots of 2400 m^2. The plants were irrigated by the net sprinkler system, with sprinklers installed net of 15 x 15m. For definition of the irrigation depth, estimated evapotranspiration data were used via the Penman Monteith Method, based on data collected at an automatic agro-meteorological station of the brand name Metos, model Micrometos 300. The products used for phytosanitary control and respective programs of use are found in Table 1. The commercial products PrioriXtra and Opera were pulverized using a Jacto Arbus 2000 brand atomizer with flow of 400 l/ha. The following evaluations were made: a) leaf rust infection in the leaves, per plot, from February to June 2006 (after the control grouped reached a 10% infection rate. For leaf evaluations, 100 leaves were collected, from the two sides of the coffee tree, in 6 repetitions per plot); b) cercospora infection on the leaves, from February to June 2006; c) cercospora infection in the beans. Monthly evaluations were made beginning in February, when the infection began to grow in the control group. In each evaluation, 100 leaves were collected at random in 20 central plants for each repetition, in the 3rd and 4th pairs of the branches on the lower third of the coffee trees, determining the percentage of leaves with live pustules and cercospora infection. The results were submitted to statistical analysis, with application of the variance T-test and comparison of the averages by the Tukey test, at a 5% probability. In addition to control of leaf rust, the plots were harvested during the period June/July of 2006.

Table 1. Products, dosages, application times and equipment in the programs, Farm School (Uniube), Uberaba – MG, December / 2005 to June / 2006.

Treatments	Doses (Kg/l/ha)	Equipment	Application Times
1) Azoxystrobin + Cyproconazole + Nimbus*	0,5 l/ha + 0.5%	Arbus 2000 l	15/Dec 2005
	0,5 l/ha + 0.5%	Arbus 2000 l	15/Feb 2006
	0,5 l/ha + 0.5%	Arbus 2000 l	15/Apr 2006
2) Azoxystrobin + Cyproconazole + Nimbus*	0,75 l/ha + 0.5%	Arbus 2000 l	15/Dec 2005
	0,75 l/ha + 0.5%	Arbus 2000 l	15/Mar 2006
3) Azoxystrobin + Cyproconazole + Nimbus*	1,00 l/ha + 0.5%	Arbus 2000 l	15/Dec 2005
	0,50 l/ha + 0.5%	Arbus 2000 l	15/Mar 2006
4) Epoxiconazole + pyraclostrobin	1,5 l/ha	Arbus 2000 l	15/Dec 2005
	1,0 l/ha	Arbus 2000 l	15/Mar 2006
5) Control	-	-	-

** Nimbus - 2 l/ha*

RESULTS AND DISCUSSION

After 5 evaluations, it was noted that the leaf rust increased considerably in the control group, as it did during the last 4 years, beginning in March, as per Table 2. All the treatments by March 2006 showed satisfactory control of the disease, in spite of the high infection in the control group, at 50%. Beginning in May 2006, the only treatment that was effective in the control of the disease was the PrioriXtra applied 3 times (Treatment 1), a performance which remained satisfactory until June. In May and June, the control group hit a high level of infection, 99 and 100%, respectively. The other treatments (2, 3 and 4) were all statistically inferior to treatment 1. Based on these results, it is possible to infer that chemical control of coffee tree leaf rust is essential to obtain satisfactory results with coffee growing. According to Matiello and Almeida (2006), its substitution is made difficult by the fact that the majority of coffee growers opted for susceptible varieties, with high productivity and vigor. With regards to cercospora on the leaves, a similar superiority of Treatment 1 was noted, with an infection rate of just 1.5% in the leaves, compared with 15.3, 17.8, 35.3 of treatments 2, 3 and 4, and high infection of the control group, which reached 88.5% (Figure 1).

Table 2. Results of leaf rust infection from February to June 2006, Farm School – Uniube, Uberaba, MG.

Treatments	Infection (% of leaves with leaf rust)				
	Feb/06	Mar/06	Apr/06	May/06	Jun/06
1) PrioriXtra + Nimbus (0,50 + 0,50 + 0,50)	0,5 a	0.5 a	0.5 a	0.5 a	1.3 a
2) PrioriXtra + Nimbus (0,75 + 0,75)	0,5 a	1,0 a	3.5 a	8,0 b	20.3 b
3) PrioriXtra + Nimbus (1,00 + 0,50)	0,5 a	0.5 a	1,0 a	12,0 b	24.0 b
4) Opera + Nimbus (1,50 + 1,00)	1,0 a	2,0 a	3,0 a	25,0 b	44.3 c
5) Control	6,0 a	15,0 b	50,0 b	99,0 c	100.0 d
C.V. (%)	12,0	18,0	24,0	20,0	21,0

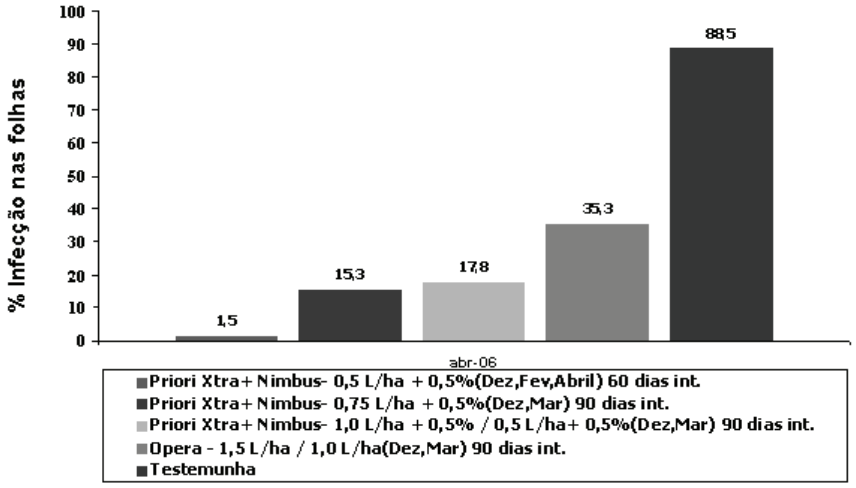


Figure 1. Results of the evaluations of cercospora inspection in leaves under different treatments, Farm School Experimental Field, Uniube, Uberaba – MG.

In the evaluation of cercospora in the beans, the best treatment was three applications of PrioriXtra at 0.5 l/ha (Treatment 1), as per Table 3. The dosage of 0.5 l/ha is recommended by Matiello and Almeida (2006), although the authors do not indicate the time or the number of applications.

Table 3. Results of cercospora infection in coffee tree beans, Farm School – Uniube, Uberaba, MG.

Treatments	% of healthy beans	% of affected beans	% of dry /rotten beans
1) PrioriXtra + Nimbus (0.50 + 0.50 + 0.50)	93.3	2.0	4.37
2) PrioriXtra + Nimbus (0.75 + 0.75)	86.2	6.8	7.0
3) PrioriXtra + Nimbus (1.00 + 0.50)	89.4	5.4	5.2
4) Opera + Nimbus (1.50 + 1.00)	87.5	7.4	5.1
5) Control	36.5	37.0	26.4

Figure 2 presents the average number of beans per production of a liter of coffee. Note the superiority of treatment 1 (567 beans) compared to the others, especially the control (913 beans/1 liter of coffee).

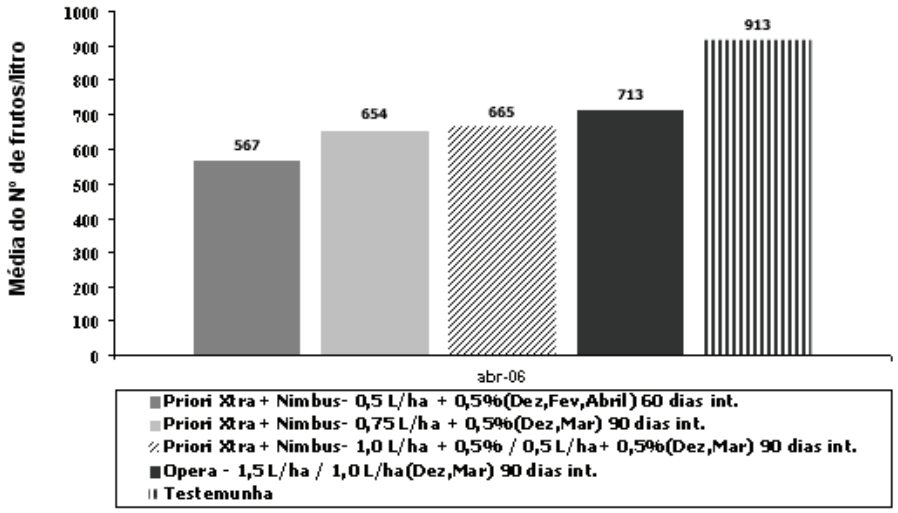


Figure 2. Average beans per liter of coffee, for different treatments.

CONCLUSION

Under the conditions of this experiment, it can be concluded that the best treatment option for coffee tree leaf rust and cercospora is through the application of the product PrioriXtra in 3 doses of 0.5 l/ha, in 60 day intervals, beginning in December.

REFERENCES

Matiello J.B., Almeida S.R. A ferrugem do cafeeiro no Brasil e seu controle. Varginha: Fundação Procafé, 106p. 2006.

Pinto N.F.J De A. Fungigação e nematigação. In: Costa, E.F. Da, Vieira R.F., Viana P.A. Quimigação: aplicação de produtos químicos e biológicos via irrigação. Brasília: EMBRAPA, 1994. cap. 9.p. 229-248.

Santinato R., Fernandes A.L.T. Cultivo do cafeeiro irrigado em plantio circular sob pivô central. Belo Horizonte: O Lutador, 2002. 252 p.

Zambolim L., Vale F.X.R., Pereira A.X., Chaves G.M. Manejo integrado das doenças do cafeeiro, In: Zambolim L. I Encontro sobre produção de café com qualidade, 1999. p. 134-215.

Efficiency of Cyproconazole + Thiamethoxan, Within a Program of Use, Applied Via Drip Irrigation Water in the Control of Coffee Leaf Rust

A.L.T. FERNANDES[1], C.A.V. BECKER[2], R. DE OLIVEIRA SILVA[1]

[1]University of Uberaba and FAZU, Uberaba, MG, Brazil
[2]Syngenta, São Paulo, SP, Brazil

SUMMARY

Leaf rust, caused by the fungus *Hemileia vastatrix* can be found in all fields cultivated in Brazil, with losses in regions that are favorable to the disease above 35%. Under conditions of prolonged drought, this can exceed 50% (Zambolim et al., 1999). According to Santinato and Fernandes (2005), the disease may be controlled genetically and chemically. Chemical control, according to Zambolim et al. (1999), may be done preventively, with contact fungicides, of which cupric fungicides are the most effective, or through the use of systemic fungicides applied to the foliage or the ground. The forms of soil application of these products may be for GR formulations with granulators and for WG formulations in liquid form using stem/manual equipment (localized application on the plant stems) or a mechanized bar adapted to PH or to atomizers. Another method of application is through the irrigation system itself, using a technique known as fungigation and insectigation, used more often in central pivot irrigation system with localized sprinklers and drip. Almeida Pinto (1994) has noted that the application of fungicides and nematicides via irrigation water is common in countries with highly technical irrigated agriculture. However, in Brazil, there is little research data available currently, and this technique has been adopted without adequate scientific backing. In order to evaluate the practicality and agronomic efficiency of the application of fungicide and insecticide products via irrigation water in the chemical control of leaf rust and (*Hemileia vastatrix*), as well as the influence of these products with nitrogenated and potassic fertilizers, research was set up at the Research and Teaching Farm, at the University of Uberaba, Minas Gerais, Brazil, between November 2003 and July 2005 (two harvests) on a plantation of *Catuaí Vermelho* coffee irrigated by the drip system. After the evaluation, the following conclusions were reached: a) even in years of high leaf rust infection, control of leaf rust is viable with the use of Cyproconazole + Thiamethoxan via irrigation water within a use program; b) both formulations of Cyproconazole + Thiamethoxan tested were highly efficient in the control of leaf rust and were superior to the standard use program (Triadimenol + Disulfoton / Tebuconazole – 2 times / Aldicarb); c) the addition of fertilizer ($N + K_2O$) in the application of Cyproconazole + Thiamethoxan and Thiamethoxan, via irrigation water, led to an improvement in disease control; d) the GR formulation of Cyproconazole + Thiamethoxan in the program of use was slightly superior to the WG formulation; and) the programs of use with Cyproconazole + Thiamethoxan and Thiamethoxan were superior to the standard program of use in control of leaf rust; f) in the first production, all programs were superior to the control, with superiority for programs Cyproconazole + Thiamethoxan and Thiamethoxan (WG and GR), with 46.4 and 47.0 beneficiated sacks/ha, respectively.

INTRODUCTION

Coffee tree leaf rust can be found in all coffee plantations in Brazil, with losses in regions favorable to the disease, in excess of 35%, which may, under prolonged drought conditions, exceed 50% (Zambolim et al., 1999). According to Santinato; Fernandes (2002), it is caused by the fungus *Hemileia vastatrix*, and the principal damage caused are early loss of leaves and drying of branches, which, as a result, do not produce beans the following year. If not controlled, with intense and successive attacks, it can lead to high losses in production and to decline of the plantations, requiring high investments from producers to replenish them. According to Santinato and Fernandes (2002), control of this disease can be genetic or chemical. Chemical control, according to Zambolim et al. (1999) can be done preventively, with contact fungicides, of which the cuprics are the most effective, or through the use of systemic fungicides through via leaves or soil. The forms of soil application of these products can be done for GR formulations with granulators for the WG formulations in liquid form, using backpack/manual equipment (localized application at the base of the plants) or mechanized. Another application method is through the irrigation system, using the technique known as fungigation (sprinkler application) and insectigation, which are more often used in the center pivot system with localized emitters and drip irrigation. Almeida Pinto (1994) noted that the application of fungicides and nematicides via irrigation water is common in countries with highly technical agriculture. However, in Brazil, there is currently little research data available, and this technique has been adopted without adequate scientific backing. In coffee plantations irrigated by the drip system, the practice is not to use products applied through irrigation water, due to a lack of conclusive experimental data. Thus, in order to: a) evaluate the efficiency of cyproconazole + Thiamethoxan and Thiamethoxan used in isolation, applied through drip irrigation water, within a use program in the control of coffee tree pests and diseases; b) evaluate the effect of the addition of nitrogenated and potassic dressing on the efficiency of the mixture cyproconazole + Thiamethoxan and Thiamethoxan by the drip system; c) compare the efficiency of the formulations WG and GR of cyproconazole + Thiamethoxan and Thiamethoxan in the use programs versus the standard program (triadimenol + disulfoton / tebuconazole – 2 times / aldicarb) and d) evaluate the effect of the different use programs in the productivity of the coffee tree, the following experiment was conducted at the Experimental Field Farm School of the University of Uberaba, in Uberaba – MG.

MATERIAL AND METHODS

The experiment was conducted at the Experimental Field Farm School of the University of Uberaba, Uberaba – MG, on cultivated Red Catuai coffee trees – IAC 144; age: 7 years old; spacing: 4,0 x 0,5 m (5.000 plants per ha). Evaluations of leaf rust control were performed between February to May 2004 and February to May 2005. The experimental delineation was strips, with 5 treatments and 4 repetitions, with plots of 2400 m^2. The irrigation system used was drip irrigation with self-compensating emitters of the Netafim brand, RAM model, with unitary flow of 2.3 L h^{-1}, and spacing of 0.75 m between drippers along the planting lines. For definition of irrigation depth, estimated evapotranspiration data obtained via the Penman Monteith Method were used, based on data collected at an automatic agro-meteorological station of the brand name Metos, model Micrometos 300. The products used for phytosanitary control, within the respective treatment programs, are in Table 1. The commercial products Cyproconazole + Thiamethoxan and Actara, formulation WG, were applied via a drip irrigation system. The commercial products cyproconazole + Thiamethoxan GR, disulfoton + triadimenol and aldicarb were applied via the soil, with the use of a granulator (Granulex). The leaf fungicides were pulverized using a Jacto Arbus 2000 atomizer with a flow of 400 l/ha.

Table 1. Products, doses, application times and equipment in use programs.

Treatments	Identification of Programs	Doses (Kg/l/ha)	Equipment	Time of Application
Cyproconazole + Thiamethoxan	Café Forte	30	Granulex	nov/03 e 04
Azoxystrobin + Nimbus		0,1+0,5%	Arbus 2000 l	dez/03 e 04
Azoxystrobin + Nimbus		0,1+0,5%	Arbus 2000 l	fev/04 e 05
Thiamethoxan (10 GR)		25	Granulex	fev/04 e 05
Cyproconazole + Thiamethoxan (600 WG*)	Café Forte Revolution	1	Drip	nov/03 e 04
Azoxystrobin + Nimbus		0,1+0,5%	Arbus 2000 l	dez/03 e 04
Azoxystrobin + Nimbus		0,1+0,5%	Arbus 2000 l	fev/04 e 05
Thiamethoxan (250 WG*)		1	Drip	fev/04 e 05
Cyproconazole + Thiamethoxan (600 WG*)+N+K_20*	Café Forte Revolution with dressing	1+ **	Drip	nov/03 e 04
Azoxystrobin + Nimbus		0,1+0,5%	Arbus 2000 l	dez/03 e 04
Azoxystrobin + Nimbus		0,1+0,5%	Arbus 2000 l	fev/04 e 05
Thiamethoxan (250 WG*)+N+K_20*		1+ **	Drip	fev/04 e 05
Disulfoton + triadimenol	Standard Program (Year 1)	50	Granulex	nov/03
Tebuconazole		1	Arbus 2000 l	dez/03
Tebuconazole		1	Arbus 2000 l	fev/04
Aldicarb		25	Granulex	fev/04
Baysiston GR	5) Standard Program – New (Year 2)	40	Granulex	nov/04
Copper Hydroxide		1.7	Atomizer	dez/04
Copper Hydroxide		1.7	Atomizer	fev/05
Sphere		0.9	Atomizer	mar/05
Aldicarb		25	Granulex	fev/05
Control	-	-	-	-

**Applied via irrigation water (drip irrigation system).*
***15 kg of N and 15 kg of K_2O/ha.*

Monthly evaluations were made beginning in February, when the infection began to grow in the control group. The treatments were evaluated until May/04 and May/2005. In each evaluation, 100 leaves were collected at random in 20 central plants for each repetition, in the 3rd and 4th pairs of the branches on the lower third of the coffee trees, determining the percentage of leaves with live pustules. The results were submitted to statistical analysis, with application of the T-test of variance and comparison of the means using the Tukey test, at a 5% probability. In addition to control of leaf rust, the plots were harvested in the period June/July of 2004 and 2005.

RESULTS AND DISCUSSION

In Table 2 and Figures 1a and 1b, the results of % de infection of leaf rust, relative efficiency of control (ER%) and productivity of the different use programs can be seen, respectively, for 2004 and 2005. As of March the use programs, Café Forte, Café Forte Revolution and Café Forte Revolution with dressing, presented the best control of leaf rust, and were statistically superior to the standard program, which was only superior to the control, since during this period it presented an infection rate of 100 % in 2004 and 87% in 2005. In April 2004, the best efficiencies in leaf rust control were again seen in the programs Café Forte (ER = 97%) and Café Forte Revolution with fertilizing (ER = 95%), which were statistically superior to the program Café Forte Revolution and to the standard program, which was not statistically

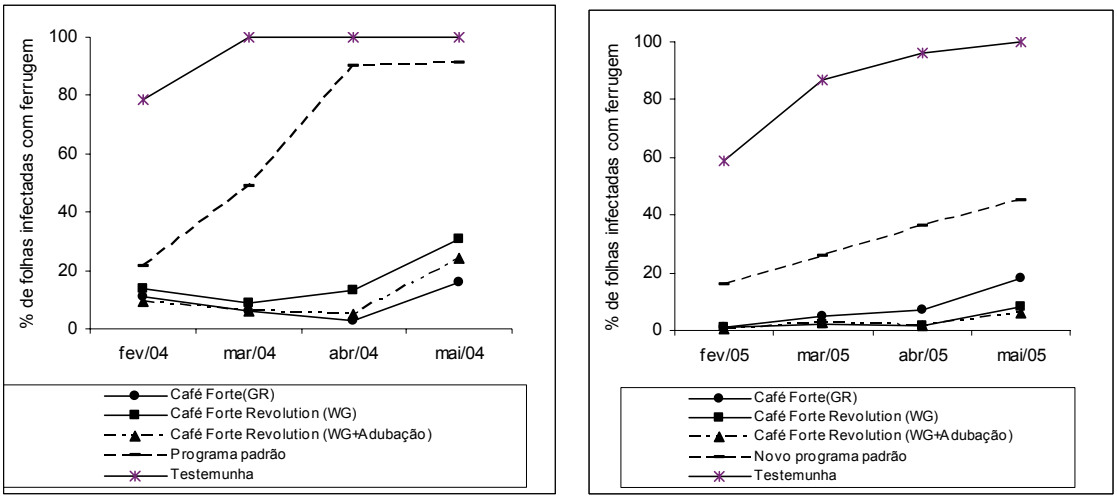
different from the control. For April 2005, the best control results were obtained with the treatments Café Forte Revolution, with and without dressing (98 and 99% of control efficiency, respectively). In the evaluation of May/04, the Café Forte program showed the best control and residual effect (84% ER), and was followed by the programs Café Forte Revolution with dressing (76% ER) and Café Forte Revolution (69% ER). For May 2005, the best results obtained were with the programs Café Forte Revolution (with and without dressing), with control efficiencies of 92 and 94%, respectively, superior to the program Café Forte (ER = 82%) and to the standard (ER = 55%).

Table 2. Infestations, relative efficiency of control and productivity, per treatment, for the years 2004 and 2005 (Experimental Field Farm School, Uberaba, MG).

Use Programs	Infection (% of leaves with leaf rust)																Production (benef. sacks/ha)	
	February				March				April				May					
	2004		2005		2004		2005		2004		2005		2004		2005		2004	2005
	%	ER	%	ER	%	ER	%	ER	%	ER	%	ER	%	ER	%	ER		
Café Forte (GR)	11a	86	1a	98	6a	94	5a	94	3a	97	7,2b	93	16a	84	18b	82	46,4	47,42
Café Forte Revolution (WG)	14a	82	1a	98	9a	91	2a	98	13b	87	1,4a	99	31b	69	8a	92	47,0	39,00
Café Forte Revol. (WG+ Dressing)	9,3a	88	0,5a	99	6a	94	3a	97	5,2a	95	1,5a	98	24b	76	6a	94	47,0	42.41
Standard Program	21,3b	73	16b	73	49b	51	26b	70	90c	10	36c	63	91c	9	45c	55	33,2	45,42
Control	78,5c	0	59c	0	100c	0	87c	0	100cd	0	96d	0	100cd	0	100d	0	24,1	2,68
C.V. (Tukey) = 12% (2004); 14% (2005)																		

E.R. = Relative Efficiency (ABBOT, 1925).

With regards to pests, it was not possible to evaluate the performance of the different programs, as they did not occur during the two years of the experiment. Also with regards to production, for the average of two harvests, superiority of the use programs Café Forte, Café Forte Revolution and Café Forte Revolution with dressing was noted, with 46.9, 43.0 and 44.7 beneficiated sacks/ha, respectively, and they were superior to the standard and control programs, which had production of 39.3 and 13.4 beneficiated sacks/ha, respectively. Pinto (1994) notes that the systemic fungicides should be preferred in relation to contact fungicides, as they are translocated in the upwards direction of the plant, and when applied to the soil, they can be absorbed by the roots, and be translocated to the air part. According to Segundo Almeida Pinto (1994), the application of fungicides through the irrigation system can bring benefits such as: savings in labor costs, good uniformity of application, little contact by operator with products, possibility of application of product at any phase of the growing cycle, less physical damage to the soil (reduction of compacting) and to the crop and reduction of production costs. Due to the fact that Cyproconazole + Thiamethoxan, with the WG formulation (granules can be dispersed in water) is efficient in the control of coffee tree leaf rust within a use program, and since they are less toxic than those commonly applied in traditional coffee plantations, the option can be made to apply these products via irrigation water.



(a) **(b)**

Figure 1. Leaf rust infection (%) in the different treatments studies: 2004; (b) 2005.

CONCLUSION

a) Control of coffee tree leaf rust is feasible with the utilization of Cyproconazole + Thiamethoxan applied via irrigation water, in use programs, even in years of high disease incidence;
b) In the control of leaf rust, the use programs Café Forte, Café Forte Revolution and Café Forte Revolution with dressing were highly efficient and superior to the standard program;
c) The addition of nitrogenated and potassic dressing to the Cyproconazole + Thiamethoxan and Thiamethoxan, in the irrigation water through the drip method led to a slight improvement in efficiency of the program;
d) The programs Café Forte and Café Forte Revolution with and without dressing presented, for the average of the two harvests, similar productivity (46.9, 43.0 and 44.7 beneficiated sacks./ha, respectively) and superior to the standard program (average of 39.3 beneficiated sacks./ha) and the control (average of 13.4 beneficiated sacks./ha).

REFERENCES

Abott W.S. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. v.18, p. 265-267, 1925.

Almeida Pinto N.F.J. Fungigação e nematigação. In: Costa E.F., Vieira R.F., Viana P.A. Quimigação: aplicação de produtos químicos e biológicos via irrigação. EMBRAPA-SPI, Brasília, p. 229-249, 1994.

Pinto N.F.J de A. Fungigação e nematigação In: Costa E.F. Da, Vieira R.F., Viana P.A. Quimigação: aplicação de produtos químicos e biológicos via irrigação. Brasília: EMBRAPA, 1994. cap. 9.p. 229-248.

Santinato R., Fernandes A.L.T. Cultivo do cafeeiro irrigado em plantio circular sob pivô central. Belo Horizonte: O Lutador, 2002. 252 p.

Zambolim L., Vale F.X.R., Pereira A.X., Chaves G.M. Manejo integrado das doenças do cafeeiro, In: Zambolim L. In: I Encontro sobre produção de café com qualidade, 1999. p. 134-215.

Early Haustoria Development in Coffee (*Coffea arabica*) - Rust (*Hemileia vastatrix*) Interaction

D.A. RAMIRO[1], J. ESCOUTE[2], A.-S. PETITOT[1], M. MALUF[3], D. FERNANDEZ[1]

[1]IRD (Institut de Recherche pour le Développement), UMR186 IRD/CIRAD/Univ. Montpellier 2, Résistance des Plantes aux Bioagresseurs (RPB), BP64501, 34394 Montpellier cedex 5, France
[2]Cirad, UMR1096 DAP, Montpellier RIO Imaging, 34398 Montpellier Cedex 5, France
[3]Embrapa Café, IAC (Instituto Agronômico de Campinas),Centro de Café 'Alcides Carvalho' - CP 28, 13.001-970 Campinas, SP – Brasil

SUMMARY

According to their lifestyle, plants parasitic fungi may be classified in necrotrophs which obtain nutrients from dead cells or biotrophs which obtain nutrients from living host tissue. A common feature of the biotrophs is the formation of specialized structures called haustoria, which are responsible for uptake of nutrients from the host cells they invaded. Avirulence (*Avr*) genes and their respective products that are recognized by the resistance proteins inside host cells may be specifically expressed in haustoria, leading to the rapid activation of defense responses against the fungus. The objective of this study was to assess the time-course of the orange rust fungus (*Hemileia vastatrix*) haustoria differentiation in coffee (*Coffea arabica*). Histological characterization of *H. vastatrix* (race II) development in Tupi (immune) and Catuai (sporulation level 4) was assessed by light microscopy examination of cross sections of infected leaf fragments stained with lactophenol blue. In both interactions, careful examination of infection sites made at 600x revealed that haustorial differentiation occurred as soon as the fungus enters stomata, much earlier than previously described in the literature. Haustorium mother cell (HMC) and haustoria (termed “pioneers haustoria”) were found in the guard and adjacent cells of the stomata, before the colonization of parenchyma had occurred. In all interactions, after pioneer haustoria differentiation, the fungus displayed the usual sequence of growth stages: anchor, new HMC and haustoria development in stomatal cells and in the parenchyma. Until 36 hours after inoculation, rust development in the two cultivars did not differ. Examination of host cell death, characteristic of the hypersensitive reaction in resistant varieties, and of defense genes regulation during rust infection are currently being examined to assess the coffee resistance responses linked to pioneers haustoria formation.

INTRODUCTION

After plant leaf penetration, rust pathogens colonize plant tissues by spreading infection hyphae that form haustorium mother cells (HMCs) involved in plant cell wall penetration and in the production of the haustorium. Through the invaginated plant cell plasma membrane, the haustorium coordinates the uptake of host water and nutrients, and also signaling between host and parasite to establish and maintain compatibility (Perfect and Green, 2001; Voegele and Mendgen, 2003; O’Connell and Panstruga, 2006). Recently, major insights have emerged from studies of haustoria-forming plant pathogens suggesting that the haustorium plays a critical role in delivering fungal effector proteins, including avirulence proteins, into the infected host cell (O’Connell and Panstruga, 2006; Catanzariti et al., 2007).

The orange rust, caused by the fungus *Hemileia vastatrix* (Berkeley and Broome), is the most severe disease of coffee plants (*Coffea arabica* L.). Plant infection process has been well documented, and involves production into the substomatal chamber of a vesicle in an anchor shape, followed by differentiation of HMCs and haustoria that primarily infect the stomatal subsidiary and guard cells, around 36-48 hours after infection (hpi) (Rijo and Rodrigues, 1977; Coutinho et al., 1993; Martins and Moraes, 1996; Silva et al., 1999). The coffee-orange rust interaction follows a "gene-for-gene" model with nine plant resistance factors that are implicated in the recognition of the corresponding virulence genes in more than 45 rust races (Rodrigues et al., 1975; Varzea and Marques, 20059). Histological observations showed that *C. arabica* resistance may be expressed by a hypersensitive reaction (HR) with cell death of stomatal and mesophyl cells (Martins and Moraes, 1996; Silva et al., 2002). Molecular analysis of genes expressed during early coffee-orange rust interactions showed that differential gene expression between compatible and incompatible interactions could be detected as early as 24 hpi (Fernandez et al., 2004). Based on the time-course of fungal development described in (Silva et al., 2002), this result suggested that specific pathogen recognition may have occurred as soon as the fungus entered the stomata, and before haustoria were produced.

To clarify these data, the objective of this study was to re-examine and precisely relate the time-course of *H. vastatrix* development with that of molecular resistance responses expression in *C. arabica*. This information will be useful to assess the time point of specific recognition of the rust pathogen by the coffee plant, which in turn should be useful for understanding coffee-orange rust interactions.

MATERIAL AND METHODS

C. arabica var. Tupi IAC1669-33 (resistant) and Catuai IAC81 (susceptible) were kept in greenhouse conditions (24 °C, 60% RH and 16h light period). For rust fungus assays, leaves of six-month-old plants were challenged with urediospores of the *H. vastatrix* isolate CIFC 1427 (race II) as previously described (Fernandez et al., 2004).

Evaluation of fungal growth in the host tissues was performed using transversal cross sections (18 μm thick) of infected leaves made with a freezing microtome (Microm HM520,Thermo Fisher Scientific) (Rijo and Rodrigues, 1977). The fragments were stained in a 70 °C warmed solution of cotton blue lactophenol (0.5%) for 2 min, washed twice in distillated water and mounted in glycerol (50%). Fungal stages inside leaf tissues were recorded from 25 to 40 infection sites per experiment, at 24, 30 and 36 hpi, with three replication (plants). Observations were made with a light microscope Leica DMRXA (Leica, Wetzlar, Germany) at 600x magnification.

RESULTS

The extent and time-course of the rust fungus development in the varieties Tupi (resistant) and Catuai (susceptible) was assessed by microscopic histological analysis. By 24 hpi, careful examination of microscopic slides unexpectedly revealed the presence of haustoria in the guard or subsidiary cell of the stomata in a number of infection sites for the two coffee varieties (Fig.1 and table 1). Results showed that haustorium production had occurred as soon as the fungus entered into the stomata, and before reaching the substomatal cavity. After appressorium formation over the stomata, the pathogen differentiated an infection hypha, named here pioneer hypha, which formed haustorial mother cells (HMC) that gave birth to a haustorium in the guard or subsidiary cell of the stomata (Fig. 1). This first haustorium produced by the rust fungus in the stomata was named by us pioneer haustorium (Figure 2).

By 24 hpi, the stages of pioneer hypha and pioneer haustoria accounted for 63% of the observations in the resistant plants and 68% in the susceptible plants (Table 1).

Table 1. Percentage of infection sites for each stages of fungal (*H. vastatrix*, race II) growth in resistant (R) and susceptible (S) plants of *C. arabica*, at different times post inoculation.

Fungal stages	Tupi (R)			Catuai (S)		
	24 hpi	30 hpi	36 hpi	24 hpi	30 hpi	36 hpi
pioneer hypha	36 a	9 b	0 c	34 a	9 b	0 c
pioneer haustorium	26 a	28 a	11 b	34 a	35 a	8 b
penetration hypha	23 b	33 a	8 c	22 ab	29 a	14 b
anchor and HMC	15 b	20 b	60 a	10 b	19 b	53 a
colonizer haustorium	0 c	10 b	21 a	0 c	9 b	25 a

Values for each fungal stage followed by the same letter were not significantly different (Tukey 5%). hpi: hours post inoculation.

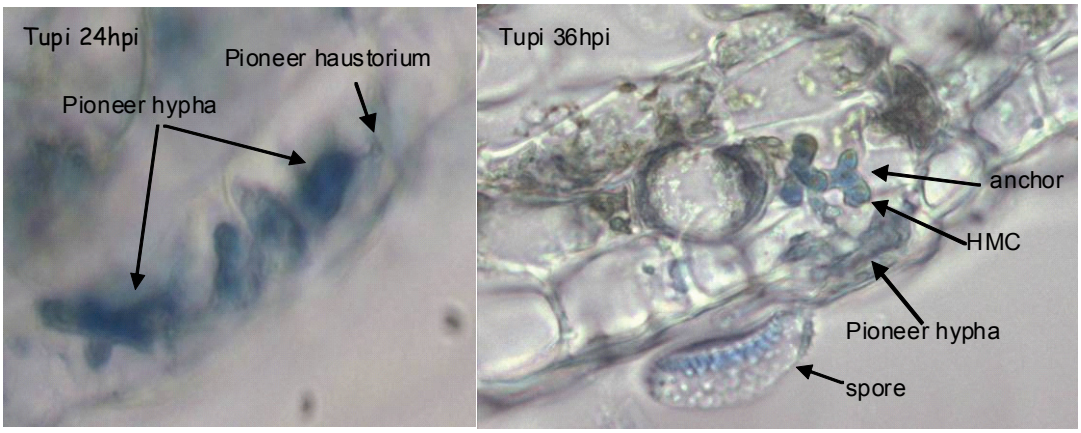


Figure 1. *H. vastatrix* race II development in Tupi leaves, 24 and 36 hpi.

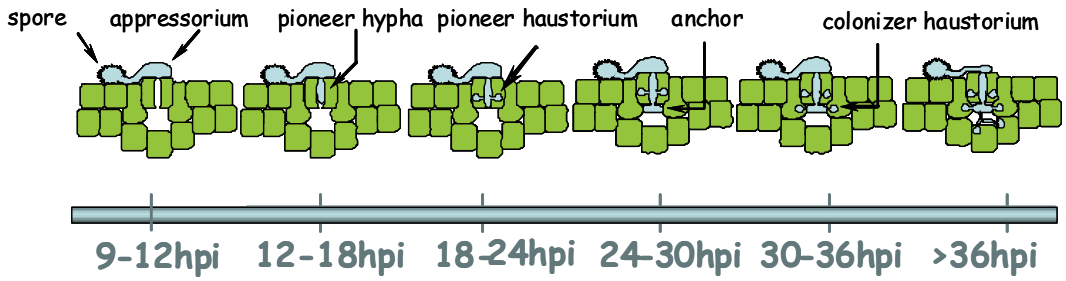


Figure 2. Schema of *H. vastatrix* race II development in coffee plants (Tupi and Catuai varieties).

Results at 30 hpi showed in 10% of infection sites that the fungus had developed an anchor with HMC and haustoria. These haustoria, named by us colonizer haustoria, were detected in adjacent and guard cells of the stomata. By 36 hpi, *H. vastatrix* had developed the first colonizer haustoria in the mesophyl cells (Table 1). Statistical evaluation of the infection process did not show any significant difference in the fungal development between leaves of resistant and susceptible cultivars until 36 hpi.

Statistical difference was evidenced between "times" (24, 30 and 36 hpi) for each fungal stage revealing the evolution of the infection process in the leaves (Table 1).

DISCUSSION

We show in this work that, unexpectedly, *H. vastatrix* appeared to produce haustoria as soon as penetrating into the stomata, at a much earlier step than previously described in the literature (Coutinho et al., 1993; Silva et al., 1999). We named the first-produced haustorium "pioneer haustorium", as opposed to the later-produced "colonizer haustorium" (Figure 3). Recent studies have shown that fungal avirulence proteins may be specifically expressed in the haustorium and recognized by disease resistance proteins within host cells (O'Connell and Panstruga, 2006; Catanzariti et al., 2007; Ridout et al., 2006). Because resistance proteins are components of the plant innate immunity system, the time-course of haustoria formation may be critical for specific host resistance responses to occur in particular plant-rust interactions. Our results provide major insights in the colonization process of the coffee orange rust fungus and suggest that the coffee molecular responses we previously registered at 24 hpi (Fernandez et al., 2004) were associated to pioneer haustorium formation. In this regard, the pioneer haustorium may play an essential role in establishing specific recognition between the coffee plant and the rust pathogen. Future analyses are directed towards defense markers genes expression to assess coffee molecular responses related to pioneer- and colonizer-haustoria production.

REFERENCES

Catanzariti A.M., Dodds P.N., Ellis J.G. 2007. Avirulence proteins from haustoria-forming pathogens. FEMS Micorbiol. Lett. 269: 181-188.

Coutinho T.A., Rijkenberg F.H.J., Van Asch M.A.J. 1993. Development of infection structures by *Hemileia vastatrix* in resistant and susceptible selections of *Coffea* and in *Phaseolus vulgaris*. Can. J. Bot. 71: 1001-1008.

Fernandez D., Santos P., Agostini C., Bon M.C., Petitot A.S., Silva M.C., Guerra-Guimarães L., Ribeiro A., Argout X. and Nicole M. 2004. Coffee (*Coffea arabica* L.) genes early expressed during infection by the rust fungus (*Hemileia vastatrix*). Mol. Plant Path., 5, 527-536.

Martins E.M.F., Moraes W.B.C. 1996. Development of *Hemileia vastatrix* in coffee plants with genetic and induced resistance. J. Phytopathol. 144: 519-526.

O'Connell R.J., Panstruga R. 2006.Tête à tête inside a plant cell: establishing compatibility between plants and biotrophic fungi and oomycetes. New Phytol., 171:699-718.

Perfect S.E., Green J.R. 2001. Infection structures of biotrophic and hemibiotrophic fungal plant pathogens. Mol. Plant Pathol. 2: 101-108.

Ridout C.J., Skamnioti P., Porritt O., Sacristan S., Jones J.D.G., Brown J.K.M. 2006. Multiple avirulence paralogues in cereal powdery mildew fungi may contribute to parasite fitness and defeat of plant resistance. *Plant Cell* 18: 2402-2414.

Rijo L., Rodrigues Jr. C.J. 1977. The infection process of *Hemileia vastatrix* in susceptible and resistant cultivars of *Coffea arabica*. In: Proceedings of the eighth International Scientific Colloquium on Coffee, 28 November-3 December, Abidjan, Ivory Coast: 509-510.

Rodrigues Jr. C.J., Bettencourt A.J., Rijo L. 1975. Races of the pathogen and resistance to coffee rust. Annu. Rev. Phytopathol. 13: 49-70.

Silva M.C., Nicole M., Rijo L., Geiger J.P., Rodrigues Jr. C.J. 1999. Cytochemical aspects of the plant-rust fungus interface during the compatible interaction *Coffea arabica* (cv. Caturra) – *Hemileia vastatrix* (race III). Int. J. Plant Sci. 160 : 79-91

Silva M.C., Nicole M., Guerra-Guimarães L., Rodrigues Jr. C.J. 2002. Hypersensitive cell death and post-haustorial defence responses arrest the orange rust (*Hemileia vastatrix*) growth in resistant coffee leaves. Physiol. Mol. Plant. Pathol. 60: 169-183.

Varzea V.M. and Marques D.V. 2005. Population variability of *Hemileia vastatrix versus* coffee durable resistance. *In* "Durable Resistance to Coffee Leaf Rust", (Zambolin L., Zambolin E.M. and Pinto Varzea V.M., Eds, Univ. Fed. Viçosa Pub., Brésil, p.285-304.

Voegele R.T., Mendgen K 2003. Rust haustoria: nutrient uptake and beyond. New Phytol. 159: 93-100.

Effectiveness of the Brocap Trap in Controlling the Coffee Berry Borer (*Hypothenemus hampei* Ferr.) in Indonesia

S. WIRYADIPUTRA[1], C. CILAS[2*], J-P. MORIN[2]**

[1]Indonesian Coffee and Cocoa Research Institute (ICCRI), 90 PB. Sudirman Street, Jember 68118, East Java, Indonesia. E-mail: soekadar@yahoo.com
[2]CIRAD-BIOS, UPR "Bioagresseurs de parennes", TA-A31/02, 34398 Montpellier Cedex 5, France. [*]E-mail: christian.cilas@cirad.fr – [**]E-mail: jpmorin45@yahoo.fr

SUMMARY

A trial on coffee berry borer (CBB, *Hypothenemus hampei*) control with the Brocap trap was conducted in a robusta coffee plantation in Lampung Province, Indonesia, from 2005 to 2007. The aim of the trial was to evaluate the effectiveness of the Brocap trap in controlling CBB in robusta coffee smallholdings. Brocap traps effectively captured CBB adults and the number of CBB caught depended on the degree of infestation in the field. In one plot, the largest number of CBB captured four months after the initial installation reached, on average, up to 1064 CBB/week/trap. After four months, the number of CBB trapped decreased drastically to fewer than 20 insects per trap per week. The effect of Brocap traps on CBB infestation was shown after 4 months, with a 22.10% to 72.62% reduction in infestation. While the Brocap traps were in place, infestation and the CBB population were significantly lower in the treatment plots compared to the control plots, even for parchment and green coffee. Green coffee production increased by 19.06% in a plot containing Brocap traps. This trial also showed that the attractant substance diffused for 137 to 156 days, hence longer than in trials conducted in El Salvador (Latin America).

INTRODUCTION

The coffee berry borer (CBB, *Hypothenemus hampei* Ferr.) is the most serious insect pest on coffee in Indonesia. It causes significant yield losses in terms of coffee production, but also reduces coffee bean quality, resulting in low productivity and the poor quality of Indonesian coffee. On average, CBB infestation on Indonesian coffee is more than 20%, and it results in yield losses of more than 10%. With an average national yield of 448.3 kg/ha/year, yield losses per hectare caused by CBB are about 50 kg/year. With a total Indonesian coffee acreage of 1.25 million hectares, the financial loss caused by CBB can reach more than 625 billion rupiahs per year (around 6.7 million USD). Globally, annual losses caused by this pest have been estimated at over $ 500 million (Anon., 2004; Vega et al., 2002).

Until now, the technology for controlling CBB in Indonesia was integrated pest management (IPM) by application of the biocontrol agent *Beauveria bassiana*, sanitation harvesting of the CBB food source (coffee berries) left on the trees and on the soil surface after harvesting, and application of insecticides if infestation is very severe. However, the efficiency of this control method is not yet entirely satisfactory. The main problem with *B. bassiana* application has been the mass production and quality control of the fungus. Since the area planted to coffee is very large, a greater quantity of *B. bassiana* is needed to achieve effective CBB control. The recommended application rate for *B. bassiana* per hectare is more than 2.5 kg of solid culture (in maize medium) per application, and it requires three applications per harvesting period. The Indonesian Coffee and Cocoa Research Institute (ICCRI) has formulated *B. bassiana* in

pure spore powder form, and the recommended rate is 100 g per ha per application. Sanitation harvesting of coffee berries after the harvest is very laborious and very difficult to carry out in some coffee areas, since harvesting occurs throughout the year. The method can only be implemented in large plantations with a good management system.

Using traps is the new CBB control method and the Brocap trap is a trap specially designed for *Hypothenemus hampei* developed by CIRAD and PROCAFE in El Salvador (Dufour, 2002; 2008). The trap is considered as a useful addition to IPM for CBB control in Indonesia. Before using the Brocap trap in Indonesia, ICCRI and CIRAD considered it important to validate the trap under local conditions. We present the results from a Brocap trapping trial for CBB control in Indonesia

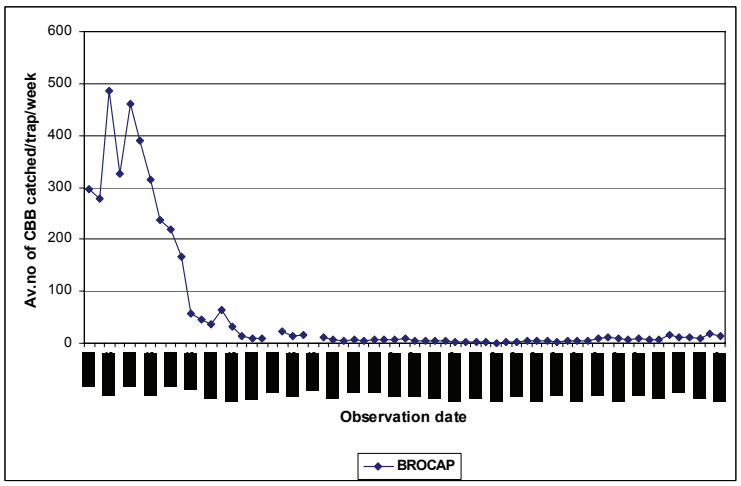
MATERIALS AND METHODS

The Brocap trap trial was conducted in Tanggamus district, in Lampung province, Indonesia. Two treatments were compared in this trial: Brocap trap versus control (without Brocap trap). Each treatment was replicated four times, so there were four locations comprising treatment and control plots. Each plot had an area of 6400 m^2 (80 m x 80 m) of robusta coffee plants and was set up with 16 Brocap traps for the treatment plots. Both treatment and control plots consisted of 16 coffee trees for observation of CBB infestation and the population. Observations focused on the CBB captured in Brocap traps, CBB infestation and the population, CBB infestation on parchment and green coffee at the final observation, and green coffee production. The CBB caught in the Brocap traps were collected and counted every week by directly counting the insects if there were fewer than 1000, and by conversion from the volume of CBB obtained. Based on previous observations, the average volume of 1000 CBB adults is 1.37 ml. So, if the volume of CBB trapped amounts to 5.7 ml, it will contain about (5.7/1.37) x 1000 = 4161 insects. The duration of attractant evaporation was also observed during the trial, to see know how long the attractant lasted under the trial area conditions.

CBB infestation was determined by counting the percentage of berries infested on four branches in sample trees or in a sample of 100 harvested berries from all trees in the plot if berries on the tree were scarce. CBB populations were observed by dissecting infested berries in the laboratory and counting all stages of CBB development. The effect of the Brocap trap treatment on green coffee production was estimated by counting 100 g of green coffee as per Delabarre (2001). The data obtained were analysed using the SAS program and were tested by the Student Newman and Keuls (SNK) test at the 5.0% level to determine the differences between treatments. CBB distribution per trap was fitted to a binomial negative law using the XLSTAT program based on the number of CBB captured on the initial observation.

RESULTS AND DISCUSSION

A large number of CBB was captured over the four months from the initial observation; in plot A, the number in one trap reached 2786 insects per week. On average, the largest number was found for the observation in July, after harvesting (Figure 1). Four months after trap installation, the number of CBB captured decreased substantially until the next harvesting season.



Av. nb. CCB caught/trap/week

Figure 1. Number of CBB captured over 14 months of Brocap trap installation in Lampung, Indonesia.

The differences between the number of CBB trapped at the outset and at the end of the trial may have been caused by the condition of the CBB population in the field. The largest population occurred when the Brocap traps were first installed, then decreased slowly, as many insects had been caught in the Brocap traps. The other reason was that the actual CBB population in the field was small due to limited food, because most of coffee berries were still at the pinhead developmental stage and were not yet suitable as food for the insects.

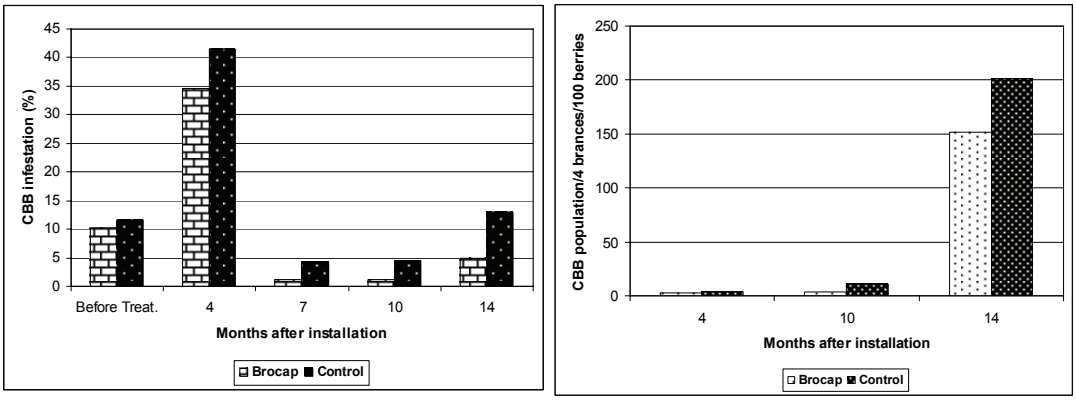
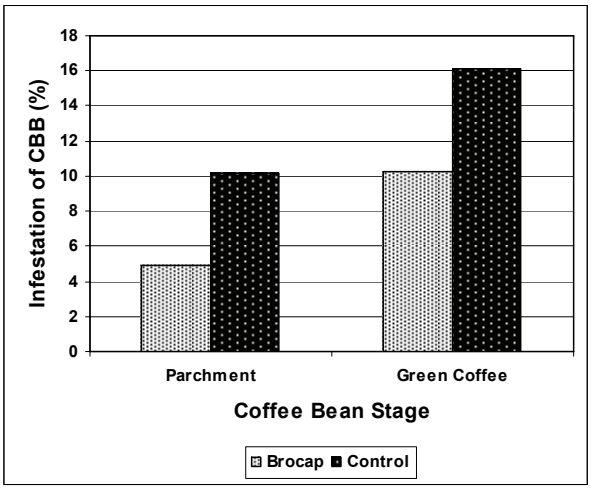


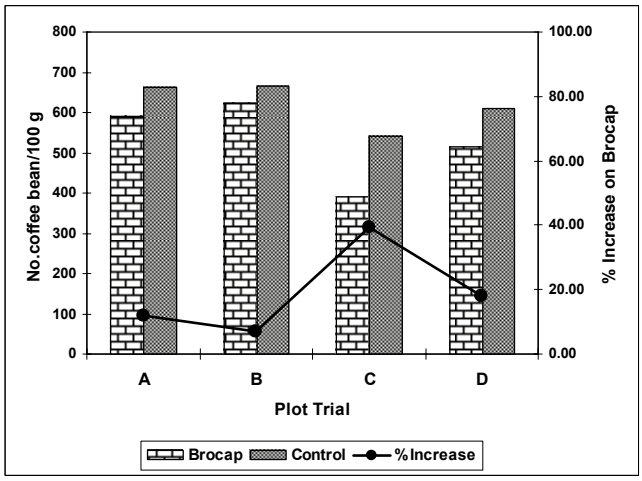
Figure 2. CBB infestation (left) and population (right) during the Brocap trap trial in Lampung, Indonesia. NS = Not Significant and (*) = Significantly different according to the SNK test at the 5% level.

The effects of Brocap traps on CBB infestation and the population are shown in Figure 2. CBB infestation before trap installation was not significantly different between the treatment and control plots. However, from four months after trap installation up to the last observation, the figures for the treatment plot were significantly lower compared to the control, both for infestation and population size.

As regards infestation on parchment and green coffee, it was found that infestation was also significantly lower in treatment plots compared to the control plots (Figure 3).



CBB infestation



Nb coffee beans/100 g
Trial plot
% increase in Brocap

Figure 3. CBB infestation on parchment and green coffee (left) and effect of Brocap trap on green coffee production (right) processed from coffee samples taken on the last observation in the Brocap trap trial in Lampung, Indonesia. Treatment and control plots were significantly different according to the SNK test at the 5.0% level.

Coffee production in the treatment plots was significantly higher than in the control plots and the production increase was, on average, 19.06%. The distribution analysis results revealed that the CBB population was fitted to a negative binomial distribution, both for the high population early in the trial and for the low population at the end of the trial. From this trial it can also be seen that the attractant diffused for 137 to 156 days with an average of 146.33 days, which was longer than in trials conducted in El Salvador (Latin America) (Dufour, 2008).

REFERENCES

Anon. (2004). Stopping the Coffee Berry Borer from Boring into Profits. http://www.ars.usda.gov/is/AR/archive/nov04/coffee1104.pdf. p.10-11.

Delabarre M. (2001). Efficacy of the BROCAP® trap under the agroclimatic conditions in El Salvador (based on the original work by Bernard Dufour). National Workshop on Hypothenemus hampei, Bangelore, India, 11-12 December 2001.12 pp.

Dufour B.P. (2008). Coffee berry borer: Triple-action integrated pest management. IICA-PROMECAFE, El Salvador. 8 pp.

Vega F.E., Frankui R.A. and Benavides P. (2002). The presence of coffee berry borer, *Hypothenemus hampei* in Puerto Rico: fact or fiction ? Journal of Insect Science, 2.13. 3 pp.

The Coffee Berry Borer (*Hypothenemus hampei*) Distribution and the Effect of Coffee Management Practices in Kenya

H.M. MUGO

Coffee Research Foundation, Entomology Section, P.O Box 4- 00232, RUIRU, Kenya.
E-mail: mugohmu@yahoo.com or crf@kenyaweb.com

SUMMARY

A survey to establish the Coffee Berry Borer (*Hypothenemus hampei*) distribution and the infestation levels was carried out in Western and Nyanza (Lake Basin region) Provinces in Kenya in January/February and June 2000. Smallholder coffee farmers growing Robusta and Arabica coffee were surveyed. Results indicated that the Coffee Berry Borer is a serious problem in the two provinces. The management levels practiced by the farmers were found to positively influence the Coffee Berry Borer infestations in the fields.

INTRODUCTION

The *H. hampei,* an indigenous coffee pest in East, Central and West Africa has spread worldwide and in many coffee producing countries it has become a major pest (Le Pelley, 1968).The first incidence of Coffee Berry Borer (CBB), *Hypothenemus hampei* (Ferri) in Kenya was reported in 1928 (Wilkinson, 1929 cited in Abasa 1975). Later on, the borer became a major pest with 80% infestation levels occurring during the peak coffee season in Western Region of Kenya (Murphy et al., 1987).

The CBB is mainly managed using insecticides. However, this practice has not been very effective because of CBB biology and feeding behaviour as well as the reported incidence of developed resistance to endosulfan (Brun *et al.*, 1989). Cultural control by stripping of all the infested berries and destroying them through deep burying or burning has been recommended (Mwangi, 1983). Biological control of CBB is another option to manage the borer when combined with proper coffee management practices. *Cephalonomia stephanoderis* and *Prorops nasuta* are reported natural enemies of CBB (Le Pelley, 1968). In Cote d'Ivore and Togo in West Africa *C. stephanoderis,* cause 50% parasitism (La Salle, 1990). In Kenya*, P. nasuta,* cause a low parasitoid level of 18% during peak infestation period (Murphy *et al.,* 1987). In view of the above fact, survey was carried out to establish the extent of the spread and infestation levels of CBB in Western and Nyanza provinces in Kenya, and the effects of coffee management practices.

MATERIALS AND METHODS

The field survey was carried out in Western and Nyanza (Lake Basin Region) provinces in Kenya in year 2000. However, at present the problem remains a major constraint to coffee production in the two provinces (Pers. Observ.). The provinces were selected based on reported heavy incidences of CBB infestation. Thirty six (36) coffee farms, four (4) per district were randomly sampled and considered as the sampling sites.

On each farm, twenty (20) coffee trees were randomly selected. One (1) bearing primary branch per tree was sampled with number of mature berries on each branch counted and their total recorded. Similarly, berries with Coffee Berry Borer attack on the same branch were counted and

recorded. A total of 100 mature coffee berries with CBB infestation per farm were picked, dissected and examined. Total number of CBB larvae, pupae and adults were counted and recorded. Number of CBB dead due to parasitoids attack or fungal infection were counted and recorded.

Based on coffee management practices (Weeding, pruning, fertilization, intercropping, pesticides use, mbuni stripping, irrigation and mulching), the farms were grouped into three categories depending on number of farm management activities practiced. Farmers carrying out at least 0-3 of the farm management practices were grouped as low managed farms while medium and high managed farms were grouped as 4-5 and 6-7, respectively.

RESULTS

The Surveyed Coffee Districts

A total of nine (9) coffee growing districts located between altitudes of 1161 to 1967 metres above sea levels (a.s.l.) were surveyed (Table 1). Three types of coffees mainly traditional varieties /Arabica (*Coffeae arabica*) (SL34, SL28 & K7), Robusta (*Coffeae canephora*) and an Arabica hybrid (Ruiru 11) were found to be grown (Tables 1).

Table 1. Surveyed Coffee growing Districts and types of Coffee grown.

Province	District	Coffee type
Western	Kakamega	SL
	Vihiga	SL, R11
	Bungoma	Ro, SL, R11
	Mt. Elgon	SL
	Busia	Ro
Nyanza	Siaya	Ro, SL
	Kisumu	SL, R11, Ro
	Kisii Central	SL, R11
	Migori	Ro, SL

Key: SL = traditional variety, R11 = Ruiru 11, Ro = Robusta.

Among the farms sampled, the percentage distribution of the coffee types varied. The traditional varieties with 47% had the highest distribution followed by Robusta and Ruiru 11 with 28 and 25.0%, respectively (Figure 1). The farming and management practices exercised by the coffee farmers varied widely. A total of 27.8, 66.7 and 5.6% of the farmers contacted practiced low, medium and high coffee management practices, respectively (Figure 2). Approximately 95% of the farmers practiced low to medium management levels.

Extent of CBB infestation and mortality levels

All the districts surveyed indicated the presence of CBB infestations. The levels of attack varied from one district to the other (Figure 3). The lowest infestation of 0.6% was recorded in Mt. Elgon District while Migori had the highest with 25.0%. Other districts; Kakamega, Vihiga, Bungoma, Busia, Siaya, Kisumu and Kisii Central had infestations of 13.9, 5.2, 3.7, 20.9, 18.1, 9.9 and 10.0 %, respectively. Except Kakamega and Migori districts, all the others districts had higher CBB mortality as compared to the infestation (Figure 3).

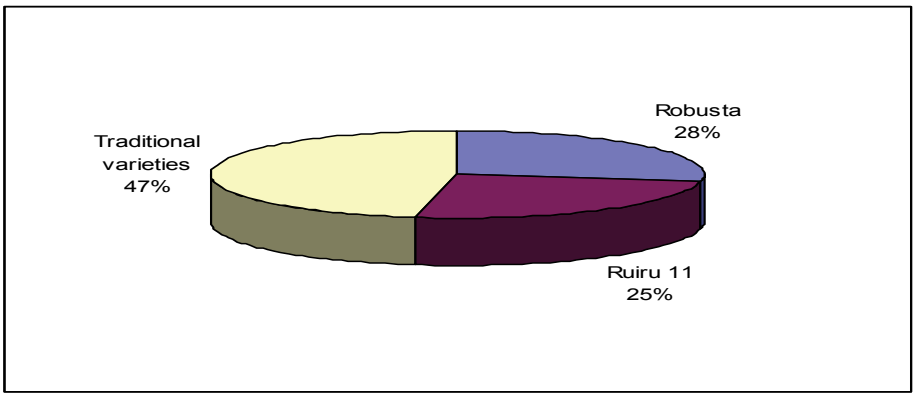


Figure 1. Distribution of coffee types in Nyanza and Western Provinces in Kenya.

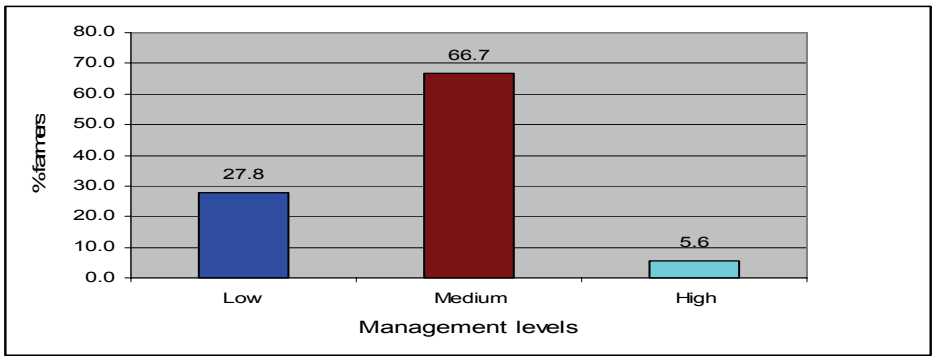


Figure 2. Percentage of coffee farmers practising various management levels in Western and Nyanza Provinces in Kenya.

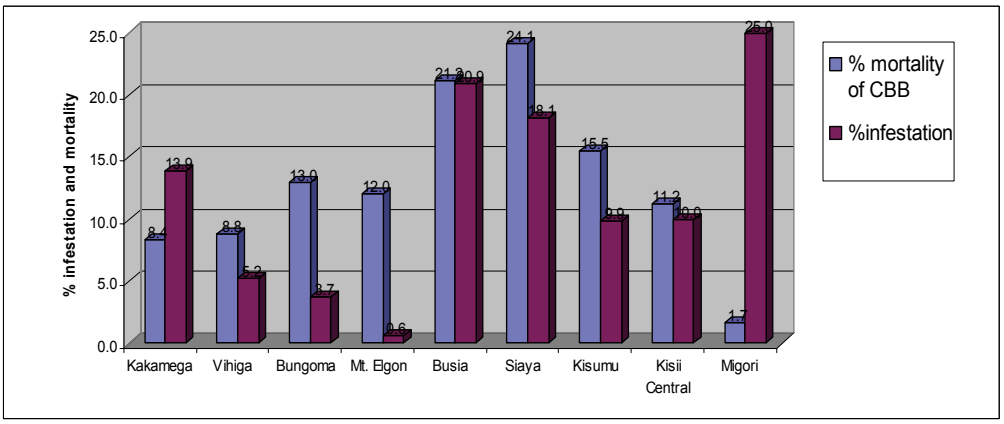


Figure 3. Mean % mortality (all stages of CBB development) and infestation of coffee berries by CBB in Western Kenya.

The management practices indicated positive effects on CBB infestation levels. The percentage CBB infestation decreased with increase in management levels while the % CBB mortality increased with increase in coffee management levels. Low managed farms recorded the highest CBB infestation of 16.7%. Highly managed farms recorded the lowest mean infestation of 1.3%. Low CBB mortality of 6.4% was recorded on low managed farms with highest mortality of 17.2% occurring on highly managed coffee farms (Figure 4).

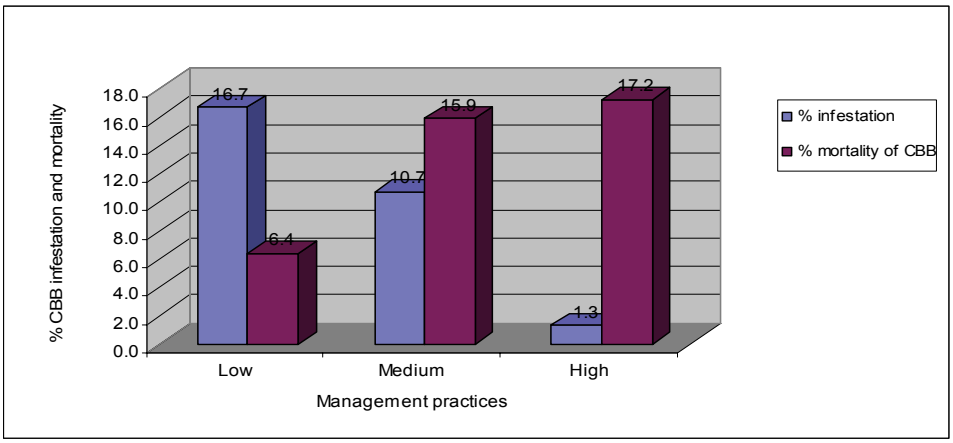


Figure 4. Effect of various coffee management practices on level of CBB parasitism (Mean % CBB mortality) in western Kenya.

DISCUSSION

The survey was carried out to establish the extent of spread and infestation levels of Coffee Berry Borer in Western and Nyanza Provinces in Kenya. During the survey it was evident that CBB is well established and a serious problem in the two Provinces. Earlier work by Rangi et al (1989) indicated the wide spread of CBB in Nyanza and Western Provinces. However, they found the problem to be more common on Robusta Coffee around Lake Victoria and on Arabica coffee in the surrounding highlands up to an altitude of approximately 1600m above sea level (M.a.s.l.). This was confirmed during this survey with CBB found to occur in all the Districts surveyed. Hargreaves (1935) reported that the CBB is common at altitudes of up to 1250m in Uganda, which confined it mainly to Robusta coffee grown at low altitudes. Rangi et al. (1989) reported that CBB occur at altitudes of up to 1880m on Arabica coffee with 1650M a.s.l. as more conducive for the establishment of this pest in Kenya. However, during this survey, CBB was found to occur at an altitude of 1967M a.s.l. on Arabica coffee, higher than previously recorded.

Rangi et al. (1989) reported that in Kenya the borer infestation could reach 80% during peak season with corresponding parasitism level of 18%. This was attributed to heavy use of pesticides, a practice with detrimental effect on the CBB natural enemies. The present study established a low mean infestation level of 11.9%. Highest mean infestation of 25.1% occurred at Migori District in Nyanza with the lowest infestation of 0.6% recorded at Mt. Elgon District in Western Province. Farms with high level of management indicated very low to no infestations while neglected farms had high CBB attack. Despite this, the Western Province generally showed that the borer infestation was rather low as compared to Nyanza. This could have been likely due to differences in mortality levels caused by the natural enemies existing in Nyanza than in Western Province.

ACKNOWLEDGMENTS

I greatly thank the United States Department of Agriculture for financial support. My thanks also goes to Messrs M Njuguna and R Ndungu from CRF Entomology Section for assisting in the field work. The paper is published with the permission of the Director of Research, Coffee Research Foundation, Ruiru, Kenya.

REFERENCES

Abasa R.O. (1975). A review of biological control of coffee insect pests in Kenya. East African Journal of Forestry and Agriculture 40(3): 292-299

Mwangi C.N. (1983). Coffee Growers Handbook. Coffee Research Foundation. 130pp. 3. Brun, L. O, Marcillaud, C; Gaudichon, V. and Suckling D M (1989): Endosulfan resistance in Coffee Berry Borer, *Hypothenemus hampei* (Coleoptera:Scolytidae) in New Caledonia, J. Econ. Entomol. 87(5) 1311-1316.

Fonseca J. P. da (1939). A broca eo Sombreamento ddos Cafezais. O. Biologicao Research, 16, 347-354.

Hargreaves H. (1935). *Stephanoderis hampei* Ferr. Coffee Berry Borer in Uganda. East African Agricultural Journal. 1: 218-224.

La Salle J. (1990). A new genus and species of Tetrastichinae (Hymenoptera: Eulophidae) parasitic on the coffe berry borer H*ypothenemus hampei* (Ferri) (Coleoptera: Scolytidae): Bulletin of Entomological Research 80 (1): p 7

Le Pelley R. H. (1968). Pests of Coffee. Longmans London, 590pp.

Rangi D.K, Murphy S.T., Nang'ayo F.L. and Moore D. (1989). Fourth and Final Progress Report on the Coffee Berry Borer Project. IDRC Centre File 3-P-84-0113 CIBC (Unpublished Report).

Murphy S.T., Donnel D.J., Mang'ayo F.L., Cross A. and Evans H.G. (1987). Second Report of the Coffee Berry Borer Project. Unpublished Report CABI Institute of Biological control. 23pp

Resistance to Leaf Rust on Coffee Cultivars Developed at Instituto Agronômico do Paraná

G.H. SERA[1], T. SERA[1], D.S. ITO[1], I.C. DE B. FONSECA[2], F.S. KANAYAMA[1], L. DEL GROSSI[1], F.L. MARANA[1], L.H. SHIGUEOKA[1]

[1]Instituto Agronômico do Paraná, PR, Brazil
[2]Universidade Estadual de Londrina, PR, Brazil

SUMMARY

The objective of this research was to evaluate the complete and incomplete resistance to rust (*Hemileia vastatrix*) in coffee cultivars developed by IAPAR. The resistance evaluation was performed for the local leaf rust population at IAPAR with high incidence in the field in August 2007. Twelve coffee cultivars from IPR 97 to IPR 108 were evaluated. 'IAPAR 59' and 'Catuaí Vermelho IAC 81' were used as resistant and susceptible standards, respectively. A complete randomized block design was used with nine replications and seven plants per plots. For the resistance evaluation a score scale varying from 1 to 5 was used, based on rust severity. The yield evaluation was based on the harvest carried out in June 2007. The cultivars IAPAR 59, IPR 97, IPR 98, IPR 104 and IPR 105 presented almost 100 % complete resistance to rust and 'IPR 99', 'IPR 101', 'IPR 102' and 'IPR 107' presented around 75% plants with complete resistance and the segregation probably occurred for one gene. Partial resistance in cultivars IPR 103 and IPR 108 was observed. 'IPR 100' and 'IPR 106' presented similar susceptibility when compared with 'Catuaí Vermelho IAC 81'.

INTRODUCTION

Coffee leaf rust, caused by the *Hemileia vastatrix* Berk. et Br. fungus, is one of the main problems for coffee cultivation because it causes high losses in yield and quality.

The most economic and ecologically correct way of controlling rust is by using resistant cultivars. However, resistance has been defeated by new races in cultivars previously considered resistant. The known resistance genes to rust are S_H1 to S_H9, that contrast with the corresponding virulence genes v1 to v9 of *H. vastatrix* (Rodrigues-Junior et al., 1975; Bettencourt, 1981; Bettencourt and Rodrigues-Junior, 1988). The existence of other genes has been confirmed in interspecific hybrids such as the Hibrido de Timor. The S_H factors promote complete resistance when they are in homozygosis and are specific for races, but when some S_H are defeated, coffee tree incomplete or partial resistance can occur (Eskes, 1989). In addition to the S_H, incomplete resistance has also been observed due to the presence of minor genes in "Icatu" and "Hibrido de Timor" plants (Eskes et al., 1990).

The Coffee Genetic Breeding Program at the Paraná Agronomic Institute (IAPAR) has developed coffee cultivars adapted to the environmental conditions of the state of Paraná since 1972. Thirteen cultivars have been obtained during this period (IAPAR 59, IPR 97, IPR 98, IPR 99, IPR 100, IPR 101, IPR 102, IPR 103, IPR 104, IPR 105, IPR 106, IPR 107 and IPR 108), registered in the Ministry of Agriculture, and four (IAPAR 59, IPR 98, IPR 99 and IPR 103) have been released and made available to coffee farmers. These coffee trees were derived from different resistance sources such as "Icatu", "Hibrido de Timor CIFC 832/2" and "Catuaí S_H2, S_H3".

The objective of this study was to assess the complete and incomplete resistance to rust in 12 coffee cultivars developed by the Paraná Agronomic Institute.

MATERIAL AND METHODS

The experiment was set up in the field at the experimental Station of the Paraná Agronomic Institute (23º 22' S, 51º 10' W) in Londrina, Paraná, Brazil. In this experiment, chemical control for rust was not carried out in the year 2007.

The resistance assessment was for the local population of rust races present at IAPAR under the high infestation conditions in the field and was carried out in August 2007 (54 months after planting).

The cultivars assessed were IPR 97 to IPR 108. The IAPAR 59 and Catuaí Vermelho IAC 81 cultivars were assessed as resistance and susceptibility standards, respectively. A complete randomized block experimental design was used with nine replications and seven plants per plot.

A scale of scores was used to assess the rust severity ranging from 1 to 5, where: 1= plants without chlorosis lesions on the leaves; 5 = more than 20 lesions with spores on the leaf and more than 35% leaves with sporulation. Plants with scores 1 and 2 were considered to have complete resistance. Plants with rust sporulation were attributed scores 3, 4 and 5. Cultivars with average rust severity score statistically equal to the cultivar IAPAR 59 and with plant frequency scores 1 or 2 among 92 to 100% of the plants were considered to have complete resistance. Cultivars with incomplete resistance were those with score 3 plant frequency (few lesions with rust sporulation) greater than 50%. Susceptible cultivars were those with average rust severity score statistically equal to the Catuaí Vermelho IAC-81 and with plant frequency with scores 4 or 5 greater 50%. The yield assessment was based on the hand harvest carried out in June 2007.

RESULTS AND DISCUSSION

The IPR 100 cultivar was statistically equal to the susceptible standard 'Catuaí Vermelho IAC 81' (Table 1). The IPR 106, IPR 103 and IPR 108 cultivars presented medium rust severity (RS) statistically less than the susceptible standard (Table 1). Most of the plants of the 'IPR 103' (69.85 %) and 'IPR 108' (67.74 %) cultivars scored 3 (few lesions with sporulation), indicating that there was incomplete resistance in these two cultivars. The resistance found in 'IPR 103' ("Catuaí" x "Icatu") probably comes from "Icatu", because different levels of partial resistance have been frequently detected in plants from "Icatu". In 'IPR 108' ('IAPAR 59' x "Catucaí") there may have been residual resistance from the genes from 'IAPAR 59' or from "Hibrido de Timor CIFC 832-2" broken by the local population of races or also due to "Icatu" genes. It is probable that the partial resistance observed in the IPR 103 and IPR 108 cultivars is durable, depending on the races present in the location. In IAPAR (Londrina, Paraná), 'IPR 103' has presented the same level of partial resistance for more than 10 years and 'IPR 108' for approximately 6 years (Sera, 2008 personal communication). In years with low yield, *H. vastatrix* severity decreases in coffee trees with incomplete resistance (Eskes, 1983; Costa et al., 2007). Therefore, 'IPR 103' and 'IPR 108' presented a good level of this resistance, because even with high yield, statistically superior to that of the resistant IAPAR 59 standard, they presented a statistically lower RS than the susceptible standard. 'IPR 106' ("Icatu") may not have partial resistance because most of the plants were score 4 (55.55%), similar to that observed for the cultivars Catuaí Vermelho IAC 81 (79.66 %) and IPR 100 (62.90 %).

Table 1. Mean rust severity scores and yield (60 kg bags/hectare) in coffee cultivars evaluated under field conditions.

Cultivar (Description) [(1)]	Rust	Yield
Catuaí Vermelho IAC 81 ('Caturra' x 'Mundo Novo')	4.233 a	39.36 a
IPR 100 ("Catuaí S_H2, S_H3")	3.677 a	37.62 a
IPR 106 ("Icatu")	3.413 b	20.09 b
IPR 103 ('Catuaí' x "Icatu")	3.096 b	40.66 a
IPR 108 ('IAPAR 59' x "Catucaí")	3.037 b	33.80 a
IPR 102 ('Catuaí' x "Icatu")	2.032 c	45.58 a
IPR 107 ('IAPAR 59' x 'Mundo Novo IAC 376-4')	1.810 c	34.55 a
IPR 99 (V. Sarchi x H. Timor)	1.778 c	27.40 b
IPR 101 ("Catuaí S_H2, S_H3")	1.540 c	27.45 b
IPR 97 (V. Sarchi x H. Timor)	1.318 d	21.81 b
IPR 104 (V. Sarchi x H. Timor)	1.079 d	26.33 b
IPR 98 (V. Sarchi x H. Timor)	1.037 d	21.15 b
IPR 105 ("Catuaí S_H2, S_H3")	1.016 d	27.78 b
IAPAR 59 (V. Sarchi x H. Timor)	1.016 d	30.35 b

[(1)] V. Sarchi = 'Villa Sarchi CIFC 971/10'; H. Timor = "Hibrido de Timor CIFC 832/2".

The IPR 99, IPR 101, IPR 102 and IPR 107 cultivars presented RS statistically lower than the IPR 106, IPR 103 and IPR 108 cultivars (Table 1). In these cultivars about 75% plants were observed with complete resistance (scores 1 and 2), therefore it is likely that they are segregating for one major gene in heterozygous condition. 'IPR 99' (Sarchimor) and 'IPR 107' (Sarchimor x Mundo Novo) probably presented fewer S_H genes than the Hibrido de Timor CIFC 832/2 (S_H5, S_H6, S_H7, S_H8, S_H9, $S_H?$) or only one of these genes segregated. Another hypothesis is that there was a new race with many virulence factors. The resistant plant frequency in the 'IPR 102' cultivar of close to 75% can be explained by the existence of some major gene in heterozygotic condition (Bettencourt and Carvalho, 1968) or associated minor genes (Eskes et al., 1990) from "Icatu" that caused complete resistance. S_H3 should be the gene that segregates in 'IPR 101' ("Catuaí S_H2, S_H3"), because coffee trees with S_H2 were susceptible at IAPAR (Sera et al., 2007). If selection were made on plants with the resistance allele(s) in homozygosis in IPR 99, IPR 101, IPR 102 and IPR 107, these would have about 100% resistant coffee trees and therefore the degree of resistance would be similar to that of the resistant standard.

Considering only plants with rust sporulation (scores 3, 4 and 5) of the IPR 99, IPR 102 and IPR 107 cultivars, respectively, 72.22%, 57.14 % and 83.33 % of these plants scored 3, indicating that there was also incomplete resistance in the plants with defeated resistance. The probable explanations for this resistance in the IPR 99, IPR 102 and IPR 107 cultivars were discussed previously for the IPR 103 and IPR 108 cultivars. The same discussions for 'IPR 108' could be applied to 'IPR 99' and 'IPR 107', because they were derived from "Sarchimor". The same hypotheses would apply to 'IPR 102' ("Catucaí") as for 'IPR 103' ("Catucaí"). Thus if the complete resistance of these cultivars was defeated there would probably be partial resistance.

The IPR 97, IPR 98, IPR 104, IPR 105 and IAPAR 59 cultivars were the most resistant (Table 1) and no susceptible plant was found in IPR 98, IPR 105 and IAPAR 59. These cultivars, with the exception of 'IPR 105' probably have the same S_H genes as the Hibrido de Timor CIFC 832/2. The complete resistance of 'IPR 105' ("Catuaí S_H2, S_H3"), was probably due to S_H3, because at IAPAR, coffee trees with S_H2 were susceptible (Sera et al., 2007).

The cultivars assessed in this study will be sent to the Centro de Investigação das Ferrugens do Cafeeiro (CIFC) in Portugal, to be inoculated with the rust race carriers of the virulence genes v1 to v9 and thus determine precisely which S_H genes these cultivars have.

REFERENCES

Bettencourt A.J. (1981) Melhoramento genético do cafeeiro: transferência de factores de resistência à *Hemileia vastatrix* Berk. & Br. para as principais cultivares de *Coffea arabica* L. Lisboa: ULTRAMAR/CIFC. 93 p.

Bettencourt A.J., Carvalho A. (1968) Melhoramento visando a resistência do cafeeiro à ferrugem. Bragantia, 27, 35-68.

Bettencourt A.J., Rodrigues-Junior C.J. (1988) Principles and practice of coffee breeding for resistance to rust and other diseases. In: Clarke, R. J.; Macrae, R. (Eds.). Coffee. London: Elsevier Applied Science, v.4. p.199-234.

Costa M.J.N., Zambolim L., Caixeta E.T., Pereira A.A. (2007) Resistência de progênies de café Catimor à ferrugem. Fitopatologia Brasileira, 32: 2, 121-130.

Eskes A.B. (1983) Incomplete resistance to coffee leaf rust (*Hemileia vastatrix*). 140 f. Doctoral thesis - Agricultural University of Wageningen, The Netherlands.

Eskes A.B. (1989) Resistance. In: Kushalappa, A.C., Eskes A.B. (Eds.). Coffee rust: epidemiology, resistance and management. Boca Raton: CRC Press. p. 171-292.

Eskes A.B., Hoogstraten J.G.J., Toma-Braghini M., Carvalho A. (1990) Race-specificity and inheritance of incomplete resistance coffee leaf rust in some Icatu coffee offspring and derivatives of Híbrido de Timor. Euphytica, 47, 11-19.

Monaco L.C. Carvalho A. (1975) Resistência a *Hemileia vastatrix* no melhoramento do cafeeiro. Ciência e Cultura, 27: 10, 1070-1081.

Rodrigues-Junior C.J., Bettencourt A.J., Rijo L. (1975) Races of the pathogen and resistance to coffee rust. Annual Review of Phytopathology, 13, 49-70.

Sera G.H., Sera T., Ito D.S., Azevedo J.A. de, Mata J.S. da, Doi D.S., Ribeiro-Filho C. (2007) Resistance to leaf rust in coffee carrying S_H3 gene and others S_H genes. Brazilian Archives of Biology and Technology, 50: 5, 753-757.

Heterosis and Simultaneous Resistance to Rust and Leaf Miner in Hybrids Carrying *Coffea racemosa* Genes

D.S. ITO[1], T. SERA[1], G.H. SERA[1], D.C. SANTIAGO[2], L. DEL GROSSI[1], F.L. MARANA[1], L.H. SHIGUEOKA[1], F.S. KANAYAMA[1]

[1]Instituto Agronômico do Paraná, Brazil
[2]Universidade Estadual de Londrina, Brazil

SUMMARY

The aim of this research was to identify coffee hybrids with heterosis and simultaneous resistance to rust and leaf miner. The field assay was established in March 2003 at IAPAR (Londrina, Brazil). Eleven F_1 hybrids with *Coffea racemosa* genes were evaluated. The yield and vegetative vigor were evaluated in March 2006, February 2007 and March 2008. Rust incidence was evaluated in July 2007 using scores from 1 to 5. The leaf miner incidence was evaluated in August 2005, January 2006 and November 2006 using scores from 1 to 5. To estimate the hybrid heterosis the formula $H = [(F_1 / C). 100] -100$ was used, where: H = heterosis; F_1 = mean F_1 hybrid yield; C = mean Catuaí yield. Almost all hybrids presented more yield and vigor than lineage cultivars. The hybrid heterosis varied from 25.00 to 59.00%. Higher yields were observed for hybrids crossed with 'IPR 98', "F_3 of IAPAR 59 x Mundo Novo", 'Tupi IAC 1669-33', 'Tupi IAC 1669-33' selection IAPAR 88480-8 and 'IAPAR 59', respectively, with 59.00 %, 52.46 %, 47.39%, 47.34% and 44.86% heterosis. Ten selected plants have potential to become hybrid cultivars, because they presented heterosis, high vegetative vigor and simultaneous resistance to rust and leaf miner.

INTRODUCTION

Currently, arabica coffee farmers use lineage cultivars propagated by seeds. F_1 hybrids present some advantages in comparison with lineages such as: more yield than lineages, the period to obtain hybrid cultivars is shorter and multiple resistance to pests and diseases is easier to obtain. The hybrids must present at least 20% heterosis and multiple resistance to reduce the high cost of cloned plants.

The hybrid heterosis can be exploited for *C. arabica* (Srinivasan and Vishveshwara, 1978; Walyaro, 1983; Ameha and Belachew, 1985; Fontes et al., 2000). Rust (*Hemileia vastatrix* Berk. et Br) is the main disease and leaf miner (*Leucoptera coffeella*) is the main pest in the hot regions of Brazilian coffees (Matiello and Almeida, 2006).

The break in resistance by new rust races has obliged breeders to pyramid many genes for durable resistance. However, this pyramiding is difficult to obtain for breeding line-type cultivars. Obtaining simultaneous resistance to leaf miner and rust is much more difficult than resistance to rust alone. Obtaining simultaneous resistance is facilitated for breeders with the use of F_1 hybrids.

Hybrid cultivar exploitation assumes mastery of a large-scale clonal multiplication methodology that can be made commercially feasible. Recently, the multiplication of *C. arabica* hybrids has become possible with the development of clonal processes via somatic embryo genesis (Sondahl et al., 1999; Etienne and Bertrand, 2001; Etienne et al., 2002).

Several new research projects in Brazil have been started to obtain coffee hybrids to make use of heterosis and incorporate resistance genes to several pests and diseases.

The objective of this study was to identify hybrids with heterosis and simultaneous resistance to rust and the leaf miner.

MATERIAL AND METHODS

The field experiment was set up on March 12, 2003 at the experimental Station at the Paraná Agronomic Institution (IAPAR) in Londrina, in 2.5 x 0.5m spacing.

The F_1 hybrid 'Tupi IAC 1669-33' x [("*C arabica* x *C. racemosa*") x 'Tupi IAC 1669-33'] or Tp x AramosaTp was used as pollinator, that is resistant to the leaf miner. The coffee trees used as mother plants were: 'IAPAR 59' (I59), 'Tupi IAC 1669-33' (Tupi), 'Icatu Precoce IAC-3282' (Icatu 3282), 'Catuaí Vermelho IAC-81' (Catuaí), 'Acaiá IAC 474-7' (Acaiá), 'IPR 98', "F_3 of IAPAR 59 x Mundo Novo" (F_3 I59 x MN), "F_3 of IAPAR 59 x Catucaí" (F_3 I59 x Ctcaí), "*C. arabica* from Ethiopia carrier of the gene S_H1 x Catuaí" (EtSh1 x Cí), 'Tupi IAC 1669-33' selection IAPAR 88480-8 (Tupi-2) and 'IPR 104'. The cultivars of the breeding line type 'IAPAR 59', 'Catuaí Vermelho IAC-81', 'Icatu Precoce IAC-3282' and 'Bourbon Vermelho' (Bourbon) were used as a standards for yield and susceptibility to the leaf miner. The latter three were susceptibility standards to rust and 'IAPAR 59' was the resistance standard.

Production and vegetative vigor were assessed in March 2006, February 2007 and March 2008. Yield was assessed visually in liters of fruit per plant. The vegetative vigor assessment was based on leaf coloring, plant size, branching and leaf size and thickness and scores from 1 to 10 were attributed where: score 1 = plants with low vegetative vigor; score 10 = plants with high vegetative vigor.

Rust incidence and severity were assessed in July 2007. Scores from 1 to 5 were used, score 1 for plants without chlorosis lesions on the leaves and score 5 for plants with more than 20 lesions with spores per leaf and more than 35% of the leaves with sporulation. Coffee trees were considered resistant to rust that presented scores 1 or 2 and susceptible for scores 3, 4 and 5. Chemical control for rust was only applied in the 2006/2007 growing season.

Leaf miner incidence was assessed in August 2005, January 2006 and November 2006. A scale of scores from 1 to 5 was used, where: 1 = plants without lesions on the leaves or less than 1% leaves with small lesions (between 0.3 and 0.6 cm); score 5 = many small, medium and large sized lesions (36 to 100% leaves). Coffee trees were only considered resistant when they had scores 1, 2 or 3 in the three assessments. Plants with scores higher than 3 in at least one of the assessments were considered susceptible. Chemical control was not carried out for the leaf miner.

The formula used to estimate heterosis was $H = [(F_1/ C). 100]-100$, where: H = heterosis; F_1 = mean of the F_1 hybrid production; C = mean of the Catuaí Vermelho IAC-81 cultivar.

The selection of individual plants from the hybrids was based on heterosis, vegetative vigor and simultaneous resistance to rust and the leaf miner. Therefore, coffee trees were only selected with average production of at least three liters of fruits, average vegetative vigor of at least score 8 and with scores 1 or 2 for rust and leaf miner severity and incidence.

RESULTS AND DISCUSSION

The standards Catuaí, Icatu 3282 and Bourbon Vermelho presented 100% plants with susceptibility to rust and leaf miner. 'IAPAR 59' was susceptible to leaf miner, but 100% of the plants were resistant to rust (Table 1).

Table 1. Heterosis and resistance to leaf rust and leaf miner of F_1 hybrids evaluated at IAPAR (Londrina, PR, Brazil).

Hybrids	Vig	Yield	LR	%RLR	Lm	%RLm	H %	n
I 59 (Lineage cultivar)	6.49	1.84	1.00	100.00	5.00	0.00		15
Catuaí (Lineage cultivar)	7.49	1.97	4.93	0.00	4.33	0.00		15
Icatu 3282 (Lineage cultivar)	7.07	1.49	5.00	0.00	4.40	0.00		10
Bourbon (Lineage cultivar)	7.30	1.40	5.00	0.00	5.00	0.00		10
IPR 98 x (Tp x AramosaTp)	7.88	3.14	1.60	85.00	2.93	55.00	59.00	20
(F_3 I59 x MN) x (Tp x AramosaTp)	7.70	3.01	1.75	70.00	3.23	40.00	52.46	20
Tupi x (Tp x AramosaTp)	7.62	2.91	1.45	85.00	3.45	35.00	47.39	20
Tupi-2 x (Tp x AramosaTp)	7.98	2.91	1.39	83.33	3.15	50.00	47.34	18
I59 x (Tp x AramosaTp)	7.90	2.86	1.25	90.00	2.95	40.00	44.86	20
Acaiá x (Tp x AramosaTp)	7.75	2.79	3.53	10.53	2.91	42.11	41.41	19
IPR 104 x (Tp x AramosaTp)	7.73	2.75	1.50	80.56	3.05	41.67	39.64	36
Icatu 3282 x (Tp x AramosaTp)	7.33	2.69	4.05	5.00	3.73	20.00	36.44	20
(F_3 I59 x Ctcaí) x (Tp x AramosaTp)	7.59	2.63	2.95	65.00	3.21	35.00	33.15	40
Catuaí x (Tp x AramosaTp)	7.79	2.57	3.80	2.50	2.82	55.00	30.51	40
(EtSh1 x Cí) x (Tp x AramosaTp)	7.52	2.47	2.90	30.00	3.31	32.50	25.04	40

Vig = vegetative vigor mean; Yield = mean yield; LR = mean leaf rust incidence; % RLR = percentage of plants resistant to leaf rust; Lm = mean of leaf miner incidence; %RLm = percentage of plants resistant to leaf miner; H % = heterosis estimated based on Catuaí yield; n = number of evaluated plants.

All hybrids presented higher yield than lineage cultivars. Only the hybrid 'Icatu 3282' x (Tp x AramosaTp) presented less vigor than lineage cultivar Catuaí. The hybrid heterosis varied from 25.04 to 59.00%. Higher yields for hybrids crossed with IPR 98, F_3 I59 x MN, Tupi, Tupi-2 and I59 were observed, respectively, with 59.00%, 52.46%, 47.39%, 47.34% and 44.86% heterosis. Other authors found heterosis varying from 6.90 to 119.64 % (Walyaro, 1983), 18.00 to 60.00% (Ameha and Belachew, 1985) and 58.00 to 330.00% (Fontes et al., 2000).

The frequencies of plants with complete resistance to rust were higher for the hybrids crossed with I59 (90.00%), Tupi (85.00%), IPR 98 (85.00%), Tupi-2 (83.33%) and 'IPR 104' (80.56%).

The frequencies of plants with resistance to leaf miner varied from 20 to 55 %, indicating that the resistance genes *Lm1* and *Lm2* of Tp x AramosaTp is in heterozygous condition.

'IAPAR 59' is resistant to *Meloidogyne exigua* (Salgado et al., 2005). Therefore, the hybrids carrying the 'IAPAR 59' genes presented resistance to rust and leaf miner and have resistance to this nematode. These hybrids resistant to leaf miner can have partial resistance to drought, such as other coffees derived from *C. racemosa* (Medina-Filho et al., 1977). The hybrid

(EtSh1 x Cí) x (Tp x AramosaTp), carrying the gene S_H1, is resistant to bacteriosis caused by *Pseudomonas syringae* pv. *garcae* and presented heterosis.

Plants of the hybrids 'I59' x (Tp x AramosaTp), 'Tupi' x (Tp x AramosaTp), 'IPR 98' x (Tp x AramosaTp), ("F_3 I59 x MN") x (Tp x AramosaTp), (F_3 I59 x Catucaí) x (Tp x AramosaTp), 'Tupi-2' x (Tp x AramosaTp) and 'IPR 104' x (Tp x AramosaTp) were selected, because they presented heterosis, high vegetative vigor and simultaneous resistance to rust and leaf miner. Ten selected plants have potential to become hybrid cultivars, because they presented economic advantages for cloning. The hybrids carry more than 90% *C. arabica* genes, but tests must be conducted to verify the cup quality.

REFERENCES

Ameha M., Belachew B. (1985) Heterosis for yield in crosses of indigenous coffee selected for yield and resistance to coffee berry disease II - at first three years. Acta Horticulturae (ISHS), v. 158, p.347-352.

Etienne H., Anthony F., Dussert S., Fernandez D., Lashermes P., Bertrand B. (2002) Biotechnological application for the improvement of coffee (*Coffea arabica* L.). In Vitro Cell Dev. Biol. Plant, v. 38. p. 129-138.

Etienne H., Bertrand B. (2001) Trueness to type and agronomic characteristics of *Coffea arabica* Trees micropropagated by the embryogenic cell suspension technique. Tree Physology, v. 21. p. 1031-1038.

Fontes J.R.M., Cardoso A.A., Cruz C.D., Pereira A.A., Zambolim L., Sakiyama N.S. (2000) Study of combining ability and heterosis in coffee. In: Sera T, Soccol CR, Pandey A, Roussos S (Eds.). Coffee Biotechnology and Quality. Dordrecht: Kluwer Academic Publishers. p. 113-121.

Matiello J.B., Almeida S.R. (2006) A ferrugem do cafeeiro no Brasil e seu controle. 98 p.

Medina-Filho H.P., Carvalho A, Medina D.M. (1977) Germoplasma de *Coffea racemosa* e seu potencial no melhoramento do cafeeiro. Bragantia, 36(1): 43-46.

Salgado S.M.L., Resende M.L.V., Campos VP (2005) Reprodução de Meloidogyne exigua em cultivares de cafeeiros resistentes e suscetíveis. Fitopatologia Brasileira, 30(4): 413-415.

Sondahl M.R., Sondahl C.N., Gonçalves W. (1999) Custo comparativo de diferentes técnicas de clonagem. In: Proceedings of 3rd International seminar on biotechnology in the coffee agroindustry, 1999, Londrina, Brazil. Proceedings ... Londrina: IAPAR/IRD. p. 59-65.

Srinivasan C.S., Vishveshwara S. (1978) Heterosis and stability for yield in arabica coffee. Indian Journal of Genetics & Plant Breeding, 38(3): 416-420.

Walyaro D.J. (1983) Considerations in breeding for improved yield and quality in arabica coffee (*Coffea arabica*), PhD Thesis, Agricultural University, Wageningen.

Coffee Disease Risk Analysis: How Epidemiology Knowledge Could Help in Assessing and Preventing Disease Invasion

D. BIEYSSE[1*], C. CILAS[2], J. MOUEN[3], P. MUSOLI[4], J. AVELINO[2]

[1]CIRAD, UMR BGPI, TA A-54/K, 34398 Montpellier Cedex 5 France.
[*]E-mail: daniel.bieysse@cirad.fr
[2]CIRAD, UPR Bioagresseurs de pérennes, TA A-31/02, 34398 Montpellier Cedex 5, France
[3]IRAD, Station Polyvalente de Foumbot, BP 665 Bafoussam, Cameroon
[4]NARO-COREC, PO BOX 185, Mukono, Uganda

SUMMARY

Plant diseases can be classified into two types: (i) the co-evolved diseases which have evolved with the host in its centre of origin and (ii) the new encounter diseases which have resulted from the adaptation of a native pathogen to an introduced crop. Coffee is under the threat of both kinds of diseases mainly due to increasing South-South trade. This situation could lead to the introduction to new territories of co-evolved diseases like Coffee Wilt Disease (CWD caused by *Fusarium xylarioides*) confined so far to Africa, or of encounter diseases like Coffee Berry Disease (CBD caused by *Colletotrihum kahawae*) only present in Africa and the American Leaf Spot Disease (ALSD caused by *Mycena citricolor*) located in America only. Moreover, the recent occurrence of *Xylella fastidiosa* on coffee in Brasil in 1995, causing the Coffee Leaf Scorch, points out the possibility of the emergence of new encounter diseases on the crop. Through the analysis of the historical expansion pathways of the main coffee diseases, their current distribution and the epidemiological knowledge on the pathosystems, we propose different strategies in order to prevent the introduction of diseases to new territories and to decrease the risk of wide and severe expansions in case of an introduction. A special emphasis will be given to CBD, CWD and ALSD.

INTRODUCTION

Coffee is under threat of two kinds of diseases: co-evolved diseases, which have evolved with the host in its centre of origin, such as Coffee Wilt Disease, and new-encounter diseases, resulting from the adaptation of a native pathogen to an introduced crop, such as Coffee Berry Disease (only in Africa), and American Leaf Spot Disease (only in America).

DISEASES ORIGIN AND CROP LOSSES IMPORTANCE

Given the damage they cause (harvest losses, tree death, disrupted vegetative system), these 3 diseases hold back production and the replanting or extension of plantations.

COFFEE WILT DISEASE

Coffee Wilt Disease (CWD caused by *Fusarium xylarioides,* teleomorph *Gibberella xylarioides*) is a vascular disease inducing tree death in 2 to 24 months. Runoff water or upkeep operations are conducive to small-scale disease dispersal inside plots. CWD was first reported on *Coffea excelsa* in the Central African Republic in 1927 and subsequently developed on *C. canephora* in the Democratic Republic of Congo (DRC, ex-Zaïre) and Ivory Coast, where losses of more than 50% were reported. After a programme of replanting with

resistant *C. canephora*, the disease became less serious, until its re-emergence in DRC in the 70s. Contemporary lines of expansion over large distances have been mainly due to road transportation of infected material to Uganda (1992), then Tanzania (1997).

COFFEE BERRY DISEASE

Coffee Berry Disease (CBD due to *Colletotrichum kahawae*) is a disease specific to C. arabica berries which induces 20 to 50% of crop losses. *C. kahawae* is an aerial pathogen transmitted by free water and splashing over short distances. Lines of expansion over longer distances follow roads or are due to accidental transportation of infected planting material over long distances; CBD is considered to be a new-encounter disease as it appeared for the first time in Kenya in 1922, outside the centre of origin of *C. arabica,* which is located in Ethiopia.

AMERICAN LEAF SPOT DISEASE

Mycena citricolor is a gemmiferous fungus with a broad spectrum of hosts, which probably existed on the American continent even before coffee was introduced. On coffee, American Leaf Spot Disease (ALSD) occurs on branches, leaves, and fruits. It causes heavy losses in Central America, especially in Costa Rica. Short horizontal distances for gemmae dissemination by splashing (< 60 cm) have been reported, suggesting that disease dispersal has occurred through human activities and by the introduction of infected plants in disease-free areas.

DISCUSSION

An analysis of the lines of expansion of these 3 diseases reveals a modest effect of natural factors on pathogen dispersal. The dispersal is mainly due to human activities. Especially, transportation of infected planting material over large distances and its accidental introduction have a major effect on disease emergence. So far, these 3 diseases have been confined to a single continent.

The risk of introducing one of these pathogens on a free disease continent is a serious threat to coffee culture, both economically and in terms of environmental protection. Consequently, increased surveillance needs to be developed for all producing countries, along with a quarantine service to control planting material movements, particularly in a context of intercontinental planting material exchanges.

REFERENCES

Avelino J., Cabut S., Barboza B., Barquero M., Alfaro R., Esquivel C.,Durand J.-F. and Cilas C., 2007. Topography and crop management are key factors for the development of American leaf spot epidemics on coffee in Costa Rica. Phytopathology 97: 1532-1542.

Mouen Bedimo J.A., Bieysse D., Cilas C., Notteghem J.L., 2007. Spatio-temporal dynamics of arabica coffee berry disease caused by *Colletotrichum kahawae* on a plot scale. *Plant Disease* 91 (10): 1229-1236.

Musoli C.P., Pinard F., Charrier A., Kangire A., G. M. ten Hoopen, Kabole C., Ogwang J., Bieysse D., Cilas C., 2008. Spatial and temporal analysis of coffee wilt disease caused by *Fusarium xylarioides* in *Coffea canephora.* European Joural of Plant Pathology [On line] DOI: 10.1007/s10658-008-9310-5.

The Use of Sarchimor Derivatives in Coffee Breeding Resistance to Leaf Rust

V.M.P. VÁRZEA, V.D. MARQUES, A.P. PEREIRA, M.C. SILVA

Centro de Investigação das Ferrugens do Cafeeiro − Instituto de Investigação Científica Tropical − Quinta do Marquês, 2784 − 505 Oeiras, Portugal

SUMMARY

Coffee Leaf Rust (CLR) has a worldwide distribution. Research work to develop coffee varieties with resistance to this disease has been a priority of Coffee Rusts Research Center (CIFC) at Oeiras, Portugal since the early fifties. Many hybrids were synthesized at CIFC aiming to transfer resistance genes to *Hemileia vastatrix* in coffee plants of Hibrido de Timor (HDT) to commercial short size varieties like Caturra and Villa Sarchi. One of the hybrids, CIFC H361 = Villa Sarchi CIFC 971/10 x HDT CIFC 832/2 was supplied by CIFC, around 1970, to different coffee research Institutes in coffee growing countries. In F2 and further generations these populations were named by UFV as "Sarchimor". Several cultivars were recently released from these populations: Tupi, Obatã (IAC, Brazil); IAPAR 59, IPR 97, IPR 98, IPR 104 and IPR 107 (IAPAR, Brazil); IHCAFE-2004 (IHCAFE, Honduras); Chandragiri (CCRI, India); different lines of "Sarchimor" T5296 (Central America); Limaní (EEA, Puerto Rico). Studies carried out by CIFC, IRD and CIRAD in France, CCRI in India, and also by different coffee research institutions in Latin America showed variability in derivatives of CIFC H361 populations. Different levels of introgression to *Coffea arabica* as well as resistance to *H. vastatrix*, *Colletotrichum kahawae* and *Meloidogyne* spp., can be found in these populations. The coffee plants CIFC HDT 832/2 and H361 showed, at CIFC, resistance to all known rust races (physiologic group A). To avoid the decreasing of resistance to coffee leaf rust during the selection process as well as to find coffee genotypes with more resistance genes in "Sarchimor" test crosses are being used at IAPAR. Some aspects to be considered in the selection process of new coffee varieties: **a)** "Sarchimor" is a given name to released coffee cultivars, selected lines as well as to other coffee genotypes from CIFC H361 derivatives; **b)** One coffee plant, a population or a cultivar with resistance to coffee leaf rust in one particular region give no indication about its resistance genetic background and its behaviour in other regions; **c)** Screening to rust carried out at CIFC in some advanced materials from CIFC H361 showed different levels of resistance. It is possible to find coffee lines with resistance to all the rust isolates of CIFC's collection including coffee lines with very low level of resistance. Some "Sarchimor" resistant lines/cultivars to rust from South America showed no resistance when confronted, at CIFC, with some rust races from other continents; **d)** *H. vastatrix* showed a very high ability to increase its virulence in presence of resistant cultivars; **e)** The populations of "Sarchimor" must be manipulated carefully by breeders to avoid the release of coffee cultivars with very low spectra of resistance to coffee leaf rust; **f)** The production of hybrids between Sarchimor and other coffee genotypes with the objective to increase the number of resistance genes without screening tests to rust is not recommended because it can cause the reverse effect.

INTRODUCTION

The coffee leaf rust, if not handled properly may threaten the coffee production in a given country or region. The severe damages caused by the disease, aggravated by inexistent or

deficient agronomic practices, will have a negative impact on the social, economical stability of the areas where coffee growing is a main occupation and an important source of revenues. The topographical and economic impossibilities of using chemical control as well as the disadvantages of its application which are manifold, led the Centro de Investigação das Ferrugens do Cafeeiro (CIFC), also known as Coffee Rusts Research Centre, Oeiras, Portugal, to establish its research lines with the main purpose of securing varieties of *Coffea arabica* L. with satisfactory degree of resistance to *Hemileia vastatrix* Berk et Br.

Several thousands of accessions of coffee plants from different species and origins were characterized in the CIFC according to their spectra of resistance to the 45 physiologic rust races of the pathogen and grouped in 41 physiologic groups (Bettencourt and Rodrigues, 1988; Várzea and Duarte, 2005). The physiologic groups include plants which are totally resistant to the known physiologic races (group A), those which are entirely susceptible (group F), and those which are intermediate (C, D, E, G, H, 2, 3, etc).

A decisive step on the research program of the CIFC was the discovery of Hibrido de Timor (HDT), a population where most of the plants belong to group A, i. e., resistant to all the known rust races (Bettencourt and Rodrigues, 1988). This population resulted of a natural cross between *C. arabica* and *Coffea canephora* Pierre ex Froehner can be easily bred with *C. arabica* because it possesses the same number of chromosomes of this species (2n = 4x = 44).

ORIGIN OF SARCHIMOR

The first seed of HDT from Timor was received at CIFC in 1957 and only 2 plants could be obtained from this batch of several thousands of seeds (entry code CIFC HDT832/1 and CIFC HDT 832/2. These plants showed resistance to all the known rust races and many hybrids were done at CIFC to introduce rust resistance from these two HDT coffee plants to traditional susceptible coffee cultivars. From the hybrid H361 (Villa Sarchi CIFC 971/10 x CIFC HDT 832/2) five plants were selected: H 361/1,2,3,4 and 5 (group A). The selected material of this generation was the starting point for further improvement and selection. Seed from these plants was distributed to many countries in seventies. In Brazil, the material received from Oeiras through the Universidade Federal de Viçosa (UFV) and Instituto Agronómico de Campinas (IAC) has been object of intensive studies not only in these institutions but in other Brazilian Research Institutes like Instituto Agronómico do Paraná (IAPAR), MAPA/PROCAFÉ (ex-IBC) as well as in other coffee growing countries in Latin America and India. In Brazil, the general designation of Sarchimor was suggested to the germplasm from the hybrid CIFC H361 (Pereira et al., 2005). In Brazil the F2 introductions UFV 349, IAC 1668 from H361/1 and UFV 350, IAC 1669 from H361/4 are well known. The Cachimor designation resulted from the backcrossing between the hybrid CIFC H529 (yellow Caturra CIFC 1637/56 x CIFC H361/3). This material was introduced in the UFV as UFV 351. The selected plants UFV 351-30 and UFV 351-33 originated, in generation F2, the progenies UFV 1001 and UFV 1002, respectively. The subsequent generations have been intensively studied in the State of Minas Gerais, and it is very likely that new cultivars will be achieved (Pereira et al., 2005).

CULTIVARS RELEASED FROM SARCHIMOR

The cultivar Obatã IAC 1669-20, officially released by IAC in 2000 (Fazuoli et al., 2006), was originated by a natural cross (hybridisation) from coffee plant (F2) of H361/4 with a coffee plant of the variety Catuaí Vermelho. The cultivar Obatã Amarelo IAC4739 was a natural cross of Obatã IAC 1699-20 and Catuaí Amarelo. The cultivar Tupi IAC 1669-33 was

also released officially by IAC in 2000 after pedigree selections were carried out up to F6, like in Obatã (Fazuoli et al., 2006).

In 1975, the introduction by IAPAR of F3 generation of LC 1669 from IAC originates the progenie 75163-22. From this progenie was released the cultivar "IAPAR-59". Some years ago some more cultivars were released by IAPAR like "IPR 97", "IPR98", "IPR104", "IPR107", "IPR 108", "IPR99" (Sera et al., 2002; sera et al., 2005). The Brazilian cultivars "Acauã", "Katipó"," Siriema" released by MAPA/PROCAFÉ (ex-IBC) were also "Sarchimor" derivatives (Matiello and Carvalho, 2005).

The LC 1669 (IAC) was introduced in Central America in F3 generation and selected the T5296-170 and T5296-184 with the common designation of "Sarchimor T5296" (Bertrand et al., 1999).

The variety "Chandragiri" was released in 2007 in India from CIFC H361 (F2) introduced in the gene bank of Central Coffee Research Institute (CCRI) during the year 1975.

The varieties "Limani" (Puerto Rico), "IHCAFE" 2004 (Honduras) are also originated from "Sarchimor" population.

In some regions in Brazil as well as in many other coffee growing countries the Sarchimor population is cultivated under the ordinary designation of "Sarchimor".

VARIABILITY ON SARCHIMOR POPULATION

In Sarchimor population there is a lot of variability to agronomic characteristics (Bertrand et al., 1999; 1997; Sera et al., 2003; Silveira et al., 2003), resistance to *Hemileia vastatrix* (Várzea et al., 2002; 2005; Silva et al., 2006) *Colletotrichum* spp. (Silva et al., 2006; Várzea et al., 2002; Sera et al., 2007), *Pseudomanas syringae pv. Garcae* (Petek et al., 2006; Ito et al., 2008), and *Meloidogyne* spp. (Bertrand et al., 1999; Itoet al., 2008; Bertrand et al., 2001; Noir et al., 2003; Anthony et al., 2003; 2005; Fazuoli et al., 2002; Muniz, 2007).

Despite best efforts, coffee leaf rust problems continue. The rust screening carried out at CIFC on Sarchimor population, using a collection of rust races from different coffee growing countries, showed progenies with resistance to all known rust races (group A), as well as progenies with very low spectra of resistance and some of them with very high level of segregation to susceptibility (group E) like Caturra, Catuaí and Villa Sarchi. On the other hand all the original coffee plants H361 from the CIFC's collection exhibit resistance to all the known races.

The work carried out by Sera and collaborators (Sera et al., 2007) entitled "*Selection for durable resistance to leaf rust using test-crosses on IAPAR-59 and Tupi IAC 1669-33 cultivars of Coffea arabica*" gives a good contribution to the evidence of variability of Sarchimor to rust resistance. The main conclusions of these authors are:

"*Many plants of the 'IAPAR-59' and 'Tupi IAC 1669-33' presented more defeated resistance genes by the rust races present at IAPAR than others of these cultivars or the genes were in heterozygous that ought to be avoided to use in future crossings.*

In coffee plants considered with resistance to rust, test-crosses must be made to verify which plants presented less defeated resistance genes and/or more genes in homozygous before crossing aiming durable resistance".

About the Indian variety Chandragiri, Santa Ram (2004-2005) mentioned: "a) *The original Sarchimor plants are still standing resistant to rust disease; b) An important caution to the growers is that Sarchimor is a hybrid genotype of Arabica and tends to segregate into different types of plants with reference to the plant type as well as with reference to its manifested resistance to different biotic adversaries like rust pathogen".*

Prakash and collaborators (Prakash et al., 2005) also mentioned that semi-dwarf ideoptypes like Catimor, Catimor x Catuaí, Catimor x Sarchimor (generated in India) tended to show susceptibility to *H. vastatrix* after few production cycles because of the plant debilitation and dilution of resistance that was also seen on generation advancement.

The derivatives of the Catimor genotypes (CIFC 19/1 Caturra x CIFC HDT 832/1) have been under commercial cultivation in India since late 1980s with the popular name "Cauvery". In the initial years, these genotypes manifested a high degree of resistance to leaf rust pathogen. Surprisingly, there was a breakdown of resistance in this variety from 1991 (Prakash et al., 2005). This phenomenon was noticed in several coffee growing countries. Resistance breakdown is caused by high disease pressure and changes in the rust population, not by any change in the genetic resistance.

DURABLE RESISTANCE

Durable resistance, as defined by Johnson is one that has remained effective in a cultivar, during its widespread cultivation for a long sequence of generations or period of time in an environment favorable to a disease or pest. In India, China, Brazil and in other coffee growing countries it is possible to see that the derivatives of Catimor (CIFC 19/1 Caturra x CIFC HDT 832/1) lost the resistance to rust. However, in other countries the rust was not able to breakdown the resistance of the same Catimor. How long this resistance will be effective? Nobody knows, depends of the ability of rust to develop new rust races with the necessary virulent genes to infect those plants.

What will happen when "Sarchimor" will be cultivated in a large scale in many regions?

Probably it will be noticed the same phenomena that occurred before in India, Brazil and China after the introduction of Catimor! Effective durability requires a sound knowledge of the race structure of the pathogen population and an understanding of the genetic bases for resistance. The breeders involved with selection to rust resistance from "Sarchimor population" must be clear about the: **a)** number of rust races and their agresssiveness vary from region to region; **b)** coffee differentials were not yet characterized to some virulent rust races in Sarchimor; **c)** ability of rust to overcome the resistance on HDT derivatives is very high; **d)** genetic background for rust resistance of Sarchimor is not yet well known.

REFERENCES

Anthony F., Topart P., Anzueto F., Astorga C., Bertrand B. La resistencia genética de *Coffea* spp. a *Meloidogyne* spp. : identificación y utilización para la cafeicultura latinoamericana. Manejo Integrado de Plagas y Agroecología, 2003, 67, 4-11.

Anthony F., Topart P., Martinez A., Silva M., Nicole M. Hypersensitive-like reaction conferred by the *Mex-1* resistance gene against *Meloidogyne exigua* in coffee. Plant Pathology 2005**,** 54, 476-482.

Bertrand B., Aguilar G., Bompard E., Rafinon A., Anthony F**.** Comportement agronomique et résistance aux principaux déprédateurs des lignées de Sarchimor et Catimor au Costa

Rica. Agronomic perfomance and resistance to the main pests of Sarchimor and Catimor lines in Costa Rica. Plantations, Recherche, Développement, 1997, 4, 312-321.

Bertrand B., Aguilar-Vega G.J., Bompard Santacreo R., Anzueto F. El mejoramento genético en America Central. In Desafios de la Cafeicultura en Centroamerica; Bertrand B., Rapidel B., Eds.; San José, C.R.: IICA. PROMECAFÉ: CIRAD:IRD:CCCR. França; 1999, 407-456.

Bertrand B., Anthony F., Lashermes P. Breeding for resistance to *Meloidogyne exigua* in *Coffea arabica* by introgression of resistance genes of *Coffea canephora.* Plant Pathology, 2001, 50, 637-643.

Bettencourt A.J., Rodrigues Jr. C.J. Principles and practice of coffee breeding for resistance to rust and other diseases. In Coffee Agronomy; Clarke R.J, Macrae R., Eds.; Vol. 4; Elsevier Applied Science Publishers LTD, London, 1988, 199-234.

Fazuoli L.C., Medina Filho H.P., Gonçalves W., Guerreiro Filho O., Silvarola M.B. Melhoramento do cafeeiro: variedades tipo Arábica obtidas no Instituto Agronómico de Campinas. In O Estado da Arte de Tecnologias na Produção de Café; Zambolim L. Ed., Universidade Federal de Viçosa, Viçosa, Brasil; 2002, 163-215.

Fazuoli L.C., Gonçalves W., Toma-Braghini M., Silvarolla M.B. TUPI RN IAC 1669-13: a coffee cultivar resistant to *Hemileia vastatrix* and *Meloidogyne exigua* nematode. International Conference on Coffee Science, Montpellier, France, 11-15 September, 2006, 143-51.

Ito D.S., Sera T., Sera G.H., Grossi L.D., Kanayama F.S. Resistance to bacterial blight in arabica coffee cultivars. Crop Breeding and Applied Biotechnology 2008, 8, 99-103.

Johnson R. Practical breeding for durable resistance to rust diseases in self pollinating cereals. Euphytica 1978, 27, 529-540.

Matiello J.B., Carvalho C.H.S. Resistant cultivars to coffee leaf rust in durable resistance to coffee leaf rust. In Durable Resistance to Coffee Leaf Rust; Zambolim L., Zambolim E., Várzea V.M.P., Eds. Universidade Federal de Viçosa: Viçosa, Brasil; 2005, 443-450.

Muniz M.F.S. Variabilidade genética e biológica de *Meloidogyne exigua* e patogenicidade de *Meloidogyne* spp. em genótipos de cafeeiro. Tese de Doutoramento. Universidade Federal de Lavras. 2007.

Noir S., Anthony F., Bertrand B., Combes M.C., Lashermes P. Identification of a major gene (Mex-1) from *Coffea canephora* conferring resistance to *Meloidogyne exigua* in coffee (*Coffea arabica*). Plant Pathology, 2003, 52, 97-103.

Pereira A.A., Sakiyama N.S., Zambolim L., Moura W.M., Zambolim E., Caixeta E.T. 2005. Identification and use of sources of durable resistance to coffee leaf rust in the UFV/EPAMIG breeding program. In Durable Resistance to Coffee Leaf Rust; Zambolim, L., Zambolim, E., Várzea, V.M.P., Eds. Universidade Federal de Viçosa: Viçosa, Brasil; 2005, 215-232.

Petek M.R., Sera T., Sera G.H., Fonseca I.C.B., Ito D.S. Seleção de progênies de *Coffea arabica* com resistência simultânea à mancha aureolada e à ferrugem alaranjada. Bragantia, 2006, 65, 65-73.

Prakash N.S., Ganesh D., Bhat S.S. Population dynamics of coffee leaf rust (*Hemileia vastatrix* Berk. et Br.) and recent advances in rust research in India. In Durable Resistance to Coffee Leaf Rust; Zambolim L., Zambolim E., Várzea V.M.P., Eds. Universidade Federal de Viçosa, Viçosa, Brasil. 2005, 411-442.

Santa Ram. Sarchimor: a promising new coffee variety. Magazine Published by Coffee Board. Dec 2004 - Jan 2005. http://www.indiacoffee.org/newsletter/2005/dec-jan/cover_ story. html (accessed 01.08.2008)

Sera T., Alteia M.Z., Petek M.R. Melhoramento do cafeeiro: Variedades melhoradas no Instituto Agronómico do Paraná. In O Estado da arte de tecnologias na produção de café, Zambolim, L., Ed.; Universidade Federal de Viçosa: Viçosa, Brasil, 2002, 217-251.

Sera T., Sera G.H.S., Ito D.S., Doi D.S. Coffee Breeding for durable resistance to leaf rust at Instituto Agronômico do Paraná. In Durable Resistance to Coffee Leaf Rust; Zambolim, L., Zambolim, E., Várzea, V.M.P., Eds. Universidade Federal de Viçosa: Viçosa, Brasil; 2005, 187-214.

Sera T., Ruas P.M., Ruas C.F., Diniz L.E.C., Rampim L., Ruas E.A., Silveira S.R. Genetic polymorphism among 14 elite Coffea arabica L. cultivars using RAPD markers associated with restriction digestion. *Genetics and Molecular Biology*, **2003**, 26, 59-64.

Sera G.H., Sera T., Ito D.S., Azevedo J.A., Mata J.S., Doi D.S., Ribeiro Filho C. Selection for Durable Resistance to Leaf Rust using Test-Crosses on IAPAR-59 and Tupi IAC 1669-33 cultivars of *Coffea arabica.* Brazilian Archives of Biology and Technology 2007, 50, 565-570.

Silva M.C., Várzea V. Guerra-Guimarães L., Azinheira H.G., Fernandez D. Petitot A-S, Bertrand B., Lashermes P., Nicole M. Coffee resistance to the main diseases: leaf rust and coffee berry disease. Brazilian Journal of Plant Physiology, 2006**,** 18, 119-147.

Silveira R.S., Ruas P.M., Ruas C.F., Sera T., Carvalho V.P., Coelho A.S.G. Assessment of genetic variability within and among coffee progenies and cultivars using RAPD markers. Genetics and Molecular Biology. 2003, 26, 329-336.

Várzea V.M.P., Rodrigues Jr. C.J., Silva M.C.M.L., Gouveia M., Marques D.V., Guerra-Guimarães L., Ribeiro A. Resistência do cafeeiro a *Hemileia vastatrix*. In O Estado da arte de tecnologias na produção de café. Zambolim, L. Ed. Universidade Federal de Viçosa: 2002, 297 - 320.

Várzea V.M.P, Silva M.C., Rodrigues Jr. C.J. Resistência à antracnose dos frutos verdes do cafeeiro. In O Estado da Arte de Tecnologias na Produção de Café*,* Zambolim, L. Ed. Universidade Federal de Viçosa, Viçosa, Brasil. 2002, 321-366.

Várzea V.M.P., Duarte D.V. Population variability of *Hemileia vastatrix* vs. coffee durable resistance. In Durable Resistance to Coffee Leaf Rust; Zambolim L., Zambolim E., Várzea V.M.P., Eds. Universidade Federal de Viçosa: Viçosa, Brasil, 2005, 53-74.

Pathogenicity of *Colletotrichum kahawae* Strains and their Effect on Resistant Arabica Coffee Varieties in Tanzania

D.L. KILAMBO[1], L. GUERRA-GUIMARÃES[2], V. VARZEA[2], A. LOUREIRO[2], M. SILVA[2], R.B. MABAGALA[3], D.J. MTENGA[1], L. MASUMBUKO[1], J.M. TERI[1]

[1]Tanzania Coffee Research Institute P.O.Box 3004 Moshi, Tanzania
[2]Centro de Investigação das Ferrugens do Cafeeiro (CIFC)/Instituto de Investigação Científica Tropical, Quinta do Marquês 2784-505 Oeiras, Portugal
[3]Sokoine University of Agriculture P.O.Box 3004 Morogoro, Tanzania

SUMMARY

Pathogenicity of 15 *Colletotrichum kahawae* strains collected from different ecosystems in Tanzania was studied under laboratory conditions. Growth rates of the strains studied at 15 °C, 20 °C, 25 °C and 30 °C indicated interaction of the strains and temperature regime. The isoenzymatic characterization of Tanzanian *C. kahawae* strains based on the activity of α-esterase, acid and alkaline phosphatases were done using the technique of isoelectric focusing electrophoresis (IEF) on polyacrylamide gels. Results revealed polymorphism among Tanzanian *C. kahawae* strains, and that of Kenya, Cameroon and Zimbabwe. Variability to aggressiveness was observed on detached green berries and hypocotyls inoculated with different strains. There was no evidence of physiological races among tested isolates of *C. kahawae* strains and the coffee genotypes used. Post-penetration growth of *C. kahawae* in hypocotyls measured at 24, 48 and 72 hrs was higher in susceptible genotypes than in resistant ones. The inhibition of fungal growth in the resistant genotypes was associated with autofluorescence of the host cell walls and deposition of callose around intracellular hyphae. Resistance of Lyamungu coffee hybrids showed reaction variation to *C. kahawae* strains from different geographic origins.

INTRODUCTION

Coffee berry disease (CBD) caused by *Colletotrichum kahawae* Waller and Bridge sp. nov. (Waller et al., 1993) causes 30 to 90% yield loss of *Coffea arabica* commercial cultivars in Tanzania (Ngulu et al., 1998).

Studies to detect variability of *C. kahawae* were conducted in different countries. In Kenya coffee genotypes were tested using *C. kahawae* strains and pathotypes (physiological races) was not observed (van der Vossen et al., 1976). Moreover, differential interactions between host and *C. kahawae* populations were seldom found in Ethiopia (Eshetu and Waller, 2003). Várzea et al. (2002b) reported variability in the aggressiveness of *C. kahawae* populations attributed to geographic origins. Rodrigues et al. (1992) and Várzea et al. (1993) reported variation in both aggressiveness and virulence, rate of sporulation and growth of *C. kahawae.* It remains the fact that information on the pathogenic variability of *C. kahawae* has important implications for the development of resistant coffee cultivars.

Previous histological studies showed that resistance of Hibrido de Timor derivatives were characterized by restricted fungal growth associated with hypersensitive-like host cell death, thickness and autofluorescence of host cell walls and early accumulation of phenolic

compounds (Silva et al., 2006). The coffee Improvement Programme in Tanzania puts strong emphasis in developing coffee varieties resistant to CBD.

This study reports on variation of pathogenicity in *C. kahawae* in Tanzania using growth rate analysis at different temperature regimes, reaction on green coffee detached berries, isoenymes analysis, fungal post-penetration on hypocotyls tissues of *Coffea arabica* and evaluation for resistant coffee genotypes when subjected to different strains of *C. kahawae*.

MATERIALS AND METHODS

Growth rate and pathogenicity determination of *Colletotrichum kahawae* strains

Strains of *C. kahawae* were grown on MEA in 85 mm diameter glass dishes, replicated six times and incubated in the darkness at 15 °C, 20 °C, 25 °C and 30 °C. Mean rate of colony growth in mm/24 hr were determined. Pathogenicity of the *C. kahawae* isolates were determined by inoculating 20 expanded green coffee berries of a susceptible variety Cattura according to the method developed by van der Vossen et al. (1976), and incubated at 15 °C, 20 °C and 25 °C in the incubator 100% R.H. Data recorded included percentage of infected coffee berries.

Isoenzymatic characterization

Protein extracts were obtained from fresh mycelium of *C. kahawae* strains listed in Figures 4a to 4c, according to Loureiro et al. (2006). The protein content of each sample was determined according to the Bio-Rad protein assay Kit. Proteins were analysed by isoelectric focusing electrophoresis (IEF) on a 5% (w/v) polyacrylamide gel with 2% ampholytes (Robertson et al., 1987). After running the gels were incubated in different substrate solutions according to the enzyme under study; esterases, acid phosphatases and alkaline phosphatases (Vallejos, 1983).

Histological studies

Hypocotyls of coffee genotypes 20498, 20509 and 20511 were inoculated with a conidia suspension (2 x 10^6/ml) of *C. kahawae* strains Que 2, following the technique described by van der Vossen et al. (1976), with slight modifications. Conidia germination and appressoria formation were visualized on hypocotyls surface, 24 h after the inoculation, as previously described (Silva et al., 2002). For time course studies of fungal growth, cross section of infected hypocotyls were stained and mounted with blue lactophenol and estimation of fungal hyphal length per infection site was made using a micrometric eyepiece. To detect callose deposition and autofluorescence of host cells the aniline blue and the epifluorescence tests were used, respectively (Silva et al., 2002).

Evaluation of 16 coffee genotypes for their resistance to *Colletotrichum kahawae* strains

Resistance of 16 coffee genotypes using hypocotyls was conducted under controlled conditions. A CRD arranged in Split-plot design was used in the study. Four *C. kahawae* strains represented main treatments and 16 genotypes were arranged as sub-plots with three replications. Forty hypocotyls (5-6 weeks old) from 16 coffee genotypes were spray inoculated twice with *C. kahawae* suspension; allow infection (22-24 °C) and thereafter incubation following procedure described by van der Vossen et al. (1976). Percent survivals per genotype were attributed to assessment procedures done at CIFC (Varzea et al., 2002).

Categories of reaction Disease Index Reaction (DIR) of the genotypes was determined using the following formula (Roma, 1997):

$$DIR = 25 \Sigma \frac{i\ ni}{i\quad n}$$

where: *i = numerical value of disease description*
ni = number of hypocotyls in disease description
n = number of hypocotyls in all description

Resistance categories were classified as; resistant (DIR 0 – 25), moderately Resistant (DIR 26 – 50), moderately susceptible (DIR 51 – 75), and susceptible (DIR 76 – 100).

RESULTS AND DISCUSSION

Growth rate of *Colletotrichum kahawae* strains

Results obtained showed variation in the colony growth (mm/24 hr) between strains (Figure, 1). The mean growth rate of *C. kahawae* strains was higher at 25 °C than other temperature regimes (Figure 1). Earlier findings show that optimum temperature for mycelial growth range between 21-23 °C (Nutman and Roberts, 1960a). High temperature above 30 °C does not favour growth of the fungus (Chen et al., 2003). In countries where CBD exists, infection severity increase with altitude as the disease is favoured by cooler temperatures (Phiri et al., 2001; Ngulu et al., 1998).

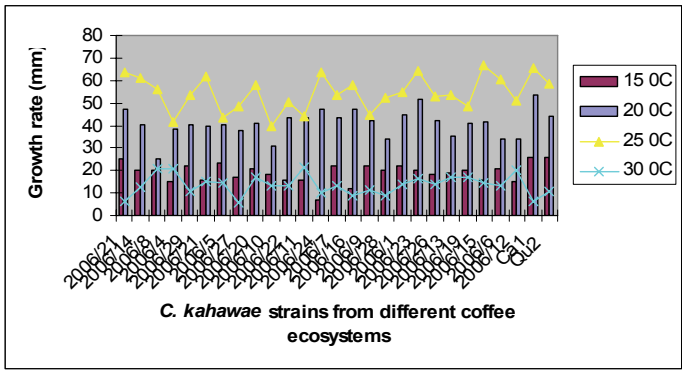


Figure 1. Growth rate (mm) of *Colletotrichum kahawae* strains from different coffee ecosystems in Tanzania at different temperature.

Characterization of pathogenicity

On green coffee berries

Percentage berry infection was higher at 15 °C than at 20 °C and 25 °C (Figure 2). Loureiro et al. (2006) when testing aggressiveness of *C. kahawae* strains in green berries found that the strains showed more aggressiveness at 15 °C than 20 °C and 25 °C. Omondi et al. (2004) confirmed that minimum temperature range (10-15 °C) can easly predispose resistant materials to CBD infection. It is known that CBD occurs mainly in high altitudes (> 1200 m.a.s.l.) in a relatively cool and wet (average of 1000 mm per annum) climate (Firman and Waller, 1977). This implies that to confirm resistance of *C. arabica* genotypes to CBD, it is worth testing at temperature ranges of 15 °C to 25 °C.

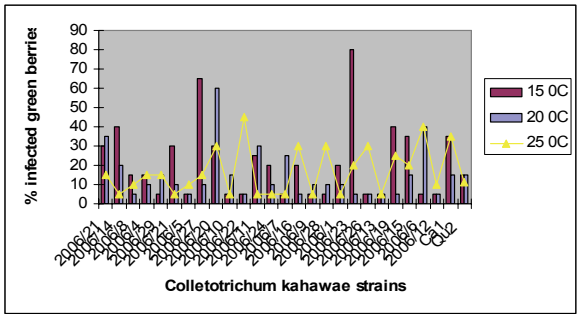


Figure 2. Pathogenicity of *Colletotrichum kahawae* strains from coffee ecosystems in Cameroon, Kenya and Tanzania on green coffee berries of cultivar Cattura at 15 °C, 20 °C and 25 °C.

On coffee hypocotyls

Pathogenicity of strain T3 was compared to *C. kahawae* strain Cam1, Que2 and Z9. Strain T3 demonstrated same level of pathogenicity to Cam1 (Figure 3). Varzea et al. (1999) reported that *C. kahawae* strain Cam1 is the most virulent strain compared to other CBD strain because of higher sporulation capacity and rapid conidial germination on the host tissues.

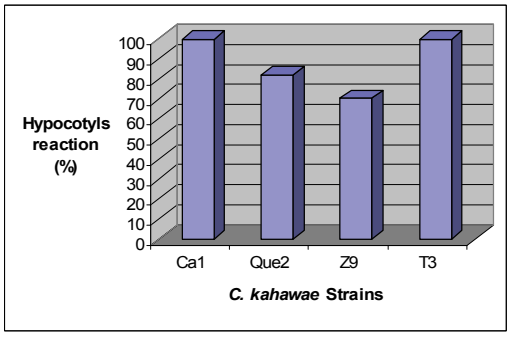


Figure 3. Percentage of dead coffee hypocotyls of cultivar N 39 caused by *Colletotrichum kahawae* strains nine days after artificial inoculation.

Variability of *Colletotrichum kahawae* strains to isozymes

From the three isoenzymatic systems analysed by IEF, a high degree of polymorphism in the isolates studied was detected by esterases zymograms among Tanzanian *C. kahawae* isolates, and that of Kenya, Cameroon and Zimbabwe (Figures 4a to 4c). On the contrary acid and alkaline phosphatases zymograms showed a low polymorphism (data not shown). Although preliminary, these studies seem to have a very high potential to characterize variability inside the isolates of *C. kahawae*. This was also stated by Loureiro *et al.* (2006) when studying isolates from different coffee producing countries.

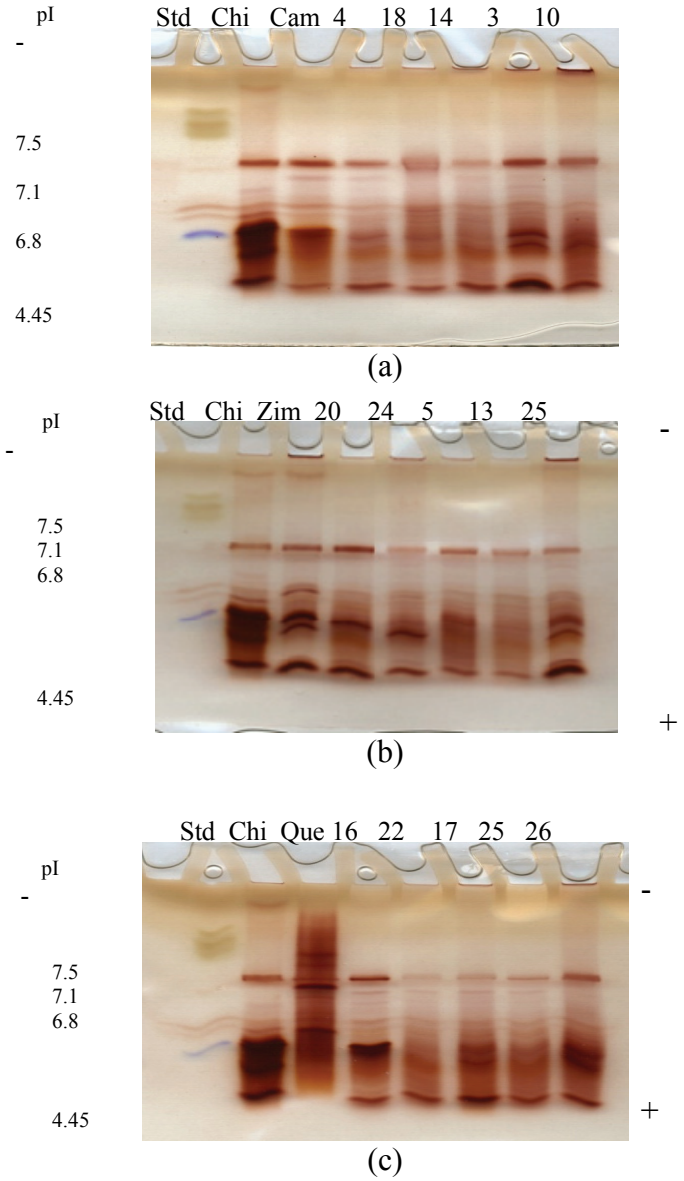


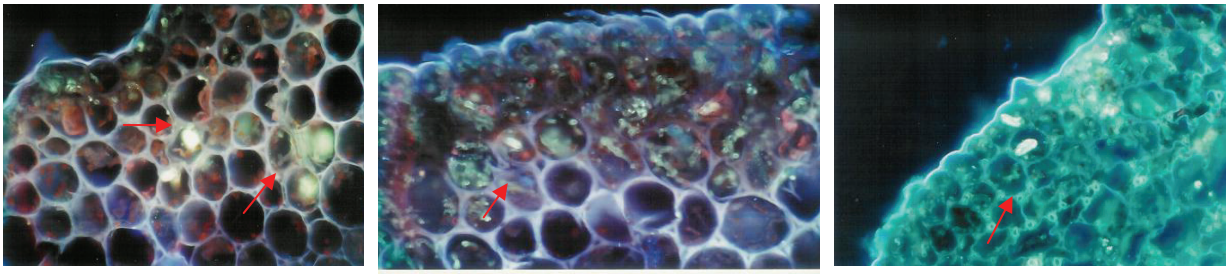
Figure 4. Isoenzymatic characterization of esterases to *C. kahawae* isolates using the technique of isoelectric focusing electrophoresis (IEF). Protein extracts were obtained from *C. kahawae* isolates from Tanzania (3,4,5,10,13,14,16,17,18,20,22,23,24,25,26), Cameroon (Cam), Zimbabwe (Zim) and Kenya (Que) and one *C. gloeosporioides* isolate fom China (Chi) grown in 50 ml of liquid medium for 10 days. Std - IEF standards broad range pI 4,45-9,6 (BIO-RAD)-. Twenty μg of protein was loaded per lane. (-) – Catode, (+) –Anode.

Histological studies-evaluation of post-penetration stages of *Colletotrichum kahawae* in coffee hypocotyls

Since conidia germination and the differentiation of melanised appressoria *in vivo* appeared to be readily accomplished on the different coffee genotypes studied only the fungal growth inside the hypocotyls tissues was quantified. As previously described by Várzea et al. (2002b) and Silva et al. (2006) *C. kahawae* penetration occurred via melanised appressoria directly through the epidermal cell walls. After penetration the tip of the infection peg swells to form a globose infection vesicle and the susceptibility involved the intra- and intercellular

ramification of the infection vesicle in the living host cells. This period of asymptomatic biotrophy is rapidly succeeded by a phase of destructive necrotrophy associated with the appearance of symptoms and pathogen reproduction.

In the present study the evaluation of hyphal length (isolate Que2) inside host tissues, between 24 h and 72 h after the inoculation, revealed no significant differences among the 3 genotypes tested (data not shown). The hyphae were confined to the first 5-6 layers of cortex cells. At these sites it was detected early deposition of callose around some intracellular hyphae (Figures 5a-5c), and the autofluorescence of host cell walls (not shown) indicating accumulation of phenolic-like compounds (Bennett et al., 1996). These host responses corresponded macroscopically to the scab lesion.



5a **5b** **5c**

Figure 5. Light micrographs, aniline blue test (u. v. light). Callose around some intracellular hyphae (arrows) in coffee genotypes 20498, 20509 and 20511, seven days after inoculation (x 100).

A better knowledge of the *C. kahawae* variability and the factors that determine host resistance and susceptibility may help to select adequate sources of resistance to this disease, to be used in the coffee breeding programmes.

Evaluation of 16 coffee genotypes for their resistance to *Colletotrichum kahawae* strains

Results presented in Table 1 shows that the percentage of hypocotyls resisted CBD between coffee genotypes differs significantly ($P \leq 0.05$). However it shows variations of interactions between *C. kahawae* strains Ca1 (Cameroon), Que2 (Kenya), Z9 (Zimbabwe) and T3 (Tanzania) and *Coffea arabica* genotypes.

Results on the DIR of the coffee genotype also show interaction to *C. kahawae* strains. For example, while coffee genotype 20509 (L13) was resistant to *C. kahawae* strains Ca1, Que2, and Z9; it was susceptible to strain T3. Like wise, coffee genotype 20498 (L2) indicated resistance to strains Ca1, Que2 and Z9; was susceptible to T3. Coffee genotype 20510 (L14) shows susceptibility to strain Ca1, but is resistant to the three strains of *C. kahawae*. These results imply that these *C. Kahawae* strains are likely to have adapted to coffee genotypes. However, further investigations are recommended to establish existence of physiologic races of *C. kahawae* strains. Earlier findings on this subject show that variation among strains of *C. kahawae* is predominantly due to differences in the degree of pathogenicity, and physiologic races do not exists (Omondi et al., 2004; van der Vossen et al., 1976).

These studies confirms further that within Lyamungu coffee hybrids there are good sources of resistance to *C. kahawae* strains and can be used to improve the coffee breeding programme. Also in Tanzanian coffee ecosystems there exist highly pathogenic *C. kahawae* strains that can be used in resistance screening programme to CBD.

Table 1. Percentage of coffee hypocotyls per coffee genotype indicated CBD resistance to four *Colletotrichum kahawae* strains from Cameroon, Kenya, Zimbabwe and Tanzania.

Genotype/strains	Cal		Que 2		Z9		T3	
	%	DIR	%	DIR	%	DIR	%	DIR
20497 (L1)	10.4 E	37.5	81.3 C	32.9	55.8 C	36.1	59.5 C	31.5
20498 (L2)	70.7 B	25.0	84.5 BC	32.0	55.6 C	48.9	26.0 F	64.3
20499 (L3)	0.0 F	100.0	25.0 I	56.8	2.0 HI	93.8	4.4 I	90.9
20500 (L4)	47.2 C	28.0	75.0 D	28.9	17.7 G	68.0	20.9 G	46.9
20501 (L5)	0.0 F	100.0	73.9 D	28.8	37.5 D	24.9	34.3 E	37.5
20502 (L6)	0.0 F	95.8	46.7 G	35.6	5.9 H	56.8	20.8 G	47.0
20503 (L7)	4.6 F	76.7	89.1 AB	35.4	31.6 E	55.4	79.8 B	34.0
20504 (L8)	16.7 D	51.9	60.9 E	41.7	25.0 F	63.3	22.5 FG	63.0
20505 (L9)	4.0 F	92.9	36.3 H	49.9	0.0 I	88.5	10.5 H	58.4
20506 (L10)	2.4 F	93.8	72.9 D	41.9	0.0 I	91.0	48.3 D	42.7
20507 (L11)	4.0 F	75.0	60.1 E	40.8	23.9 F	72.6	35.5 E	36.5
20508 (L12)	0.0 F	100.0	87.5 AB	29.2	36.7 D	63.5	59.3 C	37.5
20509 (L13)	79.5 A	25.0	92.0 A	25.0	92.1 A	25.0	25.1 FG	63.1
20510 (L14)	4.2 F	79.9	89.2 AB	27.7	66.1 B	27.3	97.9 A	26.1
20511 (L15)	0.0 F	100.0	55.2 F	100.0	0.0 1	100.0	0.0 I	100.0
20512 (L16)	2.0 F	92.9	64.1	100.0	2.3 HI	82.5	0.0 I	96.9
DMRT	4.6		4.6		4.6		4.6	
Mean	15.4		64.4		28.3		34.0	
S.E	6.5		6.6		6.9		7.1	

Means followed by small letter within the same column do not differ significantly following mean separation by Duncan's Multiple Range Test.
DIR = Disease Intensity Reaction, S.D = Standard deviation, SE = Standard error
DIR 0-25, Resistant; 26-50, moderately resistant; 51-75, moderately susceptible; 76-100 susceptible.
Ca1, Que2, Z9 and T3 = C. kahawae strains.

CONCLUSION

These studies confirms further that within Lyamungu coffee hybrids there are good sources of resistance to *C. kahawae* strains and can be used to improve the coffee breeding programme. Also in Tanzanian coffee ecosystems there exist highly pathogenic *C. kahawae* strains that can be used in resistance screening programme to CBD.

ACKNOWLEDGEMENT

Our gratitude to CIFC, SUA and TaCRI management for direct contribution to the successful documentation of this work. Also grateful to the European Union (EU) for the financial support provided to attend the ASIC conference.

REFERENCE

Bennett M., Gallagher M., Fagg J., Bestwick C., Paul T., Beal M., Mansfield J. (1996) The hypersensitive reaction, membrane damage and accumulation of Autofluorescent phenolics in lettuce cells challenged by *Bremia lactucae*. *Plant Journal* 9:851-865.

Chen Z.J., Ribeiro A., Silva M.C., Santos P., Guerra-Guimarães L., Gouveia M., Fernandez D.R.Jr. (2003). Heat shock induced susceptibility of green coffee leaves and berries to

Colletotrichum gloeosporioides and its association to PRs and hsp 70 gene expression. Physiological Molecular Plant Pathology 63:181-189.

Eshetu D. and Waller J.M. (2003) Variation among *Colletotrichum* isolates from diseased coffee berries in Ethiopia. *Crop Protection* 22:561-565.

Firman I.D. and Waller J.M. (1971) Coffee berry disease and *Colletotrichum* diseases of coffee. Phytopathological paper no 20. CAB International 53pp.

Loureiro A., Várzea V., Guerra-Guimarães L., Ribeiro A., Silva M.C., Bertrand B.(2006). Characterization of Colletotrichum kahawae diversity. In: Proceedings of the 21th International Conference on Coffee Science (ASIC), 11-15 Sept, Montpellier, France, Pp. 1277-1283.

Ngulu F.S., Kilambo D.L., Koinange E.M.K. (1998). Effective management of coffee berry disease: An Overview of research efforts in Tanzania. In:Research and Training News letter vol. X111, nos. 1-3, 13-16pp.

Nutman F.J. and Roberts F.M. (1960a). Investigations on a disease of *Coffea arabica* caused by a form of *Colletotrichum coffeanum* Noack. I: Some factors affecting infection by the pathogen. Transactions of the British Mycological Society 43 (3):489-505.

Omondi C., Agwanda C.D., Gichuru E.K. (2004). Field expression of resistance to coffee berry disease (CBD) as affected by variation in environment and pathogen. In: Proceedings of the 20th International Conference on Coffee Science (ASIC), 11-15 October, 2004, Bangalore, India.

Phiri N.A., Hillocks R.J., Jeffries P. (2003). Incidence and severity of coffee diseases in smallholder plantations in northern Malawi. Crop Protection 20:325-332.

Robertson E.F., Dannelly H.K., Mallory P.J., Reeves H.C. (1987). Rapid isoelectric focusing in a vertical polyacrylamide minigel system. Analytical Biochemistry 167: 290-294.

Rodrigues Jr. C.J., Varzea V.M., Medeirol, E. F. (1991). Strains of *Colletotrichum coffeanum* Noack causing coffee berry disease in Angola and Malawi with characteristics different to the Kenya starin. Journal of Phytopathology 131:205-209.

Roma E. (1997) Etude de la variabilite du pouvoir pathogene chez *Colletotrichum kahawae,* agent de I' anthracnose des baies du cafeier Arabica (Pathogenic variation of Colletotrichum kahawae causaltive agent of anthracnose of green coffee berries in Arabica). Stage effectue au CIRAD-CP-UR DDC – Montipellier.

Silva M.C., Nicole M., Guerra-Guimarães L., Rodrigues Jr. C.J. (2002). Hypersensitive cell death and post-haustorial defense responses arrest the orange rust (*Hemileia vastatrix*) growth in resistant coffee leaves. Physiological and Molecular Plant Pathology 60 (4): 169-183.

Silva M.C., Varzea V., Guerra-Guimaraes L., Azinheira H.G., Fernandez D., Petitot A., Bertrand B., Lashermes P., Nicole M. (2006). Coffee resistance to the main diseases: leaf rust and coffee berry disease. Brazilian Journal of Plant Physiology 18 (1):119-147.

Vallejos E.C. (1983). Enzyme activity staining in isoenzymes in Plant Genetics and Breeding, Part A – S.D. Tanksley and T.J. Orton (Editors). Elsevier Science Publishers B.V. Amsterdam: 469-516.

van der Vossen H.A.M., Cook R.T.A., Murakaru G.N.W. (1976). Breeding for the resistance to coffee berry disease caused by *Colletotrichum coffeanum* in *Coffea arabica L*. I. Methods of preselection for resistance. Euphytica 25:733-745.

Várzea V.M.P., Rodriques Jr, Silva M.C. (2002). Loss of resistance in interspecific tetraploid coffee varieties to some pathotypes of *Hemileia vastatrix*. In: International Synposium on Durable resistance: key to sustainable agriculture, Ede-Wageningen, Holanda. 34pp.

Várzea V.M.P., Silva M.C., Rogrigues Jr. C.J. (2002b). Resistencia a anthracnose os frutos do cafeeiro. (Resistance to anthracnose of green coffee berries). (Edited by Zambolim L, O Estado da Arte de Tecnologias na Producao de Café. Universidade Federal Vicosa, Vicosa Brazil. 321-366pp.

Várzea V.M.P., Rodriques Jr C.J., Silva M.C., Pedro J.P., Marques D.M. (1999). High virulence of a *Colletotrichum kahawae* isolate from Cameroon as compared with other isolates from other regions. Proceedings of the 18th International Conference on Coffee Science. Helsink, Finland. A 131.

Waller J.M., Bridge P.D., Black R., Hakiza G. (1993). Characterization of the coffee berry pathogen, *Colletotrichum kahawae* sp. Nov. Mycological Research 97:989-994.

Effect of Temperature and Rainfall Variations on Coffee Berry Disease (*Colletotrichum kahawae*)

J.A. MOUEN BEDIMO*, D. BIEYSSE, C. CILAS AND J.L. NOTTÉGHEM

Institute of Agricultural Research for Development (IRAD), Foumbot Multipurpose Station, P.O.Box 665 Bafoussam, Cameroon. *E-mail: josephmouen@yahoo.fr

INTRODUCTION

Arabica coffee production in Africa is highly affected by Coffee Berry Disease (CBD), due to *Colletotrichum kahawae*. This disease is specific to green berries and leads to 60-80%harvest losses under conditions favourable to development of the pathogen. Agricultural practices coupled with chemical control with 8 to 12 annual fungicide applications are known to be very effective against CBD, especially in high altitude regions (> 1600 m) where farms sustaining the most damage are found. Temperature and rainfall may be very decisive for development of CBD epidemics. Consequently, an epidemiological study was conducted on smallholders' farms in Cameroon, to assess disease development dependence upon variations of these factors.

Figure 1. Heavy CBD attack on a Arabica coffee branch.

METHODOLOGY

An experiment was carried out in the smallholder's plantation consisted in coffee trees of *Coffea arabica* cv. Jamaïca, located at Santa (05°47.190N, 10°09.672E, and 1750 m above sea level), a region with high CBD incidence in North-West Cameroon. 100 vigorous, high-yielding coffee trees were chosen randomly in that plantation for disease monitoring over two successive years (2004-2005). Weekly observations were made on three plagiotropic branches at different positions in each coffee tree canopy (top, middle, and bottom). They consisted in counting (a) the total number of berries, (b) the number of new infected berries, marking them with small labels to avoid counting again in subsequent assessments, and (c) the number of old infected berries.

Daily climatic data (rainfall, minimum and maximum temperatures) were recorded every year. Weekly climatic parameters obtained from these date were used for cross correlations with disease severity.

Figure 2. Diseased berries labelled during observations.

RESULTS

Cross-correlations between disease severity and climatic parameters recorded weekly over two successive years (2004-2005), showed a significant increase of disease severity depending on decreasing temperatures (minimum or maximum).

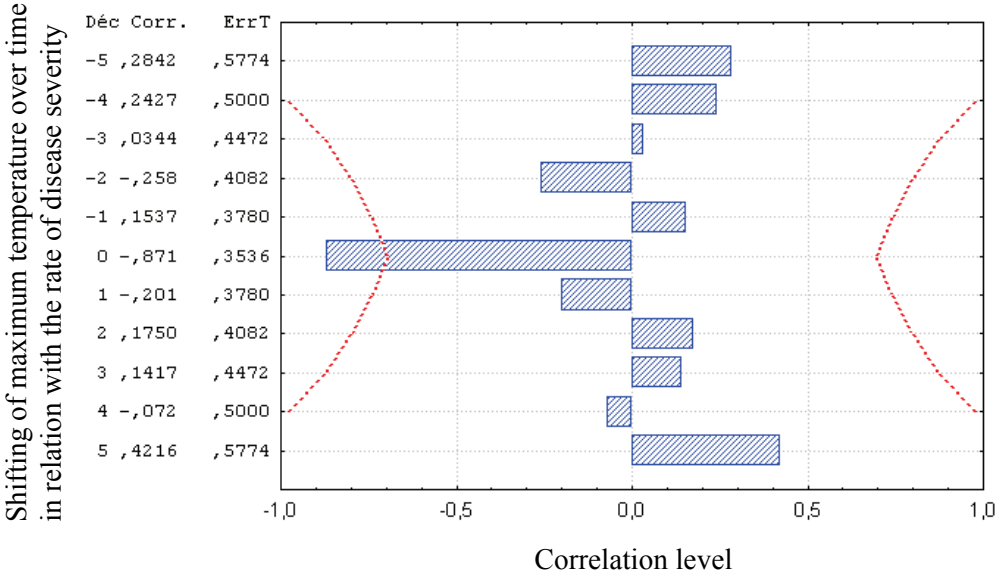


Figure 3. Correlogram of maximum temperatures with new infected berries (the blue histogram represents the disease severity rate and the red line, the signification level at 5%.

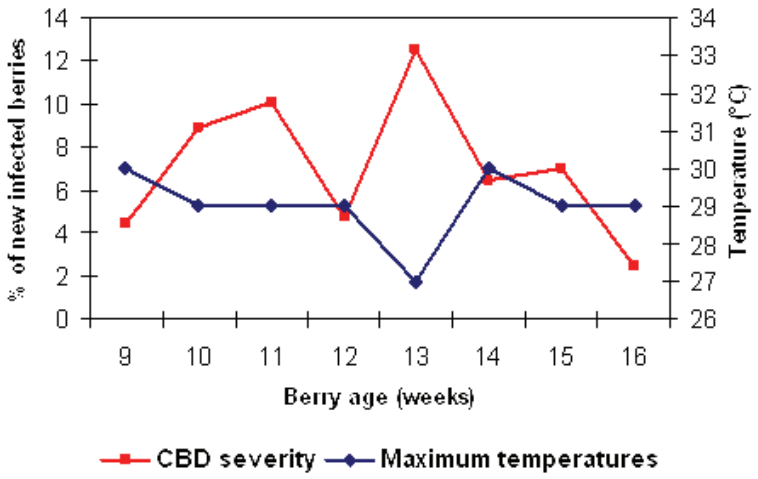


Figure 4. Changing of disease severity over time depending on maximum temperatures.

They also indicated a high variation of disease severity depending on the number of rainy days during berry growth. However, no significant correlation was found with the quantity of rainfall over the two years of observations.

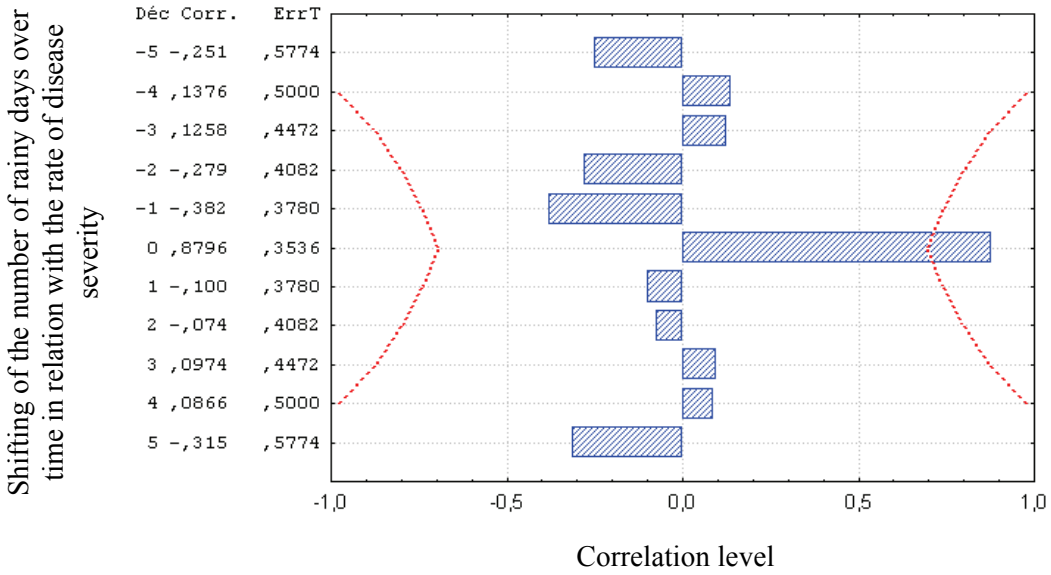


Figure 5. Correlogram of the number of rainy days with new infected berries (the blue histogram represents the disease severity rate and the red line, the signification level at 5%.

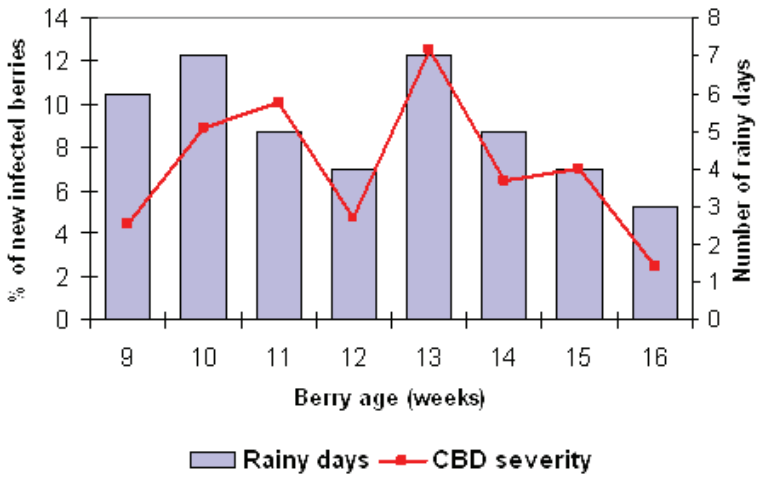


Figure 6. Changing of disease severity over time depending on the number of rainy days

CONCLUSION

Temperatures and rainfall distribution appear to be the key climatic parameters that favour the development of CBD epidemics. The present results suggest that these parameters might be very useful for setting up predictive models enabling optimization of effective control of CBD in areas with high disease incidence.

Recent Leads in Breeding and Field Strategies Adopted for Achieving Durable Rust Resistance in Coffee *(Coffea arabica)* in India

N. SURYA PRAKASH[1], SUNDARESHA[2], K. ROOHE[2], B. MUNISWAMY[2], K. BHASKAR REDDY[1], S.S. BHAT[3], H.L. SREENATH[2], A.S. RAM[3], C.S. SRINIVASAN[4], N. RAMAMURTHY[1], JAYARAMA[3]

[1]Regional Coffee Research Station, Coffee Board, R.V. Nagar – 531 133, A.P, India
[2]Biotechnology center, Coffee Board, Mysore – 570 006, Karnataka, India
[3]Central Coffee Research Institute (CCRI), Coffee Board, Coffee Research Station - 577 117, Karnataka, India
[4]Joint Director (Rtd), CCRI, Coffee Board of India

SUMMARY

Coffee leaf rust caused by the fungus *Hemileia vastatrix* Berk. et Br is the major disease of concern for arabica coffee (*Coffea arabica* L) cultivation round the globe leading to severe crop losses if proper control measures are not practiced. Therefore, development of improved coffee cultivars with long lasting durable resistance in field, is a crucial priority in general and more particular in Indian context. Considering the fact that the resistance genes originated from diploid coffee species (*C. canephora* and *C. liberica*) seem to be more effective than those from tetraploid *C. arabica,* accumulation of the major genes responsible for the resistance in a selected genotype and selection for non-specific resistance factors are some of the suggested approaches for achieving durable rust resistance. In this direction, systematic breeding efforts have been undertaken in India and several hybrid populations were developed and established in field. The field performance of these hybrid progenies with respect to field tolerance to leaf rust, berry yields, bean quality traits and liquor profiles have been extensively studied for the last 6 years at Regional Coffee Research Station, R.V.Nagar, India and superior lines have been identified and advanced for further exploitation.

Evaluation of 12 tetraploid interspecific hybrid lines revealed that mean yields ranged from 692 kg/ha in S. 4638 to 1181 kg/ha in S.4178. Analysis of variance and Stability analysis showed significant differences between genotypes. The genotypes S. 4177 and S. 4178 recorded above unit response to the environmental index ($b > 1.0$) and low deviation sum of squares from linear regression (below 13%) indicating their superior performance in favorable environment. Data on leaf rust incidence indicated that S. 4621 has shown high mean incidence (53.37%) followed by S. 4637 and S. 4638 (34.69 and 40.36%, respectively). Minimum incidence was noticed in S. 4422 (3.56%), S. 4595 (5.21%) and S. 4596 (7.58%). The genotypes S. 4177 and S. 4178 in addition to high yields, also recorded acceptable average 100 bean weight of 16.65 and 15.23 gm, respectively. Statistical analysis showed that the genotypes differ significantly at 5% C.D for percent 'A' grade but the genotypes did not show significant differences for hundred-bean weight. On considering all the parameters, S. 4177, S. 4178 and S. 4595 are found to be the superior performers. The scope and potential of the selected progenies in deriving commercial lines for the zone are discussed.

Further, preliminary evaluation of the new F_1 hybrid lines evolved by crossing locally adaptable varieties like Sln. 4 (Agaro) and Sln. 5A (Devamachy x S.881- Rume Sudan

collection) and also the multi-line progeny established in field also recorded promising performance. Initial findings of the validation of SCAR markers for S_H3 rust resistance gene in S.795 populations revealed the potential implications of the SCAR markers for maintenance breeding of the popular Indian strain S.795 and marker assisted selection in coffee, in a broader perspective.

INTRODUCTION

Coffee is a commodity of interest world wide, especially in over 50 countries involved in its production, trade and consumption. *Coffea arabica* popularly known as Arabica coffee and *Coffea canephora* called as Robusta coffee are the two main species under commercial cultivation contributing 70% and 30% respectively of the total Global coffee production. India is one of the traditionally coffee growing countries cultivating coffee in about 0.38 million ha of which Arabica occupies 46% of the area while robusta covers 54% of the area (Anonymous 2008). Coffee cultivation is mainly confined to the Southern states of Karnataka (59%), Kerala (22%) and Tamil Nadu (8%). Coffee is also grown in few other states like Andhra Pradesh, Orissa and North Eastern states in an extent of 42,000 ha (11%). These states are considered as non-traditional areas and coffee was introduced and encouraged for cultivation in these areas with an environmental and socio-economic perspective. India produced around 4.8 million bags (2.62 lakh MT) during 2007-08, approximately 4.14% of world's total production (Anonymous 2008). The contribution of Robusta and Arabica coffees to the Country's total production is around 65% and 35%, respectively. It has been observed that, for the past few years there has been a shift towards robusta coffee cultivation because of the rising production costs, labour scarcity and other difficulties in providing timely inputs especially for disease & pest management associated with arabica cultivation.

In India, coffee leaf rust caused by the obligate parasitic fungus *Hemileia vastatrix* Berk & Br and white stem borer (*Xylotrechus quadripes* Chevrolat) are the major limiting factors for arabica coffee cultivation. The coffee leaf rust is a great threat for arabica coffee production as the climatic factors that prevail in Indian coffee tracts are favourable for high disease build up, leading to severe crop losses up to 70% in susceptible cultivars, if proper control measures are not adopted (Anonymous, 2003). In order to manage the disease, the coffee growers are mainly relying on use of prophylactic and systemic fungicides and also on cultivation of rust tolerant cultivars. Considering the constant demand from coffee farming community for rust tolerant cultivars, breeding for rust resistance has been the major focus of arabica coffee improvement in India that has so far resulted in several new coffee cultivars manifesting varying levels of resistance to leaf rust. However, the adaptive capacity of the *H. vastatrix* with ability to overcome host resistances has resulted in the gradual loss of resistance in some cultivars under field conditions. As a result, all the nine major S_H genes (S_H1 to S_H9) identified so far have been defeated by continuous appearance of new physiological races of rust pathogen (45 races to date). Only cultivars (derivatives of introgressive breeding with *C. canephora*) possessing so called `A` type of host resistance have so far remained free from leaf rust epidemics (Van der Vossen, 2005). Therefore, there is an urgent need for improving the durability of resistance to coffee leaf rust in arabica coffee cultivars and this is a crucial priority in Indian context. From the past experience, resistance genes originated from diploid coffee species seem to be more effective than those of tetraploid *C. arabica*. Hence, the combined use of resistance genes of diploid species in *C. arabica* varieties is one of the available options for achieving durable resistance. Thus, transfer of desirable genes, in particular for disease resistance, from coffee species into Arabica cultivars without affecting quality traits has been the main objective of Arabica breeding (Carvalho, 1988; Van der Vossen, 2001). Among *Coffea* species, *C. canephora* provides the main source of disease and pest resistance traits not found in *C. arabica*, including coffee leaf rust (*H. vastatrix*), Coffee

Berry Disease (*C. kahawae*) and root-knot nematode (*Meloidogyne* spp.). Similarly, other coffee species, *C. liberica* is of considerable interest in this respect as in early coffee breeding programmes of India, *C. liberica* was successfully used as source of resistance to leaf rust (Srinivasan and Narasimhaswamy, 1975). Considering the potential of these two species as sources of rust resistance genes, a wide array of tetraploid interspecific hybrids have been developed in India by interspecific and introgressive breeding strategies and some of these new hybrid progenies were introduced in different agro-climatic zones for evaluation of their location specific suitability. From the field evaluation data, superior performers have been short listed based on a selection index that reflect the overall performance of a genotype across the characters studied (Amaravenmathy and Srinivasan, 2003; Prakash et al., 2006) and selfed progenies of the identified lines have been further advanced for commercial exploitation.

In the present paper, we report the salient findings of the field evaluation trials of several tetraploid interspecific hybrid derivatives that were established in field plots at Regional Coffee Research Station located at R.V.Nagar in Andhra Pradesh state which cater to the needs of non-traditional areas (NTAs). Keeping in view the harsh climatic conditions (extremities in temperatures, erratic rain fall distribution) prevail in the non traditional tracts and low cultivation practices being practiced by the tribal coffee farmers who are the major stake holders of the zone, the potential of the identified varieties as the hardy material for commercial cultivation in the zone were detailed and discussed. Further, the observations on field trials initiated to manage the disease build up levels such as planting multi-lines/composite varieties to create physically homogeneous but genetically heterogeneous populations and race suppression strategy are presented. Furthermore, the initiatives on large scale validation of SCAR markers for S_H3 rust resistance gene aimed at maintenance breeding of the most popular Indian strain, S.795 are also presented and discussed.

MATERIALS AND METHODS

Field evaluation of selected hybrid lines

The plant material included in the present field evaluation study comprised of 12 tetraploid interspecific hybrid progenies established at Regional Coffee Research Station, R.V. Nagar, Andhra Pradesh, during 1997. The genotypes included five progenies of the crosses between Cauvery (Catimor) and other arabicas, three lines derived from the crosses involving Catimor and Catuai Amerello/Vermello (introduced from CIFC Portugal) and four other hybrid derivatives as detailed below.

Progenies of Cauvery (Catimor) crosses:

S.4621 - Sln.9 (HDT x Tafarikela) x Cauvery (Catimor)
S.4634 - Cauvery (Catimor) x Sarchimor
S.4637 - Ethiopian line - wild Sidamo x Cauvery (Catimor)
S.4638 - Cauvery (Catimor) x Ethiopian line - wild Sidamo
Cauvery (Catimor) x S. 881 (Rume Sudan collection)
S.4176 - F_3 of Catimor x (Catuai Amarelo & Catuai Vermelho)
S.4177 - F_3 of Catimor x (Catuai Amarelo & Catuai Vermelho)
S.4178 - F_3 of Catimor x (Catuai Amarelo & Catuai Vermelho)

Other hybrid progenies:

S.4422 - Devamachy x S. 333

S.4375 - Sln.6 (S.2828 – Robusta x Arabica hybrid) x HdeT
S.4595 - Sln.11 (Amphiploid of *C. liberica* x *C. eugenioides*) x HdeT
S.4596 - Sln.11 x (Arabica – Wallamo x HdeT)

All the genotypes were planted in compact plots at a spacing of 1.8 x 1.8 m, under a mixed canopy of shade and 300 plants per genotype were established in each plot. The plants were trained on topped single stem system and standard agronomic practices were adopted. The progenies have been evaluated for field tolerance to rust, yield and bean characteristics. Plot yields were recorded for six years from 2002 to 2007 and mean yields were subjected for comparative analysis. Observations on incidence of leaf rust disease were carried out during Oct-Nov, the peak months of disease build up. Individual plants with even few pustules were treated as susceptible for scoring the percentage of resistant/susceptible types. The level of disease build up and reaction type in the susceptible plants was also observed following the scale of Eskes and Toma - Braghini (1981 cf Eskes, 1989). Further, vigour of the plants and retention of infected leaves was also taken into consideration for assessing the resistance manifestation and average of three years data was taken into account for analysis. Washed clean coffee samples of each genotype were prepared from 6 kg of uniformly ripened fruits by following the standard method and the same was used for analysing the bean parameters like percentage of `A` grade beans (retained on 17 no sieve – 6.65 mm) and weight of 100 `A` grade beans. Average of two year's data was considered for analysis of bean quality traits. Analysis of variance was carried out for each character and if the progeny mean square was significant, critical difference was calculated. Stability analysis for yield was done following the method of Eberhart and Russel (1966).

Development of new F_1 hybrid lines by crossing locally adaptable varieties

The genotypes Sln. 4 (Agaro) and Sln. 5A (Devamachy x S.881- Rume Sudan collection) are the best adapted varieties in Andhra Pradesh state of NTAs. The Sln.5A posses several useful traits like good plant vigour, production potential even under low management and high field tolerance to rust but small bean size is the negative feature of this variety. On the other hand, Agaro is a vigorously growing variety with moderate yields and possess superior bean and liquor quality traits. But this variety manifests high susceptibility to leaf rust. Hence, with the objective of improving bean quality traits in Sln.5A and rust resistance in Sln.4 (Agaro), reciprocal crosses were effected between Sln.4 (Agaro) and three lines of Sln.5A (S.2267, S.2831, S.2971) during 2004-05 season and F_1 hybrid progenies as well as selfed progenies of the respective parents were planted in field during 2005 season which are being evaluated at present.

Field establishment of multi-line progeny for evaluation of durability of rust resistance

Based on observations on field tolerance to leaf rust, elite plants with respect to yield and field tolerance to leaf rust pathogen were marked in the populations of different semi-dwarf genotypes viz., Cauvery (Catimor), S.4176, S.4177, S.4178, S. 4640, S.4634, S.4621, S.4695 and S.881 x Cauvery. These selected plants were selfed during 2005 season and selfed progenies were field established in a single plot as multi line/composite variety. These progenies have been regularly observed for rust disease manifestation. Clean coffee samples of the parent plants were prepared, bulked and assessed for liquor profiles.

Validation of SCAR markers for S_H3 rust resistance gene - Marker assisted selection for maintenance breeding of the most popular Indian strain, S.795

Availability of 10 sequence characterized DNA markers (SSRS & SCARs) closely linked to S_H3 leaf rust resistance gene (Mahe et al., 2007) offered great scope and possibility for marker assisted breeding for leaf rust resistance in coffee. In this direction, large scale validation of these markers has been initiated for maintenance breeding of the most popular Indian arabica strain, S.795 which carry S_H3 resistance factor for coffee rust known to be present only in *Coffea liberica* (Wagner and Bettencourt, 1965). A random survey was undertaken in S.795 populations established as early as in 1950s and 1970s and plants manifesting different levels of disease build up coupled with good plant vigour and berry yields were marked. These marked plants are being used for SCAR assays as per the procedure described by Mahe et al (2007). The marker data is correlated with the rust tolerance levels observed in field. Characterization of the selected plants for their rust resistance spectra is also initiated and screening with appropriate rust races (Race I & Race VIII) has been taken up.

RESULTS AND DISCUSSION

Field evaluation of selected hybrid lines

All the genotypes evaluated in the present study exhibited vigorous vegetative growth. Depending on cross combination, plant ideotype varied between vigorous semi-dwarfs with compact stature in case of crosses involving semi-dwarf x tall arabica types (Cauvery/Catimor crosses) or tall phenotype in case of other hybrid derivatives that resulted from tall x tall combinations. Mean yield of the 12 genotypes ranged from 692 kg/ha (S.4638) to 1181 kg/ha (S.4178). Analysis of variance showed significant differences for mean yield between the genotypes (Table 1) with S. 4178 recording the highest mean (1181 kg/ha) followed by S.4177 (1015 kg/ha) and S. 4621 (923 kg/ha). Stability analysis carried out following the method of Eberhart and Russel (1966) revealed further differences between genotypes (Table 2). The genotypes S. 4177 and S. 4178 recorded above unit response to the environmental index ($b > 1.0$) and low deviation sum of squares from linear regression (below 13%) indicating their superior performance in favorable environment and reliable response. In the present study, 2002-03 and 2004-05 were most favorable years as indicated by greater mean yield across all genotypes. During these two years, S. 4177 and S. 4178 have recorded the highest yields. Similarly, 2003-04 and 2006-07 were adverse seasons for yield but S.4177 and S.4178 have recorded moderate yield even during these two years. Further, 2005-06 and 2007-08 were average years, but even during these years the yields of S. 4177 and S. 4178 were superior. Next in the order of performance, S. 4595 could be considered which recorded mean yield of 872 kg/ha. Its response to the environment is also above unity ($b = 1.23$) but deviation sum of squares is about 18%. Hence, this genotype is also expected to yield well in favorable environments. S. 4621 although recorded higher yield (923 kg/ha) than S.4595, the unit regression ($b = 1.0$), showed high deviation from linear regression (35 %) which indicates lower stability. All other genotypes recorded lower yield and stability.

Table 1. Data on Analysis of variance.

Sl. No.	Source of variation	Degrees of freedom	Mean squares	F - value
1	Years	5	1525205.2	36.38**
2	Genotypes	11	114708.2	2.74**
3	Error	55	41919.7	

The genotypes differed significantly in their field reaction to leaf rust as studied for 3 years. The data indicates that S. 4621 has shown high mean incidence (53.37%) followed by S. 4637 and S. 4638 (34.69 and 40.36%, respectively). Minimum incidence was noticed in S. 4422 (3.56%), S. 4595 (5.21%) and S. 4596 (7.58%). In S. 4177 and S. 4178, the incidence which was below 1% in 2001-02, has increased to 52% in 2005-06. This might be due to the two consecutive high yields recorded in these two genotypes in 2004-05 and 2005-06 which is known to reduce the plant vigor due to crop strain resulting in higher susceptibility to pests and diseases. But still the mean incidence of rust in these two genotypes was below the grand mean of 21% recorded across all genotypes included in the study. For statistical analysis, rust incidence recorded during two consecutive years namely 2004-05 and 2005-06 was considered and analyzed using 'arcsine' transformation values. Results show that the genotypes differ significantly for mean rust incidence with S. 4422, S. 4595 and S. 4596 recording lowest incidence (below 10%). The genotype S. 4621 recorded the highest mean incidence of 77.4% (Table 3).

Table 2. Data on Stability analysis.

Genotype	Mean Yield	Regression coefficient	Deviation S.S.(%)
S. 4621	923	1.09	34.6
S.4176	807	0.75	37.1
S.4177	1015	1.47	12.9
S.4178	1181	1.36	7.7
S.4375	799	0.97	13.5
S.4637	746	0.92	27.1
S.4638	691	0.74	17.9
Cauvery x S.881	789	0.80	25.6
S.4634	825	1.08	11.4
S.4422	718	0.66	7.7
S.4595	872	1.23	17.8
S.4596	775	0.93	16.0
C.D. 5%	**236**		
1%	**314**		

As regards to Percent of `A` grade beans and bean weight, S. 4638 recorded the highest percentage (62.57) of 'A' grade beans compared to other genotypes. The genotypes S. 4621, S. 4595 and S. 4596 recorded low 'A' grade beans (30 to 40%). The high yielding genotypes in this study, S. 4177 and S. 4178 have also recorded acceptable percentage of 'A' grade beans (58 to 61%). The genotype S. 4637 recorded maximum value for 100 bean weight (17.54 gm) followed by S. 4595 (17.30 gm) while Cauvery x 881 recorded lowest bean weight of 14.74 gm. The genotypes S. 4177 and S. 4178 in addition to recording high yield, also recoded acceptable average 100 bean weight of 16.65 and 15.23 gm, respectively. Statistical analysis showed that the genotypes differ significantly at 5% C.D. (value = 15.25) for percent 'A' grade. However, for hundred-bean weight, the genotypes did not show significant differences (F value not significant). The evaluation of liquor revealed FAQ (Fair Average Quality) to FAQ+ rating for the cup in all the genotypes except in S.4621 and S.4634 where the rating was below FAQ. The liquor profiles of the majority of the genotypes were classified as fair body, fair+ to Good acidity and fair flavour of the liquor.

Thus, on considering all the parameters, S. 4177 and S. 4178 appear to be moderate to high yielding and stable genotypes with desirable bean grade and bean weight at this location. However, rust could be a problem on these genotypes especially in high yielding years which

needs to be addressed with suitable fungicidal spray schedule. On the other hand, S. 4595 with moderate yield, fair stability for yield, low rust incidence and acceptable bean weight ranks next in spite of having relatively low percent of 'A' grade beans.

Table 3. Analysis of variance for the character leaf rust incidence.

Genotype	Mean Incidence % (two consecutive years)
S. 4621	77.4
S.4176	30.7
S.4177	28.3
S.4178	22.5
S.4375	28.3
S.4637	43.7
S.4638	46.5
Cauvery x S.881	17.1
S.4634	27.1
S.4422	5.4
S.4595	7.8
S.4596	9.3
F - Value	**
C.D. 5%	**26.9**
1%	**38.1**

Based on 'arcsine' transformation values.

The data of the present study clearly established the potential of the new genotypes and the findings are of immense use in recommending suitable varieties for cultivation in Non traditional areas (NTAs). The Andhra Pradesh and Orissa are the major coffee growing states in NTAs in India and the climatic factors prevailing in these coffee tracts are relatively harsh compared to the traditional areas. Further, socially and economically backward tribal farmers are taking up the coffee cultivation in these areas by adopting very low management practices without any plant protection measures. From the earlier studies, some of the interspecific hybrid derivatives like Sln.5A (Devamachy x S.881- Rumesudan collection), Sln.6 (Robusa x arabica hybrid) and Sln.11 (Amphiploid of *C. liberica* x *C. eugenioides*) are reported to be hardy and recorded promising performance even under low management practices, in this zone. However, inspite of several positive attributes like, high field tolerance to leaf rust, adoptability to low rainfall areas due to drought hardy nature and consistent production, Sln.11 could not be exploited due to small bean size (Dharmaraj, 1985; Gopal, 1985, Reddy et al 1984; Reddy et al., 1985). In this context, the better performance of S.4595, a derivative of Sln.11 x HDT recorded in the present study, provides scope for its exploitation. Ganesh et al (2002) evaluated the performance of S.4595 and its back cross progenies with HDT and reported the promising potential of the back cross progeny of S.4595 to HdeT, with respect to yield, field tolerance to leaf rust, bean and liquor quality traits. Recently, Prakash et al (2006) indicated the suitability of S.4595 genotype for cultivation in tribal sector in NTAs based on a preliminary evaluation trial, which is further confirmed from the findings of this study. Considering these findings, elite plants in the progeny of S.4595 were marked and selfed progenies were established in field for use as seed blocks in future.

Further, the top performers, S. 4177 & S. 4178 could be recommended for cultivation in Orissa state where progressive private entrepreneurs are involved in coffee cultivation. However, considering the proven performance of semi-dwarf genotypes in Orissa state and keeping in view the likely possibilities for increase in rust susceptibility levels especially in

high cropping years, cultivation of a composite line, a mixture of the better performers is the better option for achieving durability of rust resistance in Orissa zone.

Development of new F_1 hybrid lines by crossing locally adapted varieties:

In general all the F_1 hybrid progenies exhibited vigorous growth and hence the first crop is allowed during current year. Observations were recorded on five morphological characters viz., stem girth, bush diameter, number of primary branches, length of longest primary, number of nodes on longest primary and two reproductive/yield contributing characters namely number of fruiting nodes on primary & number of fruits per node and also the field tolerance to rust.

From the preliminary data, it is apparent that the F_1 hybrid progenies exhibited superior growth characteristics and yield component characters compared to respective parental progenies. Among the F_1 hybrid progenies, the progenies of the crosses where Sln.4 (Agaro) was used as female parent are found superior than the corresponding reciprocal crosses where Sln.4 (Agaro) was used as male parent (pollinator). Among different hybrid progenies, the progeny of the cross Agaro-1 x S. 2971 recoded maximum values for stem girth (12.56 cm), bush radius (121.27 cm), length of longest primary (133.4 cm), number of nodes per longest primary (21) and number of fruits per node (22.93) while Agaro-2 x S.2831 progeny recorded maximum number of fruiting nodes per primary (12.73). Data on first yields has to be recoded in the forthcoming harvesting season during November-December 2008. As regards to the field tolerance to rust, all the F_1 progenies were free from rust during the period of the report except in selfed progenies of Sln.4 (Agaro), wherein 63.64% of plants manifested leaf rust disease. Eventhough the data is too preliminary, the F_1 progenies looks promising.

Field establishment of multi-line progeny for evaluation of durability of rust resistance

Growing multi-lines/composite variety is one of the proven strategies for durable rust resistance in coffee. Observations on manifestation of rust resistance indicated that, all the parent plants used as component lines of the multi-line have been free from the disease since 1997, the year of their field establishment. All the selfed progenies established as multi-line progeny during 2006 season also remained free from the disease. These progenies need to be further monitored with respect to durability of rust resistance. The clean coffee samples of different component lines of the composite variety were pooled and observed for physical characteristics. The sample looked homogeneous with nearly 50% of the beans standing on screen 17 (6.65 mm -`A` grade). Cupping of the sample revealed FAQ+ to Good cup with fair body, fair+ to Good acidity and fair flavour of the liquor. Hence, in case durability of resistance is established, the seed mixture of the component lines could be given for cultivation as composite variety.

In Colombia, the multi-line strategy was successfully adopted in releasing the composite variety ‘Colombia’ which manifested durable resistance to rust for several years. However, as per recent reports although there has been a gradual increase in rust susceptibility levels of these populations, the multi-line strategy proved very effective in not only imparting durable resistance but also in checking the development and buildup of new virulent rust races in Colombia (Alvarado 2005). Further, considering the need for achieving durable rust resistance in Brazil, Fazuoli (2005) suggested that some of the strategies for durable resistance could be use of multi-lines, composed variety (similar to multi lines) by mechanical mixture of seeds from various lines, planting lines or cultivars with diverse resistance genes (with similar or

not similar agronomic traits) in isolated strands with in the same estate. In this context the approach of the present study and the preliminary findings are very encouraging.

Validation of SCAR markers for S_H3 rust resistance gene - Marker assisted selection for maintenance breeding of the most popular Indian strain, S.795

The populations of S.795 are under commercial cultivation in India since 1947 and manifest field tolerance to leaf rust races I and II (Srinivasan and Narasimhaswamy, 1975; Ramachandran and Srinivasan, 1979) prevalent in Indian coffee tracts. This popular Indian selection carry S_H3 resistance factor for coffee rust only known to be present in *Coffea liberica* (Wagner and Bettencourt, 1965; Prakash et al., 2004). Oflate, it has been observed that most of the S. 795 plants lost their vigour with high rust build up, affecting production. Considering the fact that S. 795 is still a choice variety of the Arabica coffee growers in traditional areas, there is an urgent need to take up maintenance breeding in this variety. As S_H3 resistance gene is responsible for imparting durability of resistance in S.795 populations for several years in field, systematic monitoring and tracking of the S_H3 gene in the populations and to integrate other rust resistance genes is the crucial approach. Availability of SCAR markers for S_H3 gene facilitates this strategy. Accordingly, a maintenance breeding programme for S.795 variety has been initiated.

In order to identify suitable plants for analysis and for subsequent exploitation, a systematic survey has been undertaken in a few old plantations of S.795 established as early as in 1950s and 1970s. The plant populations were assessed with respect to vigour and field tolerance to rust and marked about 150 plants, grouped in to three groups viz. Vigour coupled with field tolerance to rust (G.1), Vigorous and moderately susceptible to rust (G.2), less vigorous and susceptible to leaf rust (G.3), for preliminary analysis. Initially, three SCAR markers (Sp-M8-SH3, Sat244, BA-124-12K-f) closely linked to S_H3 loci were tested on the selected plant population covering all three groups. Out of the there markers tested, two markers (Sat244, BA-124-12K-f) resulted in clear amplification profiles that could distinguish the presence and absence of S_H3 gene. In majority of the cases, the grouping of the plants based on field performance also correlated well with the presence and absence of S_H3 gene. Detailed characterization of plants by using selected rust races is under progress to confirm the rust resistance spectra. Thus the preliminary results of SCAR analysis are also promising with potential implications for marker assisted selection and breeding in coffee.

ACKNOWLEDGEMENTS

Special thanks are due to Dr. Jayarama, Director of Research, Coffee Board, for constant support & encouragement. The timely help extended by Dr. C. S. Srinivasan, Joint Director of Research (Rtd), Coffee Board of India, in analysing the data and for critically going through the manuscript is gratefully acknowledged

REFERENCES

Alvarado A.G. 2005. Evolution of *Hemileia vastatrix* virulence in Colombia. *In* Durable Resistance to Coffee leaf rust (Eds) Laercio Zambolim, Eunize Maciel Zambolim, Vitor Manuel Pinto Varzea, pp 99-115. (Proceedings of the 1st International Workshop on Durable Resistance to coffee leaf rust held at Vicosa, Brazil from 25-28 September 2005).

Amaravenmathy V.S. and Srinivasan C.S. 2003. Phenotypic and genotypic variations for yield and plant architecture in some hybrid progenies of arabica coffee. J. Coffee Res. 31(2): 99-105.

Anonymous. 2003. Coffee Guide, Coffee Board, Bangalore, India, 200p.

Anonymous. 2008. Database on Coffee, Coffee Board, Bangalore, India, 101p.

Dharmaraj P.S. 1985. Adaptation of of coffee selections in Andhra Pradesh. J Coffee Res. 15: 32.

Eberhart H.A. and Russel W.A., 1966. .Stability parameters for comparing varieties. Crop Sci. 6: 36-40

Eskes A.B. 1989. Resistance. In: Kushallapa, A.C and Eskes, A.B. (eds), Coffee rust: Eidemiology, Resistance and Management: 171-291. CRC Press, Boca Raton, Florida.

Fazuoli L.C., Baiao de Oliveira A.C., Toma-Braghini M., Silvarolla M.B. 2005. Identification and use of sources of durable resistance to coffee leaf rust at the IAC. *In* Durable Resistance to Coffee leaf rust (Eds) Laercio Zambolim, Eunize Maciel Zambolim, Vitor Manuel Pinto Varzea, pp 137-185. (Proceedings of the 1st International Workshop on Durable Resistance to coffee leaf rust held at Vicosa, Brazil from 25-28 September 2005).

Ganesh D., Ram A.S., Prakash N.S., Mishra M.K., Ahmed J., Jagadeesan, M., Reddy A.G.S. and Srinivasan C.S. 2002. Evaluation of *Coffea liberica* x *Coffea eugenioides* and its progenies for yield, leaf rust tolerance and quality. Proc of Placrosym XV (Plantation Crops Symposium, Mysore, India, 2002), pp 72-77.

Gopal N.H. 1985. Performance of coffee cultivars of Andhra Pradesh region with reference to drought. J Coffee Res. 15: 58.

Mahé L., Combes M.C., Várzea V.M.P., Guilhaumon C., Lashermes P. 2007. Development of sequence characterized DNA markers linked to leaf rust (*Hemileia vastatrix*) resistance in coffee (*Coffea arabica* L.). Mol Breeding DOI 10.1077/s11032-997-9112-z.

Prakash N.S., Marques D.V., Varzea V.M.P., Silva M.C., Combes M.C., Lashermes P. 2004. Introgression molecular analysis of a leaf rust resistance gene from *Coffea liberica* into C*offea arabica* L. Theor Appl Genet 109: 1311-1317

Prakash N.S., Ganesh D., Bhat S.S. 2005. Population dynamics of coffee leaf rust (*Hemileia vastatrix* Berk & Br) and recent advances in rust research in India. *In* Durable Resistance to Coffee leaf rust (Eds) Laercio Zambolim, Eunize Maciel Zambolim, Vitor Manuel Pinto Varzea, pp 411-442. (Proceedings of the 1st International Workshop on Durable Resistance to coffee leaf rust held at Vicosa, Brazil from 25-28 September 2005).

Prakash N.S., Laxmi V.V.K.M., Reddy A.G.S.M., Sreenivasan M.S., Bhaskar Reddy K., Vinodkumar P.K., Srinivasan C.S., Santaram A., Ramamurthy N., Jayarama 2006. `Breeding for durable rust resistance in coffee *(Coffea arabica)* – Field performance of some tetraploid inter-specific hybrid derivatives in India`. Proceedings of the 21st International conference on Coffee Science (ASIC 2006), held at Montpellier, France, from 11-16, Sept. 2006.

Ramachandran M. and Srinivasan C.S. 1979. Four generations of selection for resistance to race I of leaf rust in arabica cv. S.288 x 'Kents'. Indian Coffee 43(6): 159-161

Reddy A.G.S., Raju K.V.V.S.N., Dharmaraj P.S. 1984. Allopolyploidization in a spontaneously doubled hybrid of two diploid species of *Coffea*. Proc. VI Symposium on Plantation Crops, Kottayam, India, pp 31-39.

Reddy A.G.S., Raju K.V.V.S.N., Dharmaraj P.S. 1985. Breeding behavior of Ligenioides – A spontaneous amphidiploid between *Coffea liberica* and *C. eugenioides.* J Coffee Res. 15 (1 & 2): 33-37.

Rodrigues Jr C.J., Varzea V.M., Godinho I.L., Palma S., Rato R.C. 1993. New physiological races of *Hemileia vastatrix.* In: Proceedings of the 15th ASIC colloquium (Montpellier), ASIC, Paris, France, pp 318-321.

Srinivasan K.H. and Narasimhaswamy R.L. 1975 A review of coffee breeding work done at the Government coffee experiment station, Balehonnur. Indian coffee 34: 311-321

Vander vossen H.A.M. 2001. Coffee breeding practices. In: Clarke R.J and Vitzthum O.G (eds) Coffee Recent Developments − Agronomy 1 (eds), Blackwell Science Ltd, London, pp 184-201.

Vander vossen H.A.M. 2005. State-of-the-art of developing durable resistance to biotrophic pathogens in crop plants, such as coffee leaf rust. *In* Durable Resistance to Coffee leaf rust (Eds) Laercio Zambolim, Eunize Maciel Zambolim, Vitor Manuel Pinto Varzea, pp 1-29. (Proceedings of the 1st International Workshop on Durable Resistance to coffee leaf rust held at Vicosa, Brazil from 25-28 September 2005).

Varzea V.M.P. and Marques, D. V. 2005. Population variability of *Hemileia vastatrix* vs. Coffee durable resistance. In Durable Resistance to Coffee leaf rust (Eds) Laercio Zambolim, Eunize Maciel Zambolim, Vitor Manuel Pinto Varzea, pp 53-74. (Proceedings of the 1st International Workshop on Durable Resistance to coffee leaf rust held at Vicosa, Brazil from 25-28 September 2005).

Wagner M. and Bettencourt A.J. 1965. Inheritance of reaction to *Hemileia vastatrix* Berk & Br. in *Coffea arabica* L. Progress report 1960-65, Coffee Rusts Research Center, Oeiras, Portugal.

Multiple Resistances to Coffee Berry Disease, Coffee Wilt and Leaf Rust in *Coffea arabica* Populations

G. ADUGNA, C. JEFUKA, D. TEFERI, A. ZERU

Ethiopian Institute of Agricultural Research, Jimma Research Centre, P.O. Box 192 Jimma, Ethiopia. E-mail: girma.adugna@yahoo.com

SUMMARY

Coffee berry disease, coffee wilt disease (CWD) and coffee leaf rust (CLR) caused, by C*olletotrichum kahawae*, *Gibberella xylarioides* and *Hemileia vastatrix*, respectively, are the three important fungal diseases threatening coffee production in the world in general and in Africa in particular. Coffee berry disease (CBD) is still the number one disease of Arabica coffee confined to the Old World, causing up to 100% harvestable crop loss. Coffee wilt is a disease decimating the whole coffee trees irrespective of species and age, and curtailing coffee production in East and Central African countries. Leaf rust is a serious problem impacting both yield and quality of Arabica coffee throughout the world. Ethiopia as a gene pool centre endowed with enormous genetic diversities of *Coffea arabica* L., there has been great potentials to develop cultivars with multiple resistances to these major diseases. In this regard, more than 70 coffee cultivars were tested for their resistance to CBD, CWD and CLR employing seedling inoculation tests under controlled growth room and greenhouse conditions at Jimma Research Centre. Three sets of replicated experiments in randomized complete block design were carried out for each host-pathogen interactions. Seedlings were raised from each coffee cultivar in heat sterilized soil, and then inoculated following standard inoculation protocols for each disease in question. Progenies of promising cultivars were further advanced to verification plots in the fields and evaluated for CBD resistance employing attached berry test method; percentages of rusted leaves per tree was estimated visually, and the number of dead coffee trees due to CWD were also recorded per plot. Disease infection percentages were computed and statistically analyzed using SAS software after transformation. There were significant ($p < 0.01$) differences among coffee cultivars in percentages of CBD, CWD and CLR infections. Cultivars 8136 (6.9%), 7416 (15.3%), 7418 (17.7%), 74153 (17.4%), 7514 (26.8%), 7516 (16.8%), 7576 (27.8%), 75129 (21.9%), and 827 (27.8%) showed significantly low CBD severity comparable to the resistant checks 741 (19.6%) and 75227 (25.8%). Catimor line 1579 (12.7%), 20071 (15.2%) and 8136 (25.3%) were consistently resistant to coffee wilt disease. Catimor line 2179, Catuai and 8136 had no rust while 1579, 7455, 74139, 7516 and 3670 showed low rust infections (< 25%). This study showed that some coffee cultivars 8136, 7516, and 1579 had higher resistance to at least two of the diseases evidencing multiple resistances. These resistant cultivars can be utilized to control major diseases of coffee, and those possessing independent resistance to CBD, CWD and CLR can also be grown in areas where the respective disease prevail and can be used in breeding programme.

INTRODUCTION

Coffee Berry Disease, Coffee Wilt and Leaf Rust are the three important fungal diseases threatening coffee production in the world in general and in Africa in particular. Coffee Berry Disease (CBD) caused by a fungal pathogen C*olletotrichum kahawae* Waller & Bridge is the number one disease of Arabica coffee, still confined to the Old World, causing up to 100%

harvestable crop loss. CBD alone perhaps responsible for the destruction of at least one-third of the total crop that corresponds to annual loss of US$ 700 million potential revenue to African nations (Van der Vossen, 1997). It reduces coffee yield by 30 percent in Ethiopia (Tefesetewold 1995, Eshetu et al., 2000). Coffee Wilt Disease (CWD) or tracheomycosis caused by *Gibberella xylarioides* Heim & Saccas (*Fusarium xylarioides* Steyaert) is the most prevalent deadly soilborne disease of both Arabica and Robusta coffees in East and Central Africa. It was reported that income from coffee has declined by over 50% in Uganda, Ethiopia and Tanzania and the total annual losses amount to US$ 13.5 million attributed to CWD in the three affected countries (CABI, 2003). Coffee leaf rust (*Hemileia vastatrix* Berk & Br) is one of the infamous diseases in the history of Plant Pathology for decimating Arabica coffee out of production in Ceylon (now Sri Lanka). It spreads from there to all coffee producing countries with varying intensities and even to extent that justifies control with intensive fungicide sprays. Ethiopian coffee types possess at least some incomplete resistance (Kushalapa and Eskes, 1989; Mesrset, 1991) and the existence of these coffee groups enable to control the disease with tolerant cultivars.

Resistance to CBD is durable nature in that so far released coffee cultivars are as resistant as before (Arega *et al*. 2008). and durable resistance to such an important plant disease as CBD (Van der Vossen, 1997), preferably in combination with resistance to coffee wilt and leaf rust would lead to sustainable production. Coffee cultivars that possesses multiple resistances to these major diseases reduces time and costs in pyramidization (gene pyramiding) of all important traits through successive hybridization especially with the conventional breeding program of painstaking backcrossing activities. This article presents the possible occurrence of some multiple resistances to coffee berry disease, coffee wilt and leaf rust in *Coffea arabica* populations identified in intensive seedling tests and adult plant in fields.

MATERIALS AND METHODS

Seeds were prepared from 72 coffee cultivars (including highly resistant and susceptible checks to respective diseases) at various locations in southwest Ethiopia. Three sets of experiments were independently conducted in randomized complete block design (RCBD) in three replications for the three major coffee diseases (CBD, CWD and CLR) at Jimma Research Center. The seeds were sown in sterilized sandy soil in plastic boxes, and then seedling inoculation tests were undertaken following the standard procedures for each disease.

Experiment I. Testing for CBD Resistance

Seedling hypocotyl inoculation test

The modified seedling hypocotyl inoculation technique was adopted for CBD resistance test (Van der Graaff 1981, Tefesetewold 1995, Girma and Chala, 2008). The hypocotyl of each seedling was inoculated with inoculum suspension of 2 x 10^6 conidia/ml concentration by brushing the stem at soldier stage (4-6 weeks old). The inoculated seedlings were arranged on the bench in a growth room and covered with plastic sheet for 48 hours to maintain 100% relative humidity and temperature was adjusted to 21 °C that favor infection. In order to ensure infection, the seedlings were re-inoculated following the same procedure and maintained under the same conditions for further 48 hours. After three weeks incubation, the number of infected seedlings per box were scrutinized and recorded using Van der Graaff's 0-4 scales, and a disease index expressed as percentage of CBD infection was computed for each cultivar per box (Van der Graaff, 1981; Tefesetewold, 1995).

Attached berry test and disease assessment in the field

The progenies of promising coffee cultivars were advanced to verification trials in CBD hotspot areas. Attached berry test (ABT) was used to verify their levels of resistance by inoculating good number of growing berries on three branches (top, middle and bottom canopies) of a sample coffee tree (3-5 trees/plot). These inoculated branches were covered with plastic sleeves with paper bags over night to favour infection. After three weeks, the number of healthy and CBD infected berries per branch were recorded and then percentage infected berries was calculated. Besides, visual estimation of per cent CBD infection was assessed on tree base.

Experiment II. Testing for resistance to Coffee Wilt Disease

The same number of cultivars was tested for their reaction to coffee wilt. The seedlings (20/box) were inoculated at fully opened cotyledon stage (8-10 weeks old) with viable conidial suspension of the CWD pathogen (*G. xylarioid*es) isolate by stem nicking technique (Pieters and Van der Graaff 1980; Girma and Mengistu, 2000). The number of wilting seedlings per box (based on external symptoms) and days to the first symptom appearance were recorded fortnightly for 6 months, and finally percentages of dead seedlings and incubation periods (day) were used for analyses.

Experiment III. Testing for resistance to Coffee Leaf Rust

Coffee seedlings were inoculated by brushing the leaves with urediniospores of *Hemileia vastatrix* collected from specific field at Agaro in 1:5 spores to talc ratio using a fine paint brush at two pairs of true leaf stage (15 seedlings/ cultivar). After the disease development, two important parameters, rust severity and infection types were considered to measure the resistance levels of the cultivars to infection. The reaction types were recorded using 0-9 scales, where; 0 = no visible symptoms, 1-3 = variation in resistant types, 4-7 = heterogeneous reaction types with increasing sporulation and percentage of sporulating lesions, and 8-9 = highly susceptible reaction types with variations in sporulation intensity. The rust severity was measured as cumulative leaf size covered by pustules expressed as percentage of total area of inoculated leaf (Eskes and Toma-Braghini 1981).

All the data were finally transformed to angular values and analyzed using SAS statistical software.

RESULTS AND DISCUSSION

There were significant differences among the coffee cultivars tested for resistance to coffee berry disease, coffee wilt and leaf rust. In seedling hypocotyl test for CBD resistance, cultivar 7418, 74153, 74173, 7516, 75129 and 8136, showed significantly ($P < 0.05$) lower disease severity (< 20%) indicating higher CBD resistance than some of the released varieties 741 and 75227 used as checks. Cultivars 7514, 7576, 827 and 8211 were also moderately resistant to CBD infection of less than 28% which was lower than the resistant checks 74110 and 74112 (Table 1). Among the twenty three promising cultivars planted in the field and rigorously tested by attached berry test for two consecutive seasons, the severity of CBD infection was consistently lower in most of the cultivars namely, 7416, 7418, 7425, 7484, 74153, 7516, 7576 and 8136 with less than 5 to 10 mean percent infection (Table 1).

Table 1. Resistance levels of Arabica coffee cultivars to CBD in seedling hypocotyl tests in growth room at Jimma Research Center and attached berry test in the field at Gera, Ethiopia.

Coffee cultivars	[1]Seedling hypocotyl tests (actual value, %)	Transformed value	Attached berry test (actual value, %)	Transformed value
7412	38.6	38.3 o-u	11.7	15.3 d-g
7416	15.3	21.4 w-y	3.1	8.7e-h
7418	17.7	24.4 wx	0.5	3.3 h
7425	46.1	42.7 k-q	1.8	5.6 gh
7449	53.9	51.3 e-k	10.7	18.8 b-f
7480	51.7	46.4 g-o	8.5	16.1 d-g
7484	43.9	41.1 m-p	2.8	7.9 f-h
74123	50.2	45.1 i-p	7.8	14.8 d-h
74132	58.5	54.5 c-h	35.4	34.0 a
74146	56.4	51.8 d-k	15.5	22.2 a-d
74150	52.4	46.8 g-o	9.9	16.6 c-g
74153	17.4	22.4 w-y	6.4	13.8 d-h
74159	55.6	49.0 f-m	24.8	28.3 a-c
74160	49.7	44.8 j-p	15.0	20.5 b-e
74167	49.5	44.6 j-p	26.1	28.6 ab
74173	17.5	19.9 x-z	19.7	22.6 a-d
7514	26.8	29.7 u-w	7.9	11.9 d-h
7516	16.8	22.7 w-y	6.0	10.8 d-h
7576	27.9	31.0 s-w	1.1	4.9 gh
75129	22.0	27.1 v-x	7.1	14.8 d-h
75174	40.8	39.6 n-r	1.4	6.7 gh
8136	7.0	13.7 yz	3.4	10.2 e-h
8143	40.5	39.3 n-t	26.9	30.0 ab
827	27.7	31.5 r-w	nd [2]	nd
8211	27.9	31.1 s-w	nd	nd
F-35	43.0	40.9 m-q	nd	nd
741 [+]	19.6	25.8 v-x	nd	nd
7440 [+]	4.7	11.5 z	nd	nd
74110 [+]	59.8	50.9 e-k	2.6	7.1 f-h
74112 [+]	49.6	43.8 k-p	nd	nd
75227 [+]	25.8	30.4 t-w	3.6	9.8 e-h
370 [++]	66.0	58.8 b-e	nd	nd
Mean	46.1	43.2	10.5	15.3
LSD (P < 0.05)		9.2		11.9
CV		18.7		66.9

[1] Values are means of 2 years data. Means followed by the same letter(s) are not significantly different according to LSD values; the data were transformed to arcsine $\sqrt{x}$ before analysis.
[2] nd refers to no data, [+] Resistant standard varieties, [++] Susceptible checks.

There was positive correlation between seedling hypocotyl test and attached berry test values indicating that both techniques complement each other, also proving the cultivars possess consistent performance in the laboratory as well as field conditions. However, the disease severity was relatively higher in the seedling tests than it was observed in the fields and certain cultivars that showed exceptional susceptibility at seedling stage had high field

resistance, i.e., differential reactions. As indicated in Table 1, coffee cultivars like 7425, 7484, 74110 and 74112 manifested high CBD infection in the hypocotyl seedling test (susceptible at juvenile stage) while less than 5% berries of mature plant are attacked in the field. This result is in agreement with the comments made by Van der Graaff (1981) and Tefestewold (1995). The differential interactions may be attributed to variations in host defense mechanisms involved at different growth stages (hypocotyl of young seedlings *vis-à-vis* berries of adult plant).

In the second experiment, there were significantly ($P < 0.01$) low wilt severity (< 30%) recorded on seedlings of 1579, 20071 and 8136 coffee cultivars as compared to 7440 (resistant check). These cultivars exhibited infection symptoms within the longest incubation periods of 158, 153 and 150 days (Table 2), respectively, and the lowest wilt severity along with the longest incubation period implies high resistance levels to coffee wilt disease (CWD). Cultivars 214671, 8144, 7412, and 7484 showed moderate CWD infection. On the other hand, most cultivars such as Geisha, F-27, F-35, 7425, 74160, 8213, 4/70, 1185, 1785 were highly susceptible than the standard susceptible check *Cv.* SN-5 with actual seedling deaths of more than 75% (Table 2).

It was earlier reported that SN-5, 4/70, 146/71, 200/71, 201/71, 206/71 and 248/71 showed more than 85% tree losses, although death rate was the least (9%) on coffee trees of F-35 in the field (Girma et al., 2001). Also cultivar 8136 and 8144 showed significantly lower percentages of coffee tree loss (< 38%) in the field at Gera (Girma and Hindorf, 2001). The variations between coffee reactions to CWD in the fields might be ascribed to the genetic makeup, age of the cultivars, and inoculum potential of the fungus in the soil as well as cultural practices and environmental conditions prevailing at each specific locality (Girma et al., 2001).

In the third experiment, evaluating the same materials for coffee leaf rust reactions; 2179, 8136 and Catuai had almost no rusted leaves through out the study period. Seedlings of 1579, 74139, 3670, 7455 and 7516 showed significantly low rust severity ranging between 0.45 and 35% (Table 3). In the visual assessment under natural infection, high rust severity was recorded at Bebeka and Tepi than at Metu and some of the cultivars showed differences across the locations. As opposed to seedling tests (low to moderate rust infections), the highest disease severity was recorded on Catimor lines 2179 (47.6%) followed by 1579 (43.3%) at Bebeka, and there were also inconsistent reactions of the lines to the disease over years in the field under Bebeka and Tepi conditions (data not presented). Among the Catimor lines introduced to Ethiopia for having high resistance to major races of coffee leaf rust, two of them Catimor J-19 (1979) and Catimor J-21 (2179) including Geisha were best adapted to lowland areas like Bebeka and Tepi and recommended for commercial production (Bayetta *et al.* 1997) but their future dissemination should consider the possible outbreak of the major coffee diseases.

The discrepancy between the results of seedling tests and field assessments may partly be attributed to variability in rust races or appearance of more virulent races in the locality and partly to the hot humid environment that prevails in these areas that might have favored the disease epidemics. In this regard, more rust isolates must be screened on standard differential lines before using in artificial seedling tests. Further more, measuring resistance against leaf rust is time consuming and requiring high facility standards to get quantitative nature so that the screening technique, incubation conditions and scoring scales needs optimization.

Table 2. Resistance levels of coffee cultivars to coffee wilt disease at seedling stage in the glasshouse at Jimma Research Center, Ethiopia.

Coffee cultivars	Actual seedling death (%)	Transformed value	Incubation period (days)
1185	85.9	75.1 ab	90 op
1785	78.6	67.9 a-h	80 p
1579	12.7	16.9 s	157.5 a
2179	63.3	53.3 i-o	140.8 a-d
470	77.2	62.0 b-l	117.5 d-m
3670	60.9	56.0 f-n	92.5 n-p
14671	34.6	35.1 qr	122.5 d-k
20071	15.2	20.3s	152.5 ab
20671	52.8	46.1 m-q	125 d-j
8011	61.8	53.1 j-p	107.5 h-o
8133	64.2	54.1 g-n	122.5 d-k
8136	25.3	29.6 rs	150 a-c
8143	61.6	52.7 j-p	125 d-j
8144	40.2	39.1 o-r	137.5 a-e
8211	65.6	54.7 g-n	115 e-n
8213	88.7	73.1 a-d	110 g-o
8219	63.8	53.2 j-o	115 e-n
7412	43.9	42.7 n-r	140 a-d
7416	56.5	49.1 k-q	130 b-h
7418	54.8	47.5 l-q	100 k-p
7425	86.1	70.2 a-f	115 e-n
7449	61.9	52.9 j-p	125 d-j
7453	81.4	65.4 a-j	105 i-o
7455	73.9	60.1 c-m	122.5 d-k
7480	74.6	60.2 c-m	110 g-o
7484	48.2	43.5 n-r	135 a-f
74125	69.9	56.9 e-n	112.5 f-o
74150	66.0	57.1 e-n	122.5 d-k
74159	63.7	53.7 h-n	127.5 c-i
74160	90.9	76.3 ab	100 l-p
74167	77.4	62.6 b-k	112.5 f-o
7514	65.3	55.1 g-n	132.5 b-g
7516	63.9	53.6 h-n	105 i-o
7574	93.0	76.2 ab	117.5 d-m
7576	71.1	58.8 d-m	110 g-o
898	75.6	64.8 a-j	90 op
F-27	80.9	67.0 a-j	90 op
F-35	85.7	70.9 a-e	97.5 l-p
Caturra	68.9	59.2 d-m	130 b-h
Geisha	88.1	73.9 a-c	97.5 l-p
7440 $^{+}$	40.4	38.7 p-r	135 a-f
SN- 5 $^{++}$	69.7	56.7 e-n	119.17d-l
Mean	68.8	58.3	115.5
LSD ($P < 0.05$)		14.4	23.5
CV		21.8	17.9

Means followed by the same letter(s) are not significantly different from each other; the data were angularly transformed before analysis. $^{+}$ *Resistant cultivar;* $^{++}$ *susceptible check.*

Table 3. Resistance levels of coffee cultivars to leaf rust at seedling stage in the greenhouse at Jimma Research Center, Ethiopia.

Coffee cultivars	Actual rust infections (%)	Transformed value	Reaction types
8017	74.9	61.5 b-k	5.1
8019	82.4	66.9 a-h	5.0
8112	71.7	58.9 b-l	5.3
8133	74.9	64.9 a-h	5.4
8136	0.0	0.0 s	1.0
8211	65.1	55.7 c-o	4.9
8213	52.2	46.1 h-q	2.9
1185	83.7	68.7 a-g	5.2
1785	90.3	72.5 a-f	5.3
1579	20.5	24.8 q-r	1.0
2179	0.5	2.2 s	1.4
470	74.9	60.4 b-k	1.0
3670	31.7	31.1 p-r	2.6
20071	69.0	56.3 b-n	4.7
20671	55. 9	48.5 g-p	4.2
22171	42.4	40.7 k-q	4.4
7412	79.8	65.2 a-h	5.4
7416	73.3	59.0 b-l	5.1
7418	81.0	64.3 a-h	3.9
7425	61.8	52.5 e-o	4.9
7440	90.2	75.0 a-c	4.7
7449	70.4	57.3 b-m	4.6
7455	34.7	35.3 n-r	2.4
7480	46.1	55.5 c-o	5.5
74125	78.6	67.1 a-h	5.3
74139	8.2	16.0 rs	2.2
74159	40.3	38.0 l-q	3.6
74165	52.6	48.3 g-p	3.6
7514	78.8	63.6 b-I	5.3
7516	33.4	34.9 o-r	2.3
7574	67.9	56.1 b-o	5.2
75129	46.4	42.7 i-q	4.1
75227	57.7	49.7 g-p	4.5
Catuai	0	0.0 s	1.6
Caturra amarello	71.5	58.1 b-m	4.1
F-34	38.2	37.5 m-q	2.0
F-35	75.6	65.4 a-h	5.8
Geisha	71. 5	58.1 b-m	2.2
7454 [+]	88.8	71.6 a-f	7.2
Mean	63.0	53.9	
LSD ($p < 0.05$)		21.3	
CV		24.5	

Means followed by the same letter(s) are not significantly different from each other; the data were angularly transformed before analysis. [+] *Susceptible check.*

Even though the reactions of most coffee population to each disease varied, cultivars possessing adequate resistance to coffee berry disease, wilt and leaf rust were identified both in the artificial seedling tests that were proved under natural field conditions. Cultivar 8136 showed multiple type of resistances to CBD, CWD and CLR with 7, 25 and 0 percent infections, respectively. Similarly, cultivar 7418, 74153, 74173, 7516, 75129 were resistant to CBD with mean percent infection of less than 20 in the growth room and highly acceptable levels of infection (< 5%) in the field. Cultivar 7516 is also resistant to leaf rust. Coffee line 1579 is highly resistant to coffee wilt and leaf rust but very susceptible to CBD under both conditions. Thus, in localities like Bebeka and Tepi where CBD is not as such a limiting factor, wilt resistant materials with adequate tolerance to leaf rust could be effectively utilized. By virtue of its high level of resistances to the three major coffee diseases, 8136 (named as 'Merda-cheriko' after the late senior Plant Pathologist Merdassa Ejeta and the original place where it was collected Obbacherico, Gera) can be widely used in all areas where CBD, CWD and CLR are serious bottle-neck to coffee production. Individual cultivars possessing independent resistance response to each disease can also be considered in coffee breeding program. Meanwhile the host defence mechanisms and the genes that govern resistance to coffee wilt and leaf rust needs to be investigated.

ACKNOWLEDGEMENTS

The authors are very grateful to Technical Centre for Agricultural and Rural Development Cooperation ACP-EU (CTA) for sponsoring the participation of the senior author on this ASIC Conference held in (Campinas, Sao Paulo) Brazil. We appreciate the technical assistance of Pathology staff, namely Sisay T., Mamo A., Mesfin S. and Teshome T. Biyadige K. for actively conducting and successfully accomplishing the experiments.

REFERENCES

Adugna G. and Jefuka C. 2008. Resistance levels of Arabica coffee cultivars to coffee berry disease, coffee wilt and leaf rust diseases in Ethiopia. *In:* Proceedings of the 7th Crop Science Society of Ethiopia (CSSE), 22-24 May 2006, Addis Ababa, Ethiopia.

Adugna G., Hulluka M. and Hindorf H. 2001. Incidence of tracheomycosis *Gibberella xylarioides* (*Fusarium xylarioides*) on Arabica coffee in Ethiopia. J. of Pl. Dis. and Prot. 108: 136 – 142.

Adugna G. and Hindorf H. 2001. Recent investigation on coffee tracheomycosis, *Gibberella xylarioides* (*Fusarium xylarioides*) in Ethiopia. In: Proceedings of 19th International Scientific Conference on Coffee Science (ASIC). 14-18 May 2001. Trieste, Italy.

Adugna G. and Hulluka M. 2000. Cultural characteristics and pathogenicity of *Gibberella xylarioides* isolates on coffee. Pest Mgt. J. of Ethiopia 4: 11-18.

Arega Z., Demelash T., Chala J., Sisay T., Mesfin S. and Girma A. 2008. Success Stories in Managing Coffee Berry Disease in Ethiopia. Pp 239-249. In: Coffee Diversity and Knowledge; Girma A., Bayetta B., Tesfaye S., Endale T. and Taye K. (eds.). Proceedings of a National Workshop Four Decades of Coffee Research and Development in Ethiopia, 14 –17 August 2007, Addis Ababa, Ethiopia.

Bayetta B., Behailu A. and Gebramu T. 1997. Testing of F_5 lines of Catimor Hybrid Caturra vermehelo x Hybrido de Timor) for adaptation. In: Proceedings of the eight annual conference of Crop Science Society of Ethiopia (CSSE). Sebil, vol 8. 26-27 February 1997, Addis Ababa, Ethiopia.

CAB International (CABI). 2003. Surveys to assess the extent and impact of coffee wilt disease in East and Central Africa. Final Technical Report. CABI Regional Centre, Nairobi, Kenya. 149 pp.

Eshetu Derso, Girma Adugna and Teame G/Ezgi. 2000. Control of CBD by fungicides in Ethiopia. Pp. 35-46. In: Proceedings of Control of Coffee Berry Disease in Ethiopia, 13-15 August, 1999, Addis Ababa, Ethiopia.

Eskes, A.B. and Toma-Braghini, M. 1981. Assessment methods for resistance to coffee leaf rust *Hemileia vastatrix*, FAO Plant Prot. Bull., 29.

Kushalappa A.C. and Eskes A.B. 1989. Coffee rust: epidemiology, resistance and management, CRC Press.

Meseret Wondimu. 1991. Epidemiology and resistance of coffee leaf rust in Ethiopia. Ministry of Coffee and Tea Development. Addis Ababa, Ethiopia.

Owuar I.B.O. 1984. Cultivar for resistance to rust *Hemileia vastatrix* B.et. Br., in the breeding program for resistance to coffee berry disease in Kenya, in ComunicaÇÓes Simp. Fer. Caf., Out. 1983, CIFC, Portugal.

Pieters R. and Van der Graaff N.A. 1980. *Gibberella xylarioides* on Arabica coffee: evaluation of testing methods and evidence for the horizontal nature of resistance. Neth. J. Pl. Path. 86: 37-43.

Tefesetewold Biratu. 1995. Studies of *Colletotrichum* population on *Coffea arabica* L. in Ethiopia and evaluations of the reactions of coffee germplasm. PhD dissertation, University of Bonn.

Van der Graaff, N, A. 1981. Cultivar of Arabica coffee types resistant to coffee berry disease in Ethiopia. Mededelingen Land Bouwhgeschool. 81-11. 110 pp.

Van der Vossen, H.A.M. 1997. Quality aspects in Arabica coffee breeding programs in Africa. In: Proceedings of 17th ASIC conference, Nairobi, Kenya.

Variation in Resistance to Coffee Berry Disease (*Colletotrichum kahawae)* Among Germplasm Progenitors in Tanzania

D.J. MTENGA[1], S.O.W.M. REUBEN[2], V.M.P. VÁRZEA[3], P. PEREIRA[3], D.L. KILAMBO[1], J.M. TERI[1]

[1]Tanzania Coffee Research Institute (TaCRI), P. O Box 3004 Moshi, Tanzania
[2]Sokoine University of Agriculture (SUA), P.O.Box 3005 Morogoro, Tanzania
[3]Instituto de Investigação Científica Tropical. Centro de Investigação das Ferrugens do Cafeeiro (CIFC) Quinta do Marquês, 2784-505. Oeiras, Portugal

SUMMARY

The Arabica coffee improvement programme at the Tanzania Coffee Research Institute (TaCRI) has made a good progress in developing varieties with resistance to both Coffee Berry Disease (CBD) and Coffee Leaf Rust (CLR). However, some inconsistencies on the level of resistance to CBD in the progenitors used in development of the varieties was noted. This paper presents some pertinent findings obtained in the study carried out at the Tanzania Coffee Research Institute (TaCRI) and Coffee Leaf Rust Research Center (CIFC) to establish the levels of resistance within and among the progenitors in Tanzania. Two experimental trials were conducted at TaCRI from September 2006 to April 2007 and at CIFC from March to June 2007. Results showed there were considerable variations within and among varieties against most CBD isolates. Isolate Ca1 from Cameroon showed exceptionally high aggressiveness on all varieties. However, accessions VC 299 within Rume sudan and PNI 086 within Compacts varieties exhibited good resistance against this and other isolates. Variety Bourbon, a susceptible control, proved to be highly susceptible to all CBD isolates. There is a need therefore to select both within and among varieties when selecting parental materials for CBD resistance hybridization programmes.

INTRODUCTION

Coffee is one of the most important beverages in the world with a current estimated value of 10 billion US$ (Luiz et al., 2005). Coffee is also an extremely important source of national export revenue and internal cash income for farmers in many poor countries from Africa, Asia and Latin America (ICO, 2005). The two most economically important species of the genus *Coffea* are *Coffea arabica* L. and *Coffea canephora* Pierre ex Froehner. Coffee is Tanzania's largest export crop. It contributes approximately $115 million to export earning, and provides employment to some 400,000 families. About 95 percent of coffee is grown by smallholders on average holdings of 1-2 hectares, and 5 percent is grown on estates. Tanzania produces about eight hundred thousand 60-kilogram bags, or 0.7 percent of world output of 117 million bags. About two-thirds is mild arabica, and the rest is hard arabica and robusta (Baffes, 2003). Numerous production constraints have been mentioned. However, the most serious one is diseases, mainly coffee berry disease (CBD) (*Colletotrichum kahawae*, Waller and Bridge) and coffee leaf rust (CLR) *(Hemileia vastatrix*, Berk et Br). Coffee Berry Disease is an anthracnose of the green and ripening berries. Attacked berries at the green stage develop dark, brown sunken lesions while attack on ripening berries reduces quality and marketable produce (Wrigley, 1988). Together, the two diseases viz CBD and CLR control accounts for 40% of total production cost for small scale coffee producers in the country. The two diseases can effectively be controlled by intensive protective fungicide application whose costs are

prohibitive and beyond the capability of small holders. The most desirable option therefore is host resistance which offers an added advantage of being environmentally – friendly (Teri et al., 2004).

The Arabica coffee improvement programme in Tanzania has achieved a considerable success in developing CBD and CLR resistant varieties. However some inconsistencies on the levels of resistance have been observed in some of the progenitors used in the development of the varieties (Walyaro, 2004). Determination of levels of resistance in the progenitors of resistance will lead to identification of superior ones with consistent resistance, which may be used for development of improved varieties with consistent, stable and durable resistance to these diseases. The objective of the current study was therefore aimed at establishing the resistance levels within and among the main progenitors used in Tanzania against CBD.

Van der Vossen (2006) revealed that host resistance to CBD appears to be of a durable nature. This argument is based on the fact that for more than twenty years of release of resistant cultivars like Ruiru II (Kenya) and Ababuna (Ethiopia); no confirmed report of break down of host resistance under field condition had been reported. Resistance to CBD is controlled by three genes which are present in the varieties Rume Sudan (R and k), Hybrid de Timor (T) (Van der Vossen et al., 1980) while in Catimor it is controlled by a major resistance gene Ck-1 (Gichuru et al., 2006).

Conventional coffee breeding through hybridization and selection of superior progenies has achieved some success in meeting the coffee industry's needs of developing improved disease resistant varieties. Detailed screening of germplasm for variation in resistance to Coffee Berry Disease will generate information on genetic diversity present in the materials studied.

MATERIALS AND METHODS

The materials which were used in this work are three coffee varieties from Tanzania Coffee Research Institute germplasm which are the main progenitors for CBD and CLR resistance. In each variety, five accessions were evaluated as shown in Table1 below.

Table 1. Varieties and accessions studied.

Variety	Accessions
Rume sudan	VC 298, VC 299, VC 506, VC509, and VC510
Hybrid de Timor	RRC70, RRC72, VCE 1594, VCE 1587and VCE1589
Catimor/Compacts	PNI 088, PNI 086, PNI 087, CR 124 and CR127
Bourbon	N5, N39, N100, N197 and N218

Note: Bourbon is a commercial (susceptible) variety, used as a check.

SEED PREPARATION

Fully matured, red-ripe coffee berries were hand picked from trees in centre rows of each accession involved from the germplasm plot. These were pulped using a small hand pulper in separate lots for each accession. The pulper hopper was washed thoroughly using clean tap water under high pressure to ensure all the cherries from previous lot are cleaned out of the pulper before pulping the next lot. This procedure was followed for all lots. Pulped cherries from each accession were put in separate containers, placed under room temperature for 72 hours for fermentation. After 72 hours of fermentation, the cherries were washed and dried

under shed separately in partitioned wire-mesh trays for one week before they were pre-germinated to raise hypocotyls.

SOURCE OF INOCULUM

According to Cook (1973b) green infected berries with active lesions are the best source of inoculum for initial isolation because of low contamination with other non pathogenic *Colletotrichum* spp. and optimum pathogenicity of the isolates. Initial isolation of the CBD pathogen was achieved by incubating green infected berries from unsprayed susceptible variety Bourbon (N39) at 20-24 °C on moist sterilized sand in closed but somewhat ventilated plastic boxes for 10 days. This was followed by planting the conidia on 3.4% malt extract agar containing 0.04% streptomycin. A spore suspension containing 2 x 10^6 spores/ml was prepared from these pure cultures. The suspension was tested and proved spore viability in excess of 80%.

PRE-SELECTION FOR CBD RESISTANCE AT TACRI

One hundred seeds of each variety were sown out, with the parchment removed, in moist sterilized media of sand and top forest soil placed in plastic boxes with closely fitting transparent lids, keeping the boxes at normal room temperatures (20-24 °C). The seedlings were ready for inoculation when they had hypocotyl stem of 3-5 cm long, usually within 5-6 weeks after sowing out the seeds. At this stage the cotyledons are still enclosed in the testae. Just before inoculation the lid was removed from the plastic box, the seedlings sprayed with the standard CBD inoculum (2 x 10^6 spores/ml) by means of a small atomizer and the box was immediately closed again. A repeat inoculation was applied after 48 hours. The temperature of 22-24 °C was maintained for four days for successful infection, at the same time relative humidity in the boxes was maintained at 100%. This was followed by incubation period at a lower temperature of (19-20 °C) with the lids removed from the boxes to allow for normal humidity. The first symptoms became noticeable within one week after the first inoculation, but full expression of the disease susceptibility was attained at two weeks time. At the end of the incubation period seedlings were individually scored for disease symptoms developed on the hypocotyl stem using a scale with range 1- 12 (Van der Vossen et al., 1976).

COFFEE BERRY DISEASE SCREENING AT CIFC, PORTUGAL

Seed from the same coffee accessions used in Tanzania was used for this evaluation. Coffee Berry Disease isolates from Tanzania, Kenya, Zimbabwe and Cameroon were used. These isolates were maintained in malt extract agar (MEA) and preserved for long periods in agar slants. The pathogenicity of these isolates was maintained by periodic inoculation on green coffee berries and re-isolation. Pathogenicity tests were carried out by inoculation of hypocotyls according to the methodology of Van der Vossen et al. (1976), with slight modification. Six week old hypocotyls were removed from the germination pots, washed in running tap water and placed on sterilized wet nylon sponges contained in plastic trays. These trays were covered in sterilized polythene bags to preserve and maintain the humidity to almost 100%. Inoculation was done by carefully and quickly taking the trays with hypocotyls from the polythene bags and spraying them with spore suspension at a concentration of 2 x 10^6 conidia/ml. This was achieved by using an atomizer connected to a small constant pressure electric pump. The same procedure was followed for the four isolates of Tanzania, Cameroon, Zimbabwe and Kenya. One hundred hypocotyls from each accession were inoculated with each of the four isolates. The trays were put back to the polythene bags and these bags were tied up at the open end using cotton strings and taken to a chamber with temperature maintained at 22 °C for 4 days. Re inoculation was done after 48 hours using the

same procedure to ensure no escapes. After four days the trays with hypocotyls were shifted to a chamber with temperature maintained at 19 °C for the rest of the time. Duration of the experiment was 21 days after first inoculation. The disease reaction was scored as for the experiment at TaCRI where; 1 implies no visible symptoms while 2-4 indicating superficial scab or brown lesions progressively enlarging, from score 2 to 4; score 5 implies presence of small but deeper and black lesion; enlarging, coalescing, increasing in number from score 6 up to10. At scale number 10, the stem is completely giggled and the seedling is dying while score 11 and 12 indicate large part of the stem is black and shriveled completely and the seedling completely dead. The rating of the scale is as presented below:

1-4 High level of resistance.
5-6 Moderately resistant. Seedling will survive, and over come the infection.
7-9 Susceptible. In most cases, seedlings will succumb to disease.
10-12 Highly susceptible. Dead hypocotyls.

CALCULATION OF CBD SCORE MEANS

A mean grade of infection (G) was calculated for accessions in each replication as follows:

$$G = 1/N \sum_{i=1}^{4} in_i$$

Where, i is the disease class, n_i is the number of seedlings in class i and N is the total number of seedlings scored (Omondi et al., 2001).

RESULTS AND DISCUSSION

In order to increase efficiency, reduce time and cost in breeding programmes, breeders have developed screening methods in which plants as young as possible are exposed to high concentrations of specific inoculums in order to identify resistant plants or lines in segregating populations (Van der Vossen et al., 1976 and Ribeiro do Vale et al., 2001). Coffee breeding programmes are limited in progress due to the narrow genetic base of cultivated varieties especially for pest and disease resistance (Van der Vossen, 1985). However, nowadays, natural and artificial hybrids derived from *Coffea arabica x Coffea canephora* are intensively used as source of resistance to coffee berry disease caused by *Colletotricum kahawae* (Lashermes et al., 1996b). A natural hybrid, hybrid de Timor (Timor hybrid) and the artificial hybrid Catimor (Compacts) and other derivatives such as Sarchmor are some of the most widely utilized materials both as released varieties and breeding lines especially in central America (Silveira et al., 2003).

Results presented in the current investigation (Figures 1-5) are according to the format suggested by Dancer (1986). The varieties Rume sudan, Hybrid de Timor and Catimor (Compacts) showed very good resistance to coffee berry disease except against isolate Cal at CIFC Portugal. The four isolates viz. T3, Z9, Q2 and local isolate gave consistent results except Cal isolate which showed exceptionally high aggressiveness to all varieties. The unique characteristic of this isolate was observed else where (Várzea et al., 1999). However, despite the general aggressiveness of isolate Cal on all varieties, there were unique accessions within varieties that showed good levels of resistance; these include accessions within varieties that showed good levels of resistance; these include accession VC 299 within Rume sudan and accession PN1086 within Compacts variety. Variation in resistance to CBD within varieties in Rume sudan and Compacts (Catimor) have been reported somewhere else when

working with large populations of these varieties from diverse geographic origins (Agwanda et al., 1997). The unique resistance observed only on accessions VC 299 (Rume sudan) and PNI 086 (Compacts) against Cal isolate could be that for this particular isolate, there are no gene sequences in the other accessions that give gene products to confer resistance while these sequences are available in accessions VC 299 and PNI 086 in Rume sudan and Compacts respectively. Thus accessions within these varieties are not completely identical in their genetic make up indicating that some segregation is still available. This gives an opportunity for selection among accessions within varieties.

Considerable variation on resistance to CBD was observed among varieties against isolates T3, Z9, Q2 and local. However, there was no considerable variation against isolate Cal. There was noticeable variation within varieties against all isolates in varieties Hybrid de Timor and Compacts. Control variety Bourbon was uniformly highly susceptible to all isolates and hence it can be relied upon as a susceptible control. The varieties Rume sudan, a semi wild Arabica coffee variety, Hybrid de Timor a spontaneous hybrid between C. arabica x C. canephora; and Catimor (Compacts) a hybrid derived from Hybrid de Timor x Catuai are reputed for their resistance to coffee berry disease. The resistance in Rume sudan is controlled by two major genes R and k while in Hybrid de Timor is controlled by a single major gene T (Van der Vossen and Walyaro, 1980; Agwanda et al., 1997 and Omondi et al., 2001). Resistance in Catimor (Compacts) is controlled by one easily transferable Ck-1 identified recently (Gichuru et al., 2006). Variation in resistance to CBD within varieties can be explained based on two possible reasons. The first possibility may be due to possible natural out crossing with susceptible varieties in neighboring plots since even though *Coffea arabica* is self fertile specie; there is chance outcross of up to 10% (Van der Vossen, 1985). The second possibility may be due to segregation especially in hybrid varieties Hybrid de Timor and Compact (Catimor) but less so in pure lines Bourbon and Rume sudan. Thus, there is scope for selection and genetic advancement within the varieties for resistance against the CBD isolates.

The coffee berry disease isolates T3, Cal, Z9 and Q2 tested at CIFC Portugal are quite different from the local one tested at TaCRI Tanzania. However, isolate T3 is believed to have originated from Tanzania. Cal originates from Cameroon, Z9 from Zimbabwe and Q2 from Kenya. Other workers have found out that aggressiveness of the CBD isolate population shows variability between isolates from the same or different geographic origins (Van der Vossen, 1985; Omondi et al., 2001). Studies by Bieysse et al., (Unpublished data INCO-project ICA4-CT-2001-10008) on isolates from Cameroon, Kenya, Burundi, Tanzania, Rwanda, Malawi, Angola and Ethiopia concluded that the strains might be classified into 2 groups viz. East Africa and Cameroon, each geographical population showing a strong homogeneity between the isolates (Silva et al., 2006). These isolates plus several others are preserved at CIFC Portugal for world wide use in research for resistance to coffee berry disease (CBD) because the country does not produce coffee (Silva et al., 2006). General level of resistance to CBD in the evaluated progenitors Rume sudan, Hybrid de Timor and Compacts against isolates T3, Z9, Q2 at CIFC Portugal and the local one at TaCRI proved to have good resistance against the current CBD isolates in Tanzania (see also Teri et al., 2004). However, there is a possible threat from the CBD isolate Cal from Cameroon against which all the progenitors fall susceptible.

When considering selecting parents for particular crossing in developing new improved hybrids for CBD resistance, selection within varieties is important especially in Hybrid de Timor and Compacts progenitors. More sources of resistance to CBD should be prospected to improve the existing germplasm. Resistance gene deployment and management in different coffee growing zones can be tried out in Tanzania using promising accessions identified in

this study viz. VCE1589, PN1086; and also with exception of CBD isolate Cal, we have VC298, VC299, VC506, VC509 and VC510. The breeding programmes should also consider cup quality, yield and resistance to coffee leaf rust for incorporation in the same background if we are to have meaningful varieties released to farmers.

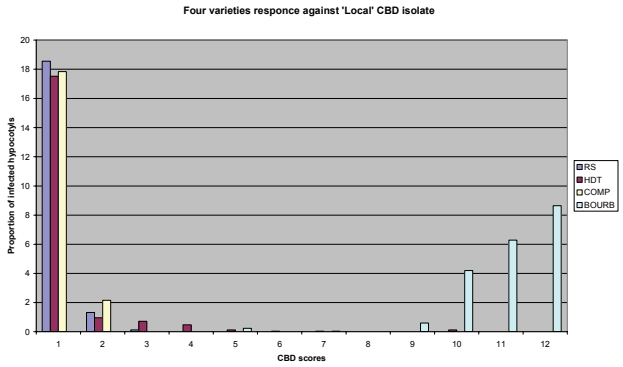


Figure 1. Coffee berry disease isolate "Local" at TaCRI Tanzania.

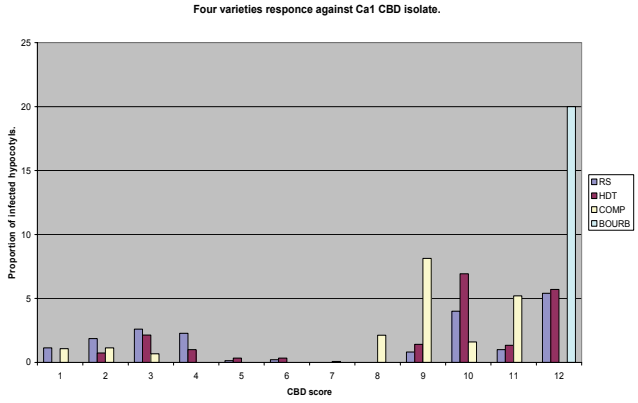


Figure 2. Coffee berry disease isolate Ca1 at CIFC Portugal.

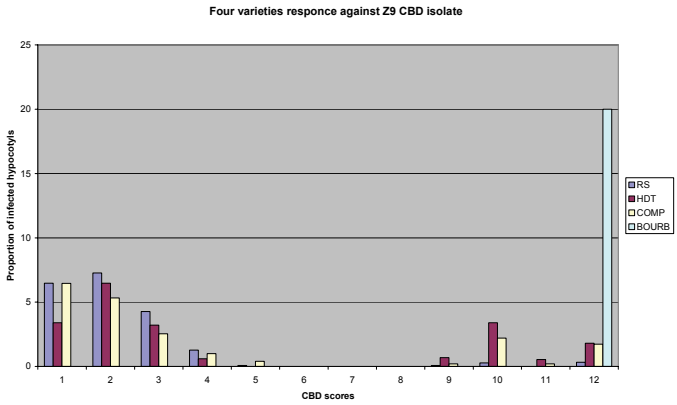


Figure 3. Coffee berry disease isolate Z9 at CIFC Portugal.

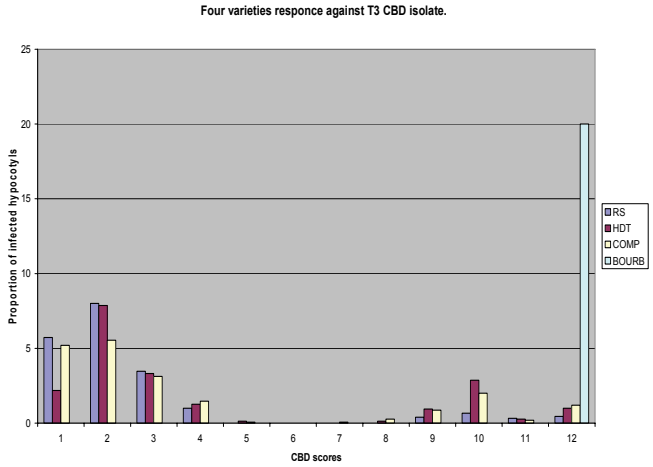


Figure 4. Coffee berry disease isolate T3 at CIFC Portugal.

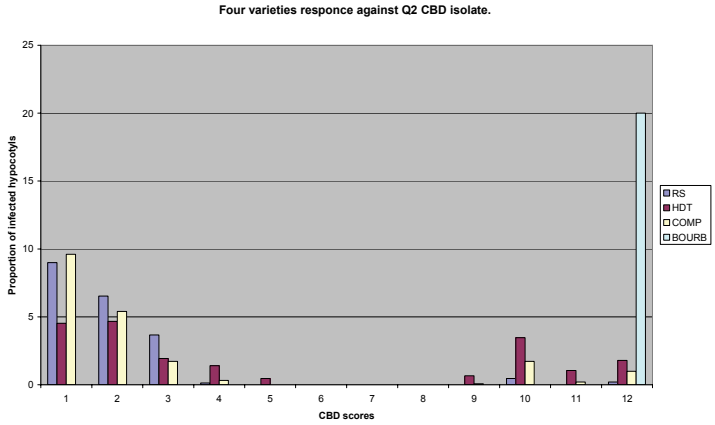


Figure 5. Coffee berry disease isolate Q2 at CIFC Portugal.

ACKNOWLEDGEMENT

We would like to record our sincere appreciation to the management of TaCRI, SUA and CIFC for their contribution to this work. We are also thankful to the European Union for their financial support.

REFERENCES

Agwanda C.O., Lashermes P., Trouslot P., Combes M.C and Charrier A. (1997). Identification of RAPD markers for resistance to coffee berry disease, *Colletotrichum kahawae* in arabica coffee. Euphytica 97: 241-248.

Baffes J. (2003). Tanzania's coffee sector: Constraints and challenges in global environment. African Region Working Paper Series No. 56, World Bank.

Cook R.T.A. (1973b). Screening coffee plants for CBD resistance. 1972/73 Kenya Coffee Research Foundation Annual report, Ruiru, Kenya. pp.66- 68.

Dancer J. (1986). The evaluation of arabica coffee cultivars for resistance to coffee berry disease: Some comments. Euphytica 35(1):125-128.

Gichuru E.K., Combes M.C., Mutitu E.W., Ngugi E.E.K., Bertrand B., Lashermes P. (2006). Characterisation and genetic mapping of a gene conferring resistance to coffee berry disease (Colletotrichum kahawae) in Arabica coffee (Coffea Arabica L). In: Proceedings of the 21st International Conference on Coffee Science (ASCI), 11-15th September, Montpellier, France, CD-ROM.

ICO (2005). Action to avoid further coffee price crises. ICO submission to the G-8 Summit, 6-8 July Gleneagles, Scotland.

Lashermes P., Trouslot P., Anthon F., Combes M.C. and Charrier A. (1996b). Genetic diversity for RAPD markers between cultivated and wild accessions of *Coffea arabica.* Euphytica 87:59-64.

Luiz C.F., Oliveira A.C.B., Masako T.B., Silvarolla M.B. (2005). Identification and uses of sources of durable resistance to CLR at the IAC. In: Durable resistance to coffee leaf rust. Edited by (Zambolim L., Zambolim E.M. and Varzea V.M.P.). Federal University of Vicosa, Vicosa, Brazil, pp. 137-185.

Omondi C.O., Ayiecho P.O., Mwangombe A.W. and Hindorf H. (2001). Resistance of *Coffea arabica* cv. Ruiru 11 tested with different isolates of *Colletotrichum kahawae*, the causal agent of coffee berry disease. Euphytica 121:19-24**.**

Ribeiro do Vale F.X., Parlevliet J.E. and Zambolin L. (2001). Concepts in plant disease resistance. Fitopatologia Brasileira (Brazillian Journal of Phytopathology). 26 (3): 577-589.

Silva M.C., Varzea V.M.P., Guerra-Guimaraes L., Azinheira H.G., Fernandes D., Petitot A.S., Bertrand B., Lashermes P. and Nicole M. (2006). Coffee resistance to the main diseases: leaf rust and coffee berry diseases. Brazillian Journal of Plant Physiology, 18 (1): 119-147.

Silveira S.R., Ruas P.M., Ruas C.F., Sera T., Carvalho V.P. and Coelho A.S.G. (2003). Assessment of genetic variability within and among coffee progenies and cultivars using RAPD markers. Brazillian Journal of Genetics and Molecular Biology. 26 (3): 329-336.

Van der Vossen H.A.M. (1985). Coffee selection and breeding. In: Coffee: Botany, Biochemistry and Production of Beans and Beverage. Edited by (Clifford M.N and Wilson K.C.) Croom Helm, London and Sydney, pp. 48-96.

Van der Vossen H.A.M. (2006). State-of-the-Art of Developing Arabica Coffee Cultivars with Durable Resistance to Coffee Berry Disease (*Colletotrichum kahawae*). In: Proceedings of the 21st International Conference on Coffee Science. (ASIC), 11-15 Sept. 2006*,* Montpellier. France. pp. 794-801.

Van der Vossen H.A.M., Cook R.T. and Murakaru G.N.W. (1976). Breeding for resistance to coffee berry disease caused by *Colletotrichum coffeanum* Noack (sensu Hindorf) in *Coffea arabica*: Methods of preselection for resistance. Euphytica 25:733-745.

Van der Vossen H.A.M. and Walyaro D.J. (1980). Breeding for resistance to coffee berry disease in *Coffea arabica*: Inheritance of resistance. Euphytica 29:777-791.

Walyaro D.J. (2004). Consultant report on coffee improvement programme at TaCRI. First mission 8-30 June EU-EDF contract, SER/STAB/FMO/TaCRI, Moshi Tanzania, 34pp.

Wrigley G. (1988). Coffee. Tropical Agriculture series. Longman Scientific and Technical Harlow, UK. 639pp.

Teri J.M., Kilambo D.L., Mtenga D.J., Nyange N.E., Nzallawahe T.S., Chipungahelo G.S., Kipokola T.P. and Kullaya I.K. (2004). Improved arabica varieties for the benefit of

Tanzania coffee producers. In: Proceedings of the 20th International conference on coffee science. (ASIC), 11-15 October 2004, Bangalore, India. pp. 1187-1191.

Várzea V.M.P., Rodrigues Jr. C.J., Silva M.C., Pedro J.P., Marques D.M. (1999). High virulence of Colletotrichum kahawae isolate from Cameroon as compared with other isolates from other regions. In: Proceedings of the 18th International Conference on Coffee Science (ASIC) Helsinki, Finland, Abstract, 131.

Towards a Variety Resistant to Coffee Wilt Disease (CWD): a Case for Robusta Coffee (*Coffea canephora*) in Uganda

P.C. MUSOLI[1], A. KANGIRE[1], T. LEROY[2], A. NABAGGALA[1], S. NAKENDO[1], S. OLAL[1], J. OCHUGO[1], C. KABOLE[1], J. PANDE[1], C. CILAS[3], A. CHARRIER[4], D. BIEYSSE[5], J.A. OGWANG[1] AND D.T. KYETERE[1]

[1]Coffee Research Centre, P.O. Box 185, Mukono
[2]Cirad, UMR PIA, Montpellier, F-34398 France
[3]Cirad-CP, UPR Bioagresseurs de pérennes, Avenue Agropolis, TA80/02 34398 Montpellier Cedex 5, France
[4] ENSAM, Place Viala, Montpellier, F-34000 France
[5]Cirad UMR BGPI, Campus International Baillarguet, TA41/K, 34398 Montpellier Cedex 5, France

SUMMARY

Coffee wilt disease (CWD), which is caused by *Fusarium xylaroides* Steyaert, the conidial stage of *Gibberella xylarioides* Hem. & Saccas, is the most serious problem of Robusta coffee (*Coffea canephora*) production in Uganda, the Democratic Republic of Congo (DRC) and Tanzania. It is also affects Arabica coffee (*Coffea arabica*) production in Ethiopia. CWD spreads quickly and widely, thus it is a threat to coffee production in all continents. It can be controlled effectively by planting resistant varieties. In Uganda CWD was first observed on *C. canephora* in 1993, in areas bordering the DRC and by 2002 it had spread to many parts of the country, where it had destroyed 44.5% of the crop (Oduor, 2005). This reduced Uganda's coffee exports by nearly 50% and led to economic loss of US dollars 80-270 million annually between 1996 and 2007. Consequently, a search for CWD resistant *C. canephora* varieties was initiated at the Coffee Research Centre (COREC) in 1997. This involved screening *C. canephora germplasm* in naturally infected fields and in artificial inoculations carried out in the screen house for resistance. The *C. canephora* genotypes both in field and screen house studies, responded quantitatively to the disease, implying that CWD resistance in *C. canephora* is controlled by many genes. 1519 completely resistant genotypes were identified through large scale germplasm screening in artificial inoculations at COREC and these were planted in mother gardens and thereafter they were cloned and planted in field evaluation trials. 167 of the clones underwent preliminary evaluation and four (4) were selected for being resistant to leaf rust and red blister disease and having good cup and bean qualities. The four selected clones were planted in on-farm multi location evaluation trials, en route to being recommended for commercial cultivation. Similarly, four (4) CWD resistant clones were selected from among many genotypes screened in naturally infected fields at COREC and were also planted on-farm multi location trials, en route for recommendation for farmer cultivation.

INTRODUCTION

Coffee wilt disease (CWD), which is caused by *Fusarium xylaroides* Steyaert, the conidial stage of *Gibberella xylarioides* Hem. & Saccas, was first reported in 1927 on *C. liberica* spp. in the Central African Republic (CAR) (Figueres, 1940). The disease subsequently destroyed this crop during 1930s to 1950s within CAR and Cameroon (Mulller, 1997). During the same period, it destroyed *C. canephora* in Ivory Coast (Delassus, 1954). Fraselle (1950) reported

observation of CWD on *C. canephora* at Yangambi in the DRC in 1948, which later became a serious problem in many parts of the country. Lejeune (1958) reported similar disease symptoms on Arabica coffee (*C. arabica)* in Ethiopia and later Kranz and Mogk (1973) confirmed that the disease on *C. arabica* was also caused by *F. xylarioides*. Later studies revealed that *F. xylariodes* strains causing CWD on *C. canephora* and *C. arabica* are different. By 1970's, the CWD had been effectively controlled in the Central and West African countries, by combined use of resistant varieties and cultural practices (Muller, 1997). However, in the 1980s, new outbreaks were reported on *C. canephora* in the DRC (Flood and Brayford, 1997), from where it spread to Uganda in 1993 and subsequently to Tanzania in 1997, affecting the same species.

In Uganda coffee is the most important cash crop and a major source of foreign currency into the country. Over 8 million Ugandans derive their livelihood directly from coffee (Uganda Bureau of Statistics-UBS, 2007). The crop contributes 18-21% of Uganda's foreign currency earnings and about 64% of the earnings from traditional export crops alone (Mugambe, 2007). Coffee has remained the lead foreign currency earner despite government's efforts to diversify to other non-traditional exports (Masiga and Ruhweza, 2007). *C. canephora* contributes 70-90% of the coffee production in Uganda and *C. arabica* contributes 10-30%. The recent devastation of *C. canephora* by CWD has significantly dented Uganda's coffee exports and thus the national economy and social welfare of people involved in its supply chain has been disrupted. Surveys conducted in 2002 found the disease in all the *C. canephora* growing areas of Uganda and on over 90% of the farms, where it had destroyed over 44% of the crop nationwide (Oduor et al., 2005). The overall effect of CWD was a significant reduction in production and export volumes, from 4.2 million 60 Kg bags of green beans exported in 1996/97 to 2.0 million bags in 2005/06, which translated into annual monitory loss of US $80-270 million (Uganda Coffee Trade Federation-UCTF, 2007). In 1997 scientists at COREC initiated a breeding programme to develop CWD resistant varieties of acceptable farm and market traits. The objective of the programme was to develop *C. canephora* varieties, which are resistant to CWD and other major coffee diseases; are high yielding and possess good bean and cup qualities.

MATERIALS AND METHODS

C. canephora germplasm was screened for CWD resistance at Kituza (Headquarters of COREC) in Mukono district, which is one of the locations where CWD was first observed in Uganda. The screening was carried out in naturally infected fields and in artificial inoculations in a screen house; followed by planting resistant genotypes in mother gardens and cloning and re-evaluating the resistant clones in the field trials.

Evaluation of *C. canephora* germplasm for CWD resistance in naturally infected field

20 *C. canephora* clones raised from rooted nodal stem cuttings and planted in the field in 1997 were carefully evaluated (figure 1). The clones included four commercial varieties (1s/2, 1s/3, 223/32 and 257s/53) and 16 hybrids selected for high yielding, good bean quality and resistance to leaf rust and red blister disease, from among various progenies of specific crosses involving various parental whose CWD resistance was not known by then. The 20 clones were planted in a randomized complete block design trial with four replications and each replicate consisting of 20 plots, each plot constituted by six coffee trees arranged in continuous patterns of 3 rows by 2 columns, spaced at 3 x 3 m. Clones Q/6/1 and Q/1/1 were only planted in replicates 1 & 2, due to insufficient planting materials. Clones H/4/1 and R/14 were only planted in replicates 1, 2 and 3, also due to insufficient planting materials. The field was maintained following routine procedure of maintaining *C. canephora* gardens.

Although CWD started affecting coffee trees in this trial in 1999, systematic assessment started in April 2001. Prior to April 2001 infected trees were uprooted and burnt on observation of first CWD symptoms as a way trying to minimizing and / or eradicate the disease from the Centre. During systematic assessment, each tree was scored for CWD severity by assessing percentage defoliation caused by the disease. The scoring was done twice in a month using a symptom severity scale of 1 to 5, where 1 = no disease, 2 = 1-25% defoliation, 3 = 26-50% defoliation, 4 = 51-75% defoliation, 5 = 76-100% defoliation. Trees in level 5 were considered dead. Percent mortality of trees was computed for each clone. Disease progress curves of each of the clones were plotted from the percentage mortality data and the genotypes were arbitrary classified into resistance classes using data of the last scoring (31 March 2006). Genotypes in resistant and moderately classes were cloned and planted in multi-location on-farm trials.

Screening germplasm through artificial inoculation

This involved inoculation of tens of thousands of open pollinated seedlings and rooted nodal cuttings of various parent genotypes obtained from germplasm plots at COREC, survivors in heavily devastated farms and wild and feral population. The plant materials were raised in the coffee nursery at Kituza following routine procedures (MAAIF) and were inoculated with a suspension of *F. xylarioides* conidia in water at concentrations of $1.3x10^6$ using root dip method (Musoli et al., 2001), when at 6-9 months old. Inoculated plants were incubated in the screen house at room temperature and watered regularly as they were monitored for CWD symptoms for at least 8-12 months. Plants that survived this long were re-inoculated using the same procedure. The re-inoculated plants were also watered and monitored for mortality for at least 8 months. All plants that survived in the second inoculation after this long, were considered resistant and were planted in mother gardens for vegetative multiplication, en route to planting in field trials for further evaluations.

Evaluation of CWD resistant clones in field trials

All CWD resistant genotypes identified in field and artificial studies were planted in mother gardens, as they became available. All the genotypes in the mother gardens were cloned and planted in on-station (Kituza) field trials. These trials were established in gardens heavily infected with CWD. The gardens were previously planted with other coffee lines, which were continuously destroyed by CWD, therefore assumed to have high concentrations of *F. xylarioides* inoculum. Each of the clones was planted in one plot constituted of a single row of 6 trees spaced at 3 m by 3 m. Besides CWD incidence, these clones were assessed for yield, incidence and severity of coffee leaf rust and red blister disease. Leaf rust and red blister diseases were scored on a scale of 1-5, where 1 = no disease and 5 is when most of the plant organs (leaf for leaf rust and fruit for red blister disease) were destroyed. Dry samples of coffee beans at 11-13% moisture content, were analyzed at the Uganda Coffee Development Authority laboratories for physical and cup qualities. Cup quality was analyzed using sensory taste by a panel of experts.

RESULTS

Evaluating *C. canephora* clones in a naturally infected field

Curves showing mortality of trees or the 20 clones evaluated for resistance against CWD in a naturally infected field are given in Figure 1. It is notable that tree mortality among the clones was at different levels when the evaluation started in April 2001 and it progressed at varying rates to varying levels by March 2006. Clone J/1/1 did not succumb to CWD throughout the assessment period. Mortality increased rapidly among clones P/3/6, H/4/1, E/3/2, C/1/7 and

B/6/2 and by March 2006 and the final mortality of these clones ranged from 87.5% for clone E/3/2 to 95.8% for C/1/7. Mortality increased very slowly on clones Q/3/4, R/1/4 and 1s/3 and by March 2006 their mortality ranged from 4.2 for clone Q/3/4 to 33.3 for clones 1s/3 and R/1/4. Clone 1s/2 appeared to be resistant until April 2002, when its trees started dying in high numbers. By March 2006, 1s/2 was among clones with very high.

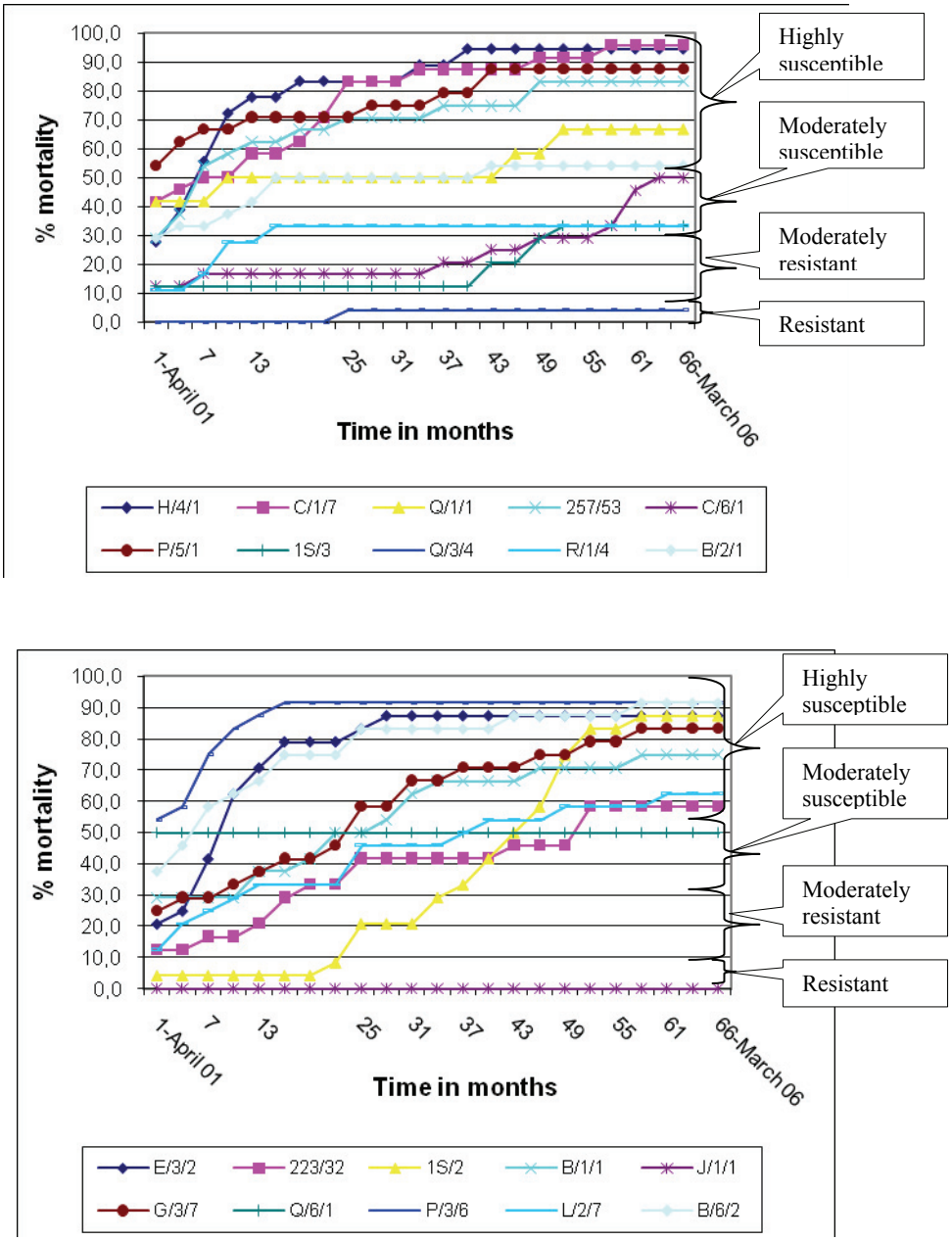


Figure 1. Progression of tree mortality of 20 *C. canephora* clones evaluated for CWD resistance in a naturally infected field.

Screening *C. canephora* germplasm in artificial inoculation

Because tens of thousand of plants were inoculated, it is impossible to present results of all the genotypes tested. Figure 2 gives comparative responses of wild, cultivated and feral populations. There were variable responses to CWD infection among and between *C. canephora* populations studied in artificial inoculations. Figure 3 presents response of 24 open pollinated seedlings inoculated in the screen house. These results are typical to what was

observed in other inoculations. Survivors of the 24 progenies are among the many that were planted in the mother gardens at Kituza for cloning en route to field evaluation.

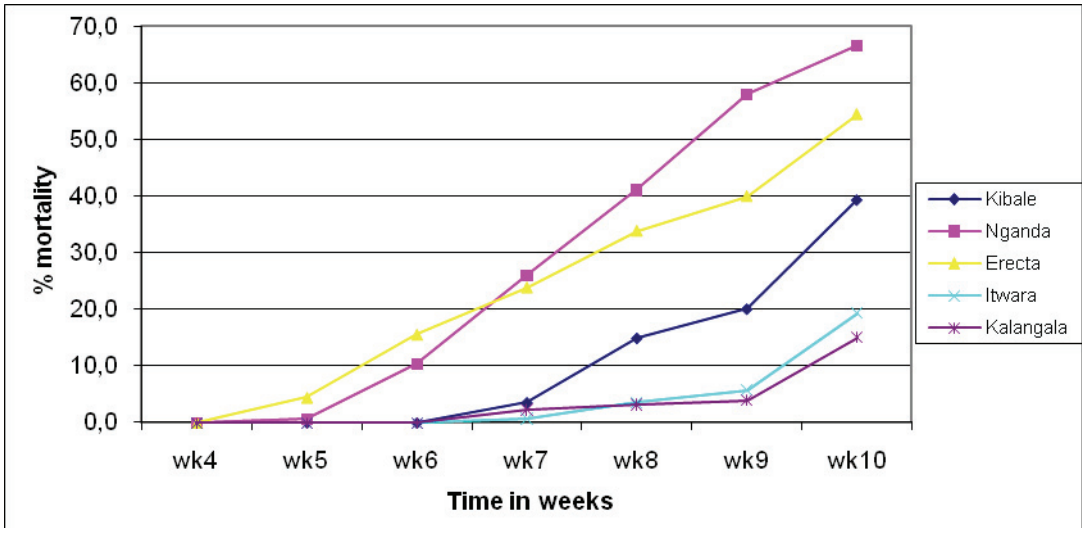


Figure 2. Response of open pollinated progenies of different *C. canephora* populations to CWD artificial inoculation with CWD pathogen.

Field evaluation of CWD resistant clones

None of the CWD resistant clones identified through artificial inoculations succumbed to CWD in the field, implying that the screening method was effective and thus the clones are effectively resistant to the disease. At the time of this report, only 167 clones had been evaluated for visual impression, quality and resistance to leaf rust and red blister diseases. Results of these assessment revealed that clones vary in their phenotypic impressions. Clone responses to leaf rust and red blister disease and their quality, is illustrated by 23 clones in Table 1. These results show that cup and bean qualities vary among the clones. Response to leaf rust and red blister diseases also varied. On the basis of the visual impression, cup and bean qualities and response to leaf rust and red blister diseases, 22 clones were selected from the on-station field trials and these together with 4 elite clones identified from the field trial, were planted in on-farm trials. Four (4) out of the 22 elite clones identified from on-station field trials plus the four (4) identified in the field trial, were proposed for limited cultivation by farmers, in the agro-ecological location, were Kituza is located. Assessment of yield of the elite CWD resistant clones commenced in 2005/06 and it will continue for at least 4-5 years.

DISCUSSION

Revelation of CWD resistance within Ugandan *C. canephora* natural populations gave ways towards the development of commercial varieties, which are resistant to the disease without involving many cycles of introgressing resistance from other species, particularly *C. arabica*. This coupled with availability of individuals with good market qualities, which are resistant to leaf rust and red blister disease, among the CWD resistant genotypes, will hasten availing of appropriate varieties for cost effective management of CWD.Revelation of the CWD resistance also removed the fear that *C. canephora* could be exterminated by CWD, although the genetic diversity is still threatened. It is feared that as farmers adopt new CWD resistant varieties, which are a product of a very narrow genetic base, they will destroy few individuals that survived CWD in farms, and as losing genes for other traits.

Table 1. Quality and resistance to leaf rust and red blister disease of 21 CWD resistant and 6 commercial *C. canephora* clones.

Source		Variety	Body	Flavour	Colour	100 bean weight	*Retained by 16/64*	*CWD resistance*	*Resistance to rust*	*Resistance to Red blister*	*Yield*
Identified through screen house tests	1	NFCT3	Fair+	Fair	Greenish brown	14.7	56.2	1	1.6	1.2	
	2	JB5109.4/5/1	Fair+	Fair-	-do-	17.6	81.4	1	1.8	1.4	
	3	Erecta unknown 20	Fair	Fair--	-do-	17.6	83.1	1	1	1	
	4	Erecta unknown 14	Fair	Fair+	-do-	17.1	87.3	1	1.5	1	
	5	261s/21/1	Fair+	Fair+	-do-	16.8	67.8	1	2	1	
	6	2/22/2	Light	Fair	-do-	16.1	87.7	1	1	2	
	7	J24/13/20/4	Fair++	Fair	-do-	17.4	86.8	1	1	1.2	
	8	Erecta unknown 11	Fair-	Fair--	-do-	17.5	93.5	1	1.5	1.8	
	9	MFCT2	Fair	Fair	-do-	13.4	33.7				
	10	J94/2/64/1	Fair+	Fair	-do-	13.8	55.8	1	2	2	
	11	MFCT1			-do-	13.1	51.4	1	1.6	1	
	12	203/32/2	Fair	Fair--	-do-	14.2	88.0	1	1.5	1	
	13	J12.01/16/1	Fair	Fair	-do-	14.4	46.8	1	1	1.2	
	14	286/1	Fair++	Fair-	-do-	13.9	70.4	1	1.2	1.2	
	15	286/2	Fair+	Fair+	-do-	15.7	92	1	1.4	1.7	
	16	2/22/12	Fair+	Fair+	-do-	19.2	95.8				
	17	J1/14/21/1	Fair	Fair+	-do-	21.1	95.4	1	1.6	1	
	18	234/37/7	Fair+	Fair	-do-	12.6	46.2	1	1	2	
Identified from field trials	1	J/1/1						1	1	1	2940
	2	Q/3/4						2	1	1	2490
	3	R/1/4						3	1	1	2210
Previous commercial lines	1	1s/3				20.5	80	3			2130
	2	1s/6				23	80	4			2240

Quality analysis: ++ is very good; + is good; - is fair, - is poor and - - is poor
Disease analysis: ++ is resistant; + is fairly resistant; - is susceptible and - - is very susceptible

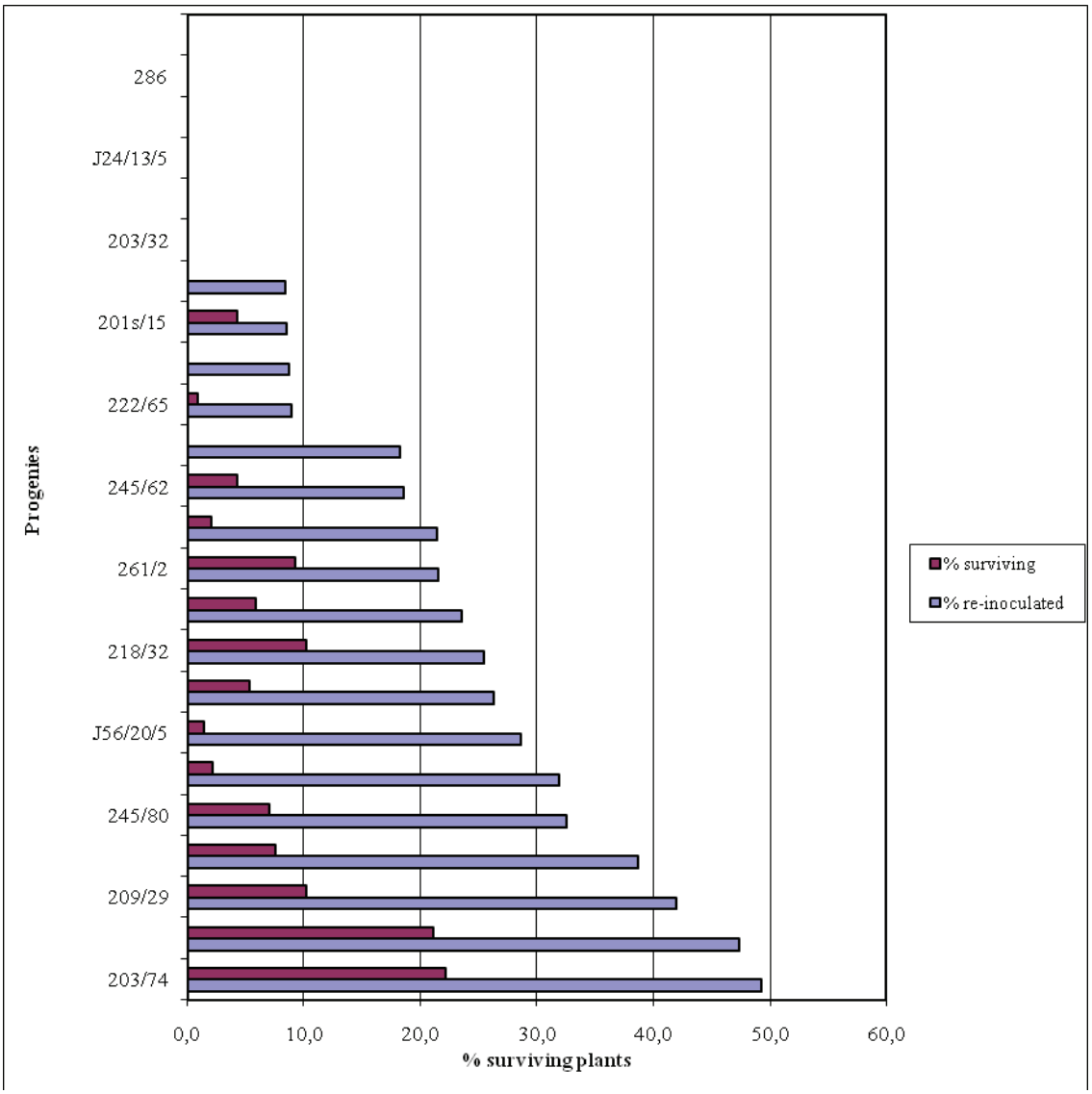


Figure 3. % survival among *C. canephora* artificially progenies inoculated with CWD pathogen in the screen house.

Although 8 elite varieties identified in this study have the potential for farmer cultivation, it should be noted that data on yield and their interaction with the environments in different *C. canephora* farming regions is not yet known. But in the mean time they can be disseminated to farmers in the agro-geographical location where Kituza is located, awaiting validation of their performance in multi-location trials. These 8 varieties might not be the best in terms of yield and bean and cup qualities and it is unthinkable that only 8 clones will suffice to support the Robusta coffee growing in the whole country. Therefore there is still need for developing more varieties. This process is continuing at COREC through identification and evaluation of other CWD resistant clones plus generation, evaluation and selection of multi-trait hybrids. Currently there are 1519 CWD resistant clones planted in the mother garden at Kituza, out of which only 167 were evaluated. The other clones are being evaluated and good performing clones shall be advanced to on-farm trials en route to being released to farmers. Through this process, many more superior varieties will be identified and supplied into the farming system to reverse the devastating effects of the CWD menace.

REFERENCES

Delassus E. 1954. La trachéomycose du caféier. Bulletin Scientifique du Ministère des Colonies, Section *Agronomie Tropicale,* 5:345-348. Delassus E. 1954. La trachéomycose du caféier. Bulletin Scientifique du Ministère des Colonies, Section Agronomie Tropicale*,* 5:345-348.

Flood J. and Brayford D. 1997. The re-emergence of *Fusarium* wilt of coffee in Africa. In: ASIC 1997 Proceedings of the International 17th Scientific Colloquium on Coffee Conference. ASIC, NAIROBI.

Fraselle J. 1950. Observations préliminaire sur une trachéomycose de *Coffea robusta*. Bulletin Agricicole, Congo Belge, XLI, 361-372.

Kranz J. and Mogk M. 1973. *Gibberella xylarioides* Heim et Saccas on arabica coffee in Ethiopia. Phytopathology*,* 78: 365-366.

Lejeune J.B.H. 1958. Rapport au Gouvernement Imperial d'Ethiopie sur la production caféière. Rapport du la FAO, Rome FAO158/3/1881. Lejeune, J. B. H. 1958. Rapport au Gouvernement Imperial d'Ethiopie sur la production caféière. Rapport du la FAO, Rome FAO158/3/1881.

MAAIF (Ministry of Agriculture Animal Industry and Fisheries) 1995. Clonal Robusta coffee hand book Part 2: Nursery management practices. Communication Centre, Entebbe.

Muller R.A. 1997. Some aspects of past studies conducted in Western and Central Francophone Africa on tracheomycosis (Cote d'Ivoire, Cameroon and Central African Republic). In: Hakiza JG, Birikunzira B, Musoli P (eds.) Proceedings of the first regional workshop on coffee wilt disease (Tracheomycosis). (pp. 15-26) International Conference Centre, Kampala, Uganda.

Masiga M., Ruhweza A. (2007). Commodity Revenue Management: Coffee and Cotton in Uganda. YOMA Consultants; International Institute for Sustainable Development. Winnipeg, Manitoba, pp. 18.

Mugambe K. (2007). The Coffee Year Book, 2006/07. Uganda Coffee Trade Federation; pp 36-38.

Musoli P., Olal S., Nabaggala A. and Kabole C. 2001. Screening Robusta coffee germplasm for resistance against coffee wilt disease. CORI progress report on coffee wilt disease research and development 1997-2000 Musoli, P., Olal, S., Nabaggala, A. and Kabole, C. 2001. Screening Robusta coffee germplasm for resistance against coffee wilt disease. CORI progress report on coffee wilt disease research and development 1997-2000.

Musoli P.C., 2007. Sources of Resistance against coffee wilt disease caused by *Fusarium xylarioides* in Uganda. Ph.D. Thesis of Montpellier II University.

Oduor G., Phiri N., Hakiza G. J., Abebe M., Asiimwe T., Kilambo D. L., Kalonji Mbuyi A., Pinard F., Simons S., Nyasse S. and Kebe I. 2005. Surveys to establish the spread of coffee wilt disease, *Fusarium (Gibberella) xylarioides*, in Africa (2004) In: ASIC 2004, Proceedings of 2Oth International Conference on Coffee Science. (pp. 1252-1255), Bangalore, India.

Variability of *Hemileia vastatrix* from Coffee Ecosystems and Resistance of Hibrido de Timor Derivatives to Aggressive Races

L.D. KILAMBO[1], V.M.P. VARZEA[2], D.J. MTENGA[2], P.P. PEREIRA[2], M.T. JAMES[1] AND L.I. MASUMBUKO[1]

[1]Tanzania Coffee Research Institute P.O.Box 3004 Moshi, Tanzania
[2]Centro de Investigação das Ferrugens do Cafeeiro (CIFC) / Instituto de Investigação Científica Tropical, Quinta do Marquês 2784-505 Oeiras, Portugal

SUMMARY

Coffee leaf rust (CLR) is a disease of economic importance in Tanzania causing yield losses averaging 66% on susceptible varieties. CLR was reported for the first time in Tanzania in 1880s. As there are possibilities for new introductions and evolution of rust races, studies were conducted to update records for existence of rust races to develop stronger breeding programme. Fifty one rust samples collected from different coffee agro-ecologies were evaluated using 18 coffee differentials. Results showed that rust race II (v5) occurred more frequently among the samples and race I (v2,5) occur less frequently. Rust races III (v1,5), XVII (?), XI (v?) and XX (v?) found in 1975 did not occurred among the samples characterized. However new rust races XXII (v5,6) and XXXIV (v2,4,5,7,? or v2,4,5,7,9,?) found out of 51 samples indicates an evolution and therefore widens possibility of increased virulence of rust races in Tanzania coffee areas. On the other hand some rust isolates characterized in the set of the coffee differentials as race I and race XXXIV are under study and show evidence to be new races. Progenies of *Coffea arabica* involving Hibrido de Timor (HdT) derivatives (*Coffea arabica* x HdT) were tested against different rust races with different spectra of virulence and aggressiveness e.g. CIFC 2191 and CIFC 5a using the method developed by d'Oliveira. Different levels of complete and incomplete resistance on HdT derivatives were found. Eleven HdT derivatives showed high percentage of plants with complete resistance. Results of variability of the coffee rust races in Tanzania and reaction of HdT derivatives to aggressive races are discussed in this report.

INTRODUCTION

Coffee leaf rust, caused by the fungus, *Hemileia vastatrix* Berk & Br was first reported in Tanzania in 1880s (Tapley, 1965). It is generally assumed that leaf rust attacked coffee as early as this crop was introduced in Tanzania in 1880s.

Although there are about 45 known physiologic races of *H. vastatrix* (Várzea and Marques, 2005), seven races were reported on 30 samples collected in different coffee ecosystems in Tanzania (Rodrigues *et al.,* 1975). The races recorded in Tanzania include race I-v2,5 (20%), II-v5 (30%), III-v1,5 (26.7%), XVII-1,2,3,5 (10%), XXIV-v2,4,5 (6.7%), XI-v? (3.3%), and XX-v? (3.3%). Out of the seven races found in Tanzania, race II was the most common race found by that time.

For breeding of coffee varieties resistant to leaf rust, the knowledge of physiologic races of *H. vastatrix* which occur in Tanzania is of critical importance. Therefore, the work in progress reported here was undertaken to identify the new races of leaf rust currently occurring in Tanzania, and evaluation of resistance in coffee selections to *H. vastatrix* races.

MATERIALS AND METHODS

Characterization of rust races

Between September 2005 and April 2006, field trips to coffee growing areas were made to collect leaf rust samples from various coffee varieties. A total of 129 rust samples; 77 of *Coffea arabica* and 52 *Coffea canephora,* were collected. Leaf rust samples as well as rust spores preserved in gelatine capsules were sent to Coffee Rust Research Centre, Oeiras, in Portugal for race identification. The methods used for race identification have been reported elsewhere (d'Oliveira and Rodrigues, 1960).

Evaluation of resistance in coffee selections to Hemileia vastatrix

One hundred progenies of *C. arabica, C. canephora* and interspecific tetraploid hybrids (Hibrido de Timor – HdT) and HdT derivatives (*C. arabica* x HdT) were tested against rust races with different spectra of virulence and aggressiveness. Isolate type CIFC 71 (race VI-v?) and isolate type CIFC 92 (race XVIII-v?) were tested to *C. canephora*. Rust races isolates CIFC 395 (race III-v1,5) and CIFC 1065 (race II-v5), CIFC 1126 (race II-v5), CIFC 1427 (race II-v5), CIFC 292a (race XXIII-v1,2,4,5) race from India with highest level of virulence and aggressiveness to HdT and HdT derivatives , CIFC 2191 (race ?-v2,5,6,7,9,?) and CIFC 5a (race ?-v2,5,7,8,9,?).

Method developed by d'Oliveira (1965) was used to evaluate resistance of coffee selections to these rust races. Leaves of the terminal node, still tender and succulent were inoculated by spreading dry rust uredospores on the lower surface of the leaves. Thirty to forty days after inoculation leaves were evaluated using a qualitative scale; Resistant (immune, flecks, necrotic spots, small tumefactions, chlorosis), Moderately Resistant (rare sporulating sori, small or medium sized pustules), Moderately Susceptible (areas with intense chlorosis, medium-sized or large pustules surrounded by chlorosis), Susceptible reaction (large sporulating pustules, heterogeneous reaction).

RESULTS AND DISCUSSION

Characterization of rust samples

The places where leaf rust samples were collected are shown in Figure 1. In the coffee samples collected, race II (v5) occurred more frequently (Várzea, 2008). This shows that race II is still the most widely distributed race in Tanzania. Races III, XI and XX found out in 1975, did not occurred among the samples characterized. This can be due to their inexistence in the coffee fields, limitation in the sampling areas or possibly evolution. However of particular importance are new races XXII and XXXIV which were recorded on samples of *Coffea canephora* Kagera. This is the first time these races have been identified in Tanzania.

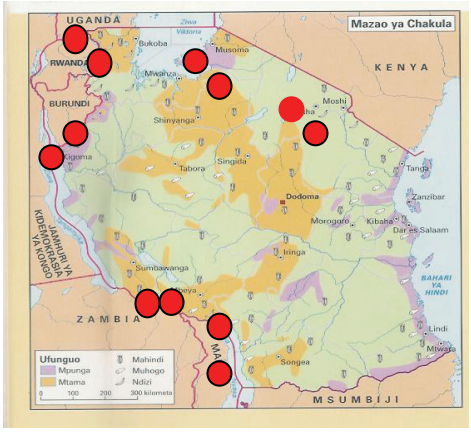


Figure 1. Map of Tanzania showing areas where rust samples were collected. ● Areas where leaf rust samples were collected.

Race XXII

This race attacks *Coffea species* with S_H genes S_H5 and S_H6, and resistant groupβ, E, R, F and N (Kushalappa and Eskes, 1989). These include *C. arabica* Matari, Bourbon and HdT, and *C. racemosa* and *C. excelsa*. This means that although this race was found in *Coffea canephora* in Tanzania, the spread of this race would be detrimental to *Coffea arabica* as well. Screening for resistance to coffee varieties existing in Lyamungu germplasm is a possibility to contain the race.

Race XXXIV

This race is identified for the first time in *Coffea canephora* in Tanzania. Várzea and Marques (2005) documented it to have pathogenic effect to *C. arabica* and some tetraploid segregants of *C. arabica* x *Coffea species* of Icatu and HdT derivatives. Similarly this race could be detrimental to *Coffea arabica* as well.

Evaluation of resistance in coffee selections to Hemileia vastatrix in Tanzania

Different levels of resistance on progenies of Tanzania *C. arabica* and HdT derivatives were found. Best progenies showed highest level of resistance are found in Table 1.

Table 1. Most resistant selections to *Hemileia vastatrix* races in Tanzania.

Progeny	***Reaction type***
(N 39 X OP 729) X HdT 1343) X N 39	Complete resistance
(N 39 X HdT 1343) X (Caturra X HdT 1343)	Complete resistance
(N 39 X Kaffa) X HdT 1343	Complete resistance
(N 39 X HdT 1343) X Illubabour	Complete resistance
(N 39 X Kaffa) X HdT 1343	Complete resistance
(N 39 X C.A 729) X HdT 1343) X HdT 1343	Complete resistance
(N 39 X HdT 1343) X Rume Sudan X Geisha	Complete resistance
(N 39 X HdT 1343) X (B.M Jam X S.6 Cioccie)	Complete resistance
(Caturra X HdT 1343) X (Kent X HdT 1343)	Complete resistance
Rume Sudan X HdT 1343	Complete resistance
N 39	Complete susceptible

Three coffee genotypes (N 39 X OP 729) X HdT 1343) X N 39, (N 39 X Kaffa) X HdT 1343 and (N 39 X HdT 1343) X Illubabour; are among the nine officially released varieties in Tanzania (Teri et al., 2004).

CONCLUSION

Rust races found in Tanzania so far will assist to shape-up Breeding programme. New races XXII and XXXIV found on *Coffea canephora* will also be tested on *Coffea arabica.* Coffee genotypes showing complete rust resistant will be recommended to improve the breeding programme or grown in areas where potential of rust disease is higher.

ACKNOWLEDGEMENT

We wish to thank Dr. Vitor Várzea of Coffee Rusts Research Centre who identified the leaf rust races and also to Prof. James Teri who permitted publication of this paper.

REFERENCES

d'Oliveira B. (1965) Introduction. *Progress Report* 1960-65, pp. 1-20. Centro de Investigação das Ferrugens do Cafeeiro, Oeiras, Portugal.

d'Oliveira B. and Rodrigues C.J. (1960) A Survey of coffee rusts. Junta de Investigações do Ultramar Centro de Investgações das Ferrugens do Cafeeiro Estacao. Estação Agronómica Nacional (Oeiras). Junta de Exprotação do Café. Pp 1-68.

Kushalappa A.C. and Eskes A.B. (1989) Resistance Reaction of Main Coffee Differentials to 31 Races of *H. vastatrix* identified at CIFC. In: Kushalapa, A. C. & Eskes, A. B. (eds). Coffee Rust: Epidemiology, Resistance, and Management. CRC Press Inc., Boca Raton, Florida, pp 198.

Rodrigues C.J., Bettencourt A.J. and Rijo L. (1975) Races of pathogen and resistance to coffee rust. Annual Review of Phytopathology 13:49-70.

Tapley, R. G. 1965. The effect of leaf rust, *Hemileia vastatrix* Bert & Br., and Leaf miners, *Leucoptera meyrick* Ghesq and *Leucoptera caffeina* Wshbn., on leaf life. Lyamungu misc. reports.

Teri J.M., Kilambo D.L., Mtenga D.J., Nyange N.E., Nzallawahe T.S., Chipungahelo G.S., Kipokola T.P. and Kullaya I.K. (2004) Improved Arabica varieties for the nenefit of Tanzania coffee producers. In: Proceedings of the 20th International Conference on Coffee Science (ASIC), Bangalore, India.

Várzea V.M.P and Marques D.V. (2005) Population variability of *Hemileia vastatrix* VS. Coffee durable resistance. In: Zambolim L., Zambolim E.M and Varzea V. M. P (eds). Durable Resistance to Coffee Leaf Rust. Universidade Federal de Vicosa Departamento de Fitopatologia Vicosa – Minas Gerais 2005.

Varzea V.M.P (2008) Scientific collaboration in fundamental and practical knowledge in the two most important coffee diseases in Tanzania (CLR and CBD) with the ultimate aim of producing varieties with durable resistance to these pathogens. Final report 2005-2007, Project number: TA STA COF 99 01. pp6.

Cultivars Evaluation *Coffea arabica* to the Parasitism of *Meloidogyne exigua*

A.L.A. GARCIA[1*], V.P. CAMPOS[2], L. PADILHA[3]

[1]Fundação Procafé, Varginha, MG. [*]Email: garcialmg@gmail.com
[2]DFP/UFLA, Lavras, MG
[3]Embrapa Café

SUMMARY

Nematodes may cause damages in Brazilians coffee plantations, depending on the species and the type of soil. The *Meloidogyne exigua* is spread in the coffee regions, especially in older farmings in South of Minas Gerais. Coffe trees in the areas with nematodes have showed reduction in their development and production. It gets worse when the plants are under stress conditions. The use of resistant cultivar is the best way to control the nematode. The aim of this work was to evaluate the behavior of C*offea arabica* cultivars when inoculated with *M. exigua* to identify resistance sources. The tests were carried out at Nematology lab in the UFLA, Lavras, MG, in greenhouse, with randomized block design, four replications and plot with three plants. The reaction of 22 coffee cultivars were evaluated with reference to the *M. exigua* using Acaiá IAC 474/19 and IAPAR 59 as resistant and susceptible controls. Coffee plant was inoculated with 5000 eggs of *M. exigua*, 80 days after transplanting. The number of galls/gr root were counted at 40 and 120 days after inoculation. To IAPAR 59 cultivar were confirmed to be immune, since no galls was observed in their roots. Catucaí Vermelho 785/15 and Acauã cultivars had segregation behavior with some resistant plants and others of reduced susceptibility. Catucaí Vermelho 36/6, Siriema, Soledade, Bem-te-vi Amarelo, Catucaí Vermelho 20/15- cv 395, Catucaí Amarelo 3-5, Franca Cultivar, Catucaí Vermelho 36/6- cv 470, Catucaí Amarelo 24/137, Sabiá Tardio, Catucaí Amarelo 2SL- cv 446, Catucaí Amarelo 3 SM, Catucaí Amarelo 20/15- 479, Icatu IAC 2944, IBC-Palma II, Canário, Catucaí Açu Vermelho, IBC-Palma I e Acaiá-IAC 474/19, were considered susceptible.

INTRODUCTION

Minas Gerais is the State that produces almost 50% of the all Brazilian coffee. The *Meloidogyne exigua* is a nematode spread in this State especially in older crops of the South of Minas Gerais. The incidence of M. exigua, as determined by studies conducted in this location, was found to be 51% of local sampling and in 37% of the samples (Campos et al., 1980). *M. exigua* penetrates in the coffee root and causes anatomical and physiological disorders, characterized by gall rounded roots. Among other damages, a decrease of coffee production was observed one year after planting and ranging from 31.4% to 50% (Arruda and Kings., 1962).

Pratical management of this parasite, as culture rotation and chemical control could be used, but planting resistant cultivar is the most efficient between the techniques of *M. exigua* controls. There is a great variability for this resistance characteristic and the species sources of resistance are already known as *C. canephora*, *C. congensis*, *C. liberica* and *C. dewevrei.* Within *C. arabica*, some cultivars such as Catucaí Vermelho 785/15, IAPAR 59, 166-13, and Acauã, Piatã, also have demonstrated resistance. The objective of this study was to evaluate the behavior of coffee cultivars in relation to the nematode parasitism *M. exígua*.

MATERIAL AND METHODS

The experiment was conducted in the Nematology section of Phypathology Department at UFLA, Lavras, MG. Seeds of 22 coffee cultivars (Table 1) were sowed in sand bed and seedling were transplanted to 72 cells trays. The randomized block design comprised four replications and three plants per plot. From 22 cultivars used, 17 were of the breeding program of Fundação Procafé. Acaiá 474/19 (susceptible) and Iapar 59 (resistant) cultivars were used as reference in the analysis of the results.

The inoculum was prepared with an *M. exigua* eggs suspension extracted from coffee roots (Hussey and Barker, 1973) in a old coffee plantation presenting a high level of nematode infestation. Each plant was inoculated 80 days after transplanting (2nd pair of leaves stage), with five ml suspension containing approximately 5000 eggs of *M. exigua.* The assessments were made 40 and 120 days after inoculation, with the help of an accountant and a digital scale. The total number of galls per root and root weight were determined and used to calculate the number of galls per gram of root (NG / g).

The data were subjected to analysis of variance and averages grouped by test-Skott Knott (1974), with SISVAR statistical program.

RESULTS AND DISCUSSION

Table 1. Number of galls per gram of root (NG/g) of 22 cultivars, *Coffea arabica*, observed after the inoculation with 5000 eggs of nematode *Meloidogyne exigua*.

CULTIVATES	40 days after inoculation		120 days after inoculation	
Iapar 59	0	a	0	a
Catucaí Vermelho 785-15	0	a	0	a
Acauã	0	a	11	b
Catucaí Vermelho 36/6	31	b	57	c
Siriema	39	b	69	c
Soledade	41	b	46	c
Bem-te-vi Amarelo	43	b	51	c
Catucaí Vermelho 20/15- cv 395	44	b	28	c
Catucaí Amarelo 3-5	45	b	72	c
Cultivar oriunda de Franca	46	b	51	c
Catucaí Vermelho 36/6- cv 470	48	b	41	c
Catucaí Amarelo 24/137	49	b	52	c
Sabiá Tardio	51	b	66	c
Catucaí Amarelo 2SL- cv 446	52	b	66	c
Catucaí Amarelo 3 SM	53	b	60	c
Catucaí Amarelo 20/15- 479	56	b	44	c
Icatu IAC 2944	57	b	31	c
IBC-Palma II	62	b	79	c
Canário	64	b	46	c
Catucaí Açu Vermelho	66	b	56	c
IBC-Palma I	70	b	68	c
Acaiá-IAC 474/19	72	b	34	c

Averages followed by same letter do not differ by statistical test Scott - Knott the significance level of 5%.

The number of galls in the first evaluation carried out 40 days after inoculation was statistically equal for the Catucaí Vermelho785/15, IAPAR 59 and Acauã cultivars (Table 1).

In the second evaluation 120 days after inoculation, the IAPAR 59 cultivar did not show any galls showing the immunity of this cultivar. On the other hand, the Catucaí Vermelho 785/15 showed an infection index near to zero (0.22/ NG/g), but there was some relationship between host and parasite. Therefore, Catuai Vermelho 785/15 was considered as resistant. In the same way, due the low level of *M. exigua* infection in the cultivar Acauã, (11.22 NG/g) led to classify it also as resistant. The number of galls index in the other cultivars ranged from 27.62 to 79.05, but again without significant difference.

Nevertheless, the different values of index of infestation (NG/gr) observed between susceptible cultivars, suggest different levels of susceptibility of cultivars to the *M. exigua.*

CONCLUSIONS

Catucaí Vermelho 785/15 and Acauã cultivars showed segregation between plants, with some exhibiting resistance and others low susceptibility to parasitism of *M. exigua.*

The cultivar IAPAR 59, under controlled conditions, is immune to *M. exigua.*

Cultivars Catucaí Vermelho 36/6, Siriema, Soledade, Bem-te-vi Amarelo, Catucaí Vermelho 20/15- cv395, Catucaí Amarelo 3-5, cultivar come from Franca, Catucaí Vermelho 36/6- cv 470, Catucaí Amarelo 24/137, Sabiá Tardio, Catucaí Amarelo 2SL-cv 446, Catucaí Amarelo 3 SM, Catucaí Amarelo 20/15- 479, Icatu IAC 2944, IBC-Palma II, Canário, Catucaí Açu Vermelho, IBC-I and Palma Acaiá-IAC 474/19 are susceptible to the *M. exigua.*

REFERENCES

Arruda and Reis (1984) Influência do *M. exígua* no desenvolvimento de lavouras. In: Congresso Brasileiro de Pesquisas Cafeeiras, 11º, Londrina – PR. Anais... p. 207-209.

Campos V.P. et al. (1980) Levantamento de nematóides parasitas do cafeeiro que ocorreram no sul de M.G. In: Congresso Brasileiro de Pesquisas Cafeeiras, 29º, Poços de Caldas. Anais... p. 440-443.

Hussey R.S., Barker K.R. (1973) A comparasion of methods for collecting inocula of *Meloidogyne* spp. Including a new technique. Plant Disease Reporter, Washington, v. 57, n. 2, p. 1025-1028.

Scott A.J., Knott M. (1974). A cluster analysis method for grouping means in the analysis of variance. Biometrics, 30, p. 507-512

Coffee Genetic Resources Under Severe Threat from Genetic Erosion in the Centres of Origin and Diversity: an Urgent Need for Conservation Measures

B. BAYETTA, J.C. SACKO

Interafrican Coffee Organisation (IACO), P.O. Box V210, Abidjan, Cote d'Ivoire

SUMMARY

Arabica (*Coffea arabica* L.) and Robusta (*Coffea canephora* Pierre ex Froehn) coffees are the two economic species which account for about 70% and 30% to the total world coffee production, respectively. All the rest 103 species are not economically important even though few of them may have some desirable attributes. Like in any other crops, continuous improvement of coffee for any desirable trait (yield, quality, disease resistance, etc.) and its sustainable production solely depends on the amount of genetic variability available. The equatorial lowland forests of West and Central Africa for *Coffea canephora* and several other species and the South Western montane rain forests of Ethiopia for *Coffea arabica* are centres of origin and diversity. Efforts made to conserve these valuable resources as *ex situ* or *in situ* are very minimal. According to some reports, Ethiopia alone possesses about 99.8 % of the total Arabica's genetic diversity. Apparently, it is an alarming situation for the future of arabica coffee industries in times of new pest out break if the genetic resources are not properly conserved, no one forget the lesson learnt from coffee leaf rust in Ceylon (now Sri Lanka) in 1869. On the other hand, the tropical rainforests harboring wild coffee genetic diversity and the cultivated traditional landraces on farmers' field are dwindling at an increasingly alarming rate than ever owing to: (1) deforestation for purposes of logging, investment or commercialization (coffee, tea, rubber, spices, cocoa, etc. plantations), resettlement of people prone to drought and land degradation, seeking more land for food crops owing to population pressure, house construction, firewood and charcoal; (2) replacement of landraces by few improved varieties; (3) competition by other crops such as tea, rubber, cocoa, chat (K*atha edulis*), spices, etc.; and (4) climatic change mainly drought. The increasing threats by human agents may indicate the lack of incentives that compensate the intended needs and cost of conservation sustained by the community or the nation. To avoid the tragedy of genetic erosion and warrant sustainable utilization, launching of a global coffee genetic resources conservation initiative, establishment of access and equitable benefit sharing agreement, development of property right systems, and presence of favourable policy are indispensable. In this initiative, the Interafrican Coffee Organization (IACO), International Coffee Organisation (ICO) and Bioversity International are relevant institutions to take the lead.

INTRODUCTION

There are about 105 species of the genus *Coffea* (Medina-Filho et al., ??). The global coffee production and its industry, however, depends on only two economic species, commonly known as Arabica coffee (*Coffea Arabica* L.) and Robusta coffee (*Coffea canephora* Pierre ex Froehn), which account for about 70% and 30% of the total world production, respectively. All species of the genus *Coffea* including the two commercial species are of tropical African origin (Bridson and Verdcourt, 1988 cited by Sondal and Van der Vossen, 2005). The

equatorial lowland forests of West and Central Africa that stretches from Guinea to Ugand are the home of diverse forms of *C.canephora*, while the natural populations of *C.arabica* are restricted to the montane rain forests of South Western Ethiopia (Berthaud and charier, 1988).The forests of Madagascar and to some extent the Mascarene Islands (Mauritius and Reunion), are the home of *Mascaro coffea* section of the genus which are characterized by low levels or complete absence of caffeine, probably a useful trait for low caffeine variety development. *Coffea liberica* is the other silent species native of the tropical forests of the present Liberia and probably the Ivory Coast (http://www.hear.org/pier/wra/pacific/ coffea_liberica -htmlwra.htm)**,** which could be of special interest as distinct specialty coffee. In addition to forests, farmers' field growing traditional landraces are the other sources of coffee gene pool.

The sustainability of the coffee industry and continuous improvement of the crop for yield, quality, disease resistance, tolerance to moisture stress, and any other desirable traits solely depends on the amount of genetic diversity available in the hands of the breeders or in the original population. Without the presence of genetic variability, any improvement endeavour is very expensive, time consuming and with little success. Despite such immense contribution of genetic diversity, much attention has not been given to the conservation and sustainable use of coffee genetic resources. Today, coffee genetic erosion is alarmingly increasing due to deforestation of coffee forest areas for conversion in to commercial (coffee, tea, cocoa, rubber, etc plantations) and subsistence agricultural land, logging and collection of wood fuel (Branson, 2008; Hennig, 2008). The demand for subsistence agriculture is increasing due mainly to increasing demand of food crops owing to population pressure and resettlement of people prone to drought and land degradation. Replacement of local landraces by few improved varieties, intensive management of forest and semi-forest coffee, competition by other crops such as Chat (Catha edulis), tea, rubber, cocoa, etc and climate change are some other factors exacerbating coffee genetic erosion in the centres of origin.

It is apparent that unless appropriate conservation measures are taken as a matter of top urgency, the valuable coffee genetic resources could be irreversibly lost in few years time jeopardizing the prospect of global coffee industry. The intension of this report is to provide a brief account of the current coffee genetic resource situation and the need for immediate conservation measures to warrant the sustainability of the coffee industry for the benefit of humankind.

THE NEED FOR URGENT CONSERVATION MEASURES

Importance of the crop

Coffee is a complex crop in that its production involves agricultural, industrial and environmental components of considerable value. It is an important agricultural commodity, second only to oil in the international trade, and over 125 million people are engaged in its production and marketing (export, roasting, processing). Globally, it generates over $12 billion annually with retail values as high as $ 70 billion. Coffee is an evergreen shade loving plant and the production of the crop and the maintenance of coffee forest areas for the sake of its management or handling are of great benefit to the protection of global environment.

High Coffee Genetic Erosion in the Centres of Origin

The tropical rainforests harbouring the wild forms and farmers field growing the cultivated landraces are currently under severe threat from genetic erosion. At present, the wild and

cultivated forms of coffee genetic resources residing in the tropical rainforests and farmers field are under severe threat from genetic erosion owing to a combination of factors:

Deforestation

Deforestation of African forests is caused by many factors. According to Branson (2008), logging causes 20-25% deforestation, conversion to commercial and subsistence agricultural land accounts for about 60%, and 90% of the 600 million people in Africa depend on wood fuel as a main source of energy. The major driving forces for conversion to agricultural land are investment programmes aimed at establishment commercial coffee, tea, rubber, cocoa, etc plantations to increase the production and productivity of forest lands; increasing demand of land for food crops owing to population pressure, and resettlement programme to rescue people that are prone to land degradation (poor soil fertility and productivity) and recurrent drought

In a forest resource assessment in Africa, the rate of deforestation per annum was estimated at 2% in West Africa with over 5% in areas of Cote d'Ivoire, Robusta coffee origin, (Branson, 2008) and about 2.5-3% in Ethiopia, arabica coffee origin (Reusing, 1998 and FAO, 2007, cited by Wikipedia). According to Hennig (2008), in Central Africa, over 90% of all logging occurs in primary forest, one of the highest ratios in the world. In Cameroon, timber generates more than a quarter of the country's revenues and about 55,000 people work in the logging sector.

Replacement of the landraces by improved cultivars

The demand for high quality, competition in export volume, problem of diseases and insect pests and low yield of the traditional cultivars and wild coffee in the forest has accelerated rapid replacement of the heterogeneous coffee land races by few narrow genetic base improved cultivars. In Ethiopia, the quantity of seeds annually distributed from CBD resistant high yielding culivars goes as high as 100 quintals with an average of 40-60Qts while preparation is underway for massive multiplication and distribution of the three hybrids recently released. Intensive management of forest and semi-forest coffee accompanied by refilling with improved varieties intended to improve the low forest coffee yield (200-250 kg/ha) is becoming a common practice which may even worsen the situation of wild coffee gene pool.

Recurrent drought and land degradation

Prolonged recurrent drought is becoming a serious threat to agriculture in Africa, more importantly in east Africa. Coffee is one of the victims of these natural calamities among agricultural crops. The Eastern part of Ethiopia which inhabits the famous Harar coffee types that recently acquired its trade mark (Getachew, 2008) is the most affected part of arabica origin by the current drought and climatic change. Land degradation is the other unprecedented threat to genetic erosion resulted mainly from long-term loss of natural vegetation and subsequent soil erosion. Reports indicate that about 65% of Africa's agricultural land suffers from soil degradation (http://www.missionariesofafrica.org/africa/). The local coffee landraces commonly found on farm lands are vastly affected by abandoning of previous farm lands due to land degradation and consequent poor productivity.

Competition by other crops

At present many other crops of equal or better economic return than coffee are coming up. Some of these crops include Rubber, tea, Chat (Katha edulis), spices and cocoa. All these

crops except cocoa are big threat to arabica coffee production in Ethiopia since they can easily flourish in all the major arabica coffee growing regions particularly in south western montane rain forests and are considered as strategic crops for coffee diversification programme. Robusta coffee is mainly competed by rubber and cocoa, particularly in some countries like Cote D'Ivoire where the economic return is about four times and 10 to 15 times than coffee (personal communication), respectively.

Minimal Conservation Efforts

Basically, there are two major conservation approaches, *ex situ* (outside their natural setting) and *in situ* (in their natural setting) which are complementary rather than substitutes. It is, however, only the *ex situ* conservation strategy that have received some attention with the major collections representing wild West African species mainly *Coffea canephora*, the eastern African species *Coffea arabica* and other diploid species, such as the subgenus *Mascarocoffea.* From the available information, it was found that there are some field gene banks in different countries all together comprising about 16,134 arabica, 8000 Robusta, 1282 Mascaro coffea accessions and some 25 diploid species without prior knowledge whether some are duplicates of one another or not (Table 1). According to some reports (Surendra, 2008), Ethiopia alone possesses about 99.8% of the total arabica's genetic diversity. In view of this report, the available accessions in field gene banks in different countries only represent a tiny fraction of the immense genetic variability existing in wild or cultivated forms in the country of origin.

Table 1. Coffee germplasm collections and *ex situ* conservation centres.

Country	Institute	Accessions (No)
Ethiopia[a]	Jimma Agricultural Research Centre (JARC)- A	5500+(6) *
	Institute of Biodiversity Conservation – A	4000
Cote d'Ivoire[b]	ORSTOM-Institut Français de recherché scientif-ique pour le development en cooperation –R	8000
Madagascar[b]	Recherche Agricolea Madagascar - MC	1282+329A
Cameroon[b]	??	1552
Kenya[b]	Coffee Research Foundation - A	634
Tanzania[b]	Tanzania Research organization - A	110
Brazil[bc]	Centro National de Recursos Geneticos - A	275+(19) *
Colombia[b]	Centro nationale de Investigaciones de cafe Pedro Uribe Mejia - A	886
Costa Rica[d]	Centro Agronomico Tropicale de Investigacion y Ensenanza - A	1992
India[b]	Central Coffee Research Institute, Kamataka - A	329
USA[b]	US Department of Agriculture - A	292

**Number of diploid species conserved; A = Arabica, R = Robusta, MC = Mascaro coffea.*
[a]Bayetta and Labouse (2006), [b]Dullo et al. (1998), [c]Medina et al (?), [d]ICO (2007).

The *in situ* method did not receive any attention in coffee except that there are some attempts in Ethiopia where three sites (Boginda-Yeba Kontir-Berhane and Geba-dogi) covering about 21,000 hectares of forest land for *arabica* and in Madegascar specifically Mauritius Island for Mascarene *Coffea* have been protected. The cultivated forms in farmers' fields also comprise considerable amount of genetic diversity which may need on-farm conservation as part of the *in situ* conservation strategy. In order to advance the present high rate of coffee genetic

erosion and capture as many genetic variability as possible, due emphasis must be given to both complementary conservation approaches

COMPLEXITY OF COFFEE GENETIC RESOURCES CONSERVATION AND USE ISSUE

The conservation of any biological diversity and its sustainable use for the benefit of present and future generation is a common concern of humankind. The different biodiversity treaties, notably the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGR) and the Convention on Biological Diversity (CBD), ratified by quite large number of countries are the direct reflections of such common interest. However, the implementation had been impeded particularly in coffee by three major parallel issues: (i) conflict of interest between the users and suppliers of genetic resources (countries of origin), (ii) internal problems inflicted in countries of origin, and (iii) characteristics of tropical rainforest areas (Fig. 1).

Conflict of interest between users and suppliers of coffee genetic resources

There is considerable lack of agreement and understanding between the users and suppliers on coffee genetic resources conservation and use issue. There is no common agreement pertaining to technical and financial support for coffee genetic resource conservation program; cost of conservation sustained by suppliers (countries of origin) are usually overlooked; access and benefit sharing agreement and the underlying property rights systems are not in place for crops or plants which are outside ITPGR. In fact, the CBD confirms access and fair and equitable benefit sharing for any genetic resources through a bilateral agreement between the country of origin providing the resources and the user under mutually agreed terms (article 15). CBD also recognizes the rights of sovereign states over their natural resources and the need to oblige to their national legislations.

Internal problems of countries of origin and diversity

Countries of coffee origin, i.e., suppliers are confronting several internal issues in the course of conservation:

Social problems

Mainly includes recurrent drought, land degradation (low productivity), increasing demand of land for food crops because of population pressure, and the resultant food insecurity which exert unprecedented pressure on the government in finding immediate solutions and rescuing insecured citizens.

High cost of conservation

The cost incurred for conservation of coffee as *in situ* and *ex situ* is too high to bear by the country of origin alone, even though it is usually overlooked by the users. In the case of *in situ*, this cost may include guarding of protected sites, supervision, management (recurrent and capital cost), foregone opportunity cost by the community, coffee yield loss due to low productivity of forest areas (200-250 Kg/ha) and other related costs. The *ex situ* conservation system also incur huge amount of cost for collection and establishment as well as maintenance of field gene banks. Analysis of *in situ* conservation cost at two sites (Boginda-Yeba and Geba-Dgi) in South Western Ethiopia that cover about 32,000 hectares indicated a cost of Ethiopian Birr (ETB) 1,159.12 or US$ 115.91 at house hold level as a result of the

foregone opportunity cost, transaction cost and hunting of wild life and cost of ETB 2,042,925.50 or US$ 204,292.55 incurred at institution level for recurrent cost, capital cost and miscellaneous costs for coordination and site management (Table 2). This study misses an important factor, yield loss due to low productivity of forest coffee, maintained for the sake of genetic conservation which could have further increased the cost of conservation.

Table 2. Annual average cost for conservation of two *in situ* sites in South Western Ethiopia. (Extracted from Assefa et al., 2008) (Note: $1=ETB 9.7).

Variables	Annual cost (ETB)		
	Collaboative[*1]	Selective[*2]	Average
Household level	1175.26	1142.99	**1,159.12**
Opportunity cost	673.14	846.00	759.57
Transaction cost	97.55	4.56	51.06
Hunting of wild life	404.57	292.43	348.50
Institution level	1,931,436	2,154,415	**2,042,925.50**
Capital cost	45,688	793,479	19,583.50
Recurrent cost	1,300,163	1,165,080	1,232,621.50
Miscellaneous cost	175,585	195,856	185,720.50
Grand total	1,932,611.26	2,155,557.99	**2,044,084.62**

[*1]*Collaborative - conservation and sustainable use of natural forest by the local community under regulated and controlled access.*

[*2]*Strict - conservation of natural forests by the local community with accesses to buffer and transition zones but strict prohibition to inter in to the core zone.*

Lack of Incentive mechanisms

There is no incentive mechanism developed that compensate the cost of conservation retained by the community for the foregone opportunity cost and the government for site management, germplasm collection and coordination in the country of origin.

Lack of favourable policies and community awareness

It is crucial that the country providing the genetic resources must have in place a favourable and adequate policy that facilitates the management and use of genetic resources. It is also important that apart from economic incentives, public awareness creation is necessary to educate the local community to at least wisely use the genetic resources.

Characteristics of Tropical Rainforest Areas

The tropical rainforest areas inhabiting wild coffee germplasm are characterized by fertile soil, dependable rainfall, high potential for logging, low productivity and covers massive area of land. These potential areas are highly tempting in solving the emerging social problems. In deed, it has been targeted as centres for logging industry, resettlement to rescue those people prone to severe drought and land degradation, increase production and productivity of these potential areas through commercialization using different crops such as coffee, tea, rubber, etc. and seeking more agricultural land for food crops for the increasing population.

The overall effect of conflicting interest between users and suppliers and the internal problems of countries of origin have accentuated the marginalization of conservation

activities with a tragic loss of coffee genetic diversity or biodiversity in general, that may result in ecological imbalance.

GLOBAL COFFEE GENETIC RESOURCES CONSERVATION AND USE STRATEGY

From the aforementioned discussions, it is apparent that the coffee genetic resources are currently under the grave threat from genetic erosion. Rescue measures are urgently needed before they are lost to mankind. In effect it is indispensable to launch a 'Global Coffee Genetic Resources Conservation Initiative' which includes the two basic complementary methods, *in situ* and *ex situ,* with the participation of all the relevant national, regional and international institutions and donor organizations. In this perspective, institutions such as Inter-African Coffee Organisation (IACO), International Coffee Organisation (ICO), and Bioversity International should take the lead in organising and facilitating the realization of the proposed initiative.

Among others, the following three major strategies could play the most important role to enhance the conservation of coffee genetic resources:

Funding of in *situ* and *ex situ* Conservation

Funding of conservation programme is the most important method of preserving coffee genetic resources. Funding is required to (a) compensate the forgone opportunity cost of the local community who mainly depend on forest product, (b) share conservation cost sustained by the suppliers, (c) build up required facilities and staff, and (d) manage the germplasm conservation programme under both *in situ* and *ex situ* systems on a sustainable basis.

Establishment of property rights

One of the major obstacles to coffee genetic resource conservation and use is absence of well established property right system. Adoption of property rights of sovereign states over their natural resources and recognition of the principle of fair and equitable benefit sharing is an important strategy to enhance collaborative and effective coffee genetic resources conservation. The United States Department of Agriculture (USDA) as a proponent of intellectual property right (IPR) stated that "IPR will allow the holders of genetic resources to reap the rewards from commercializing these resources and thus align private incentives more closely with public incentives for genetic resources conservation". In agreement, Richerzhagen and Virchow (2002) after investigating the case of arabica coffee in Ethiopia concluded that conservation and sustainable utilization of wild coffee populations will only be feasible if long-term benefit sharing will take place and envisages an amount, which is larger than the costs arising through the conservation activities.

Establishment of germplasm exchange agreement

Germplasm transfer agreement which is commonly known as material transfer agreement (MTA) is a legal instrument usually used as a means for transferring biological materials between entities, including public institutions, private companies, and countries (USDA, 2008). To day, it has become a common instrument to outline the terms for sharing genetic resources and the gains from new product development in many crops. The absence of such agreement for coffee has considerably halted effective utilization of coffee genetic resources.

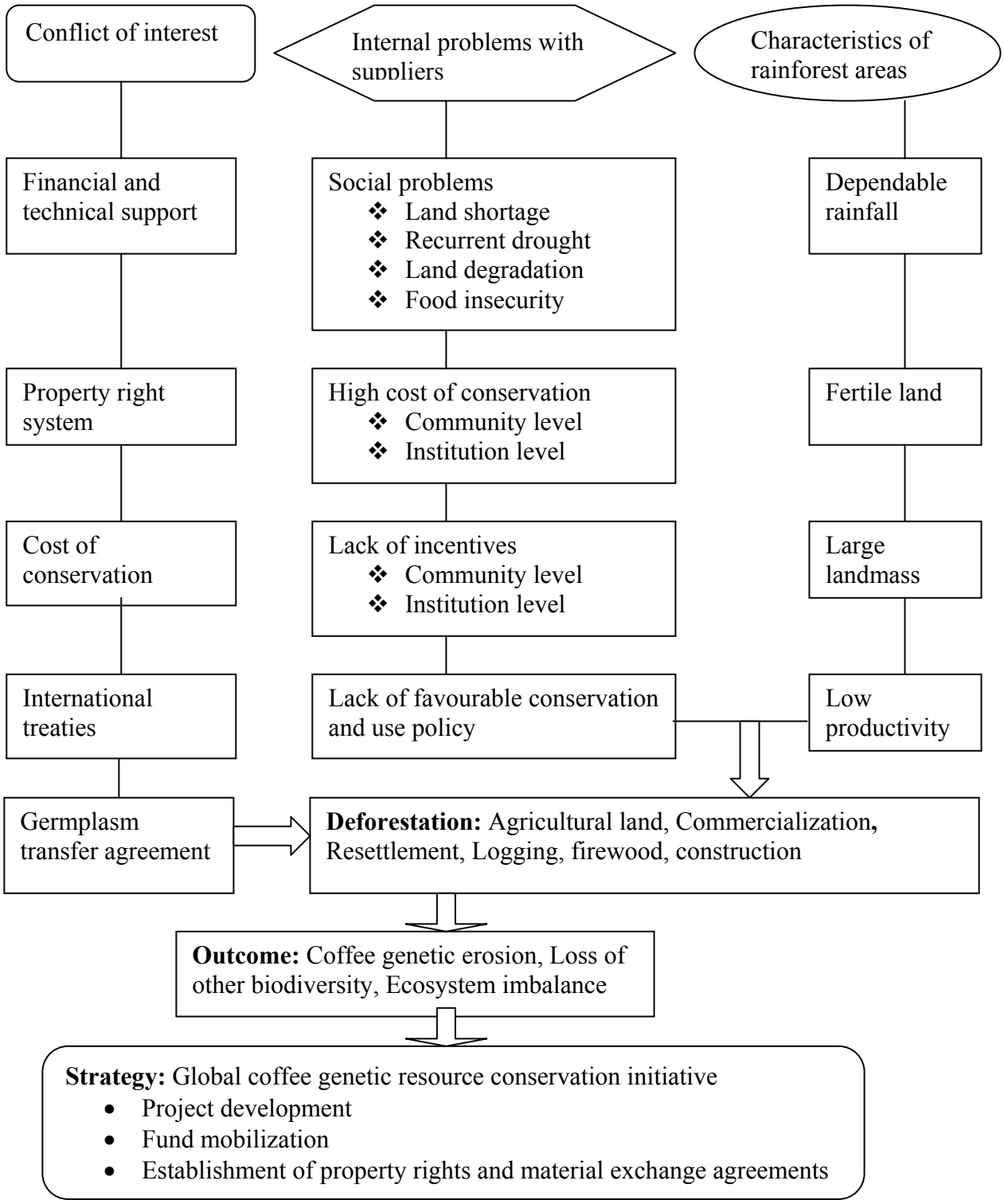


Figure 1. Complexity of coffee genetic resource conservation and mitigating strategy.

CONCLUSION

The sustainability of coffee sector and its continuous improvement directly depends on the availability coffee genetic resources that reside in the tropical rain forests of Africa in wild form and that grow in the farmers' field as traditional varieties. At present these reservoirs of coffee gene pool are dwindling at an increasingly alarming rate than ever due mainly to deforestation for various reasons, replacement of the wild and cultivated landraces by few

improved coffee varieties, competition by other crops and natural calamities mainly drought and soil erosion. The major promoters of these agents of genetic erosion are: (1) conflicting interest between users and suppliers on issues like funding, incentive mechanisms that may compensate cost of conservation, property rights and germplasm exchange agreement, (2) internal problems of suppliers that includes social problems (recurrent drought, land degradation, population pressure, and food insecurity), high cost of conservation, lack of favourable policy and awareness, and (3) the characteristics of rain forest areas itself (fertile large landmass with dependable rainfall, but low productivity) that is tempting for logging, agricultural expansion or intensification.

To reverse the tragic situation and warrant sustainable coffee sector for the benefit of mankind, it is indispensable to launch a 'global coffee genetic resource conservation initiative' without due delay. In effect, it would be pertinent to establish an ad hoc committee comprising representatives from relevant national, regional and international institutions who shall be responsible for examining and resolving all aspects of conservation problems, development of comprehensive and sustainable coffee genetic resources conservation and use project, establishment of property rights and germplasm transfer agreements based on the principle of fair and equitable benefit sharing and internal regulations of sovereign states owning the genetic resources, negotiation with multi-donors to mobilize adequate amount of fund, and lobbying of relevant organizations and authorities for the success of the conservation program for the benefit of present and future generations. In this initiative, the Interafrican Coffee Organisation (IACO), International Coffee Organisation (ICO) and Bioversity International are relevant organisation to take the lead in facilitating and coordinating the implementation of the conservation endeavours.

ACKNOWLEDGEMENT

The authors express their appreciation and gratitude to CTA (accommodation, plane ticket and registration fee) and the Inter-African Coffee Organisation (allowance) for the financial support rendered to the first author to participate in the ASIC 2008 conference held from 14 – 19 September at Campinas city, Sao Paulo state, Brazil.

REFERENCES

Afrol News. (?). Forests and deforestation in Africa.: the wasting of an immense resources. http://www.afrol.com/feature/10278

PIESP. ??. Pacific Island Ecosystems at Risk Project (PIESP): Coffea liberica risk assessment. http://www.hear.org/pier/wra/pacific/coffea_liberica-htmlwra.htm

Bellachew B. and J.P. Labouisse. 2006. Arabica coffee (*Coffea arabica* L.) local landracedevelopment strategy in its center of origin and diversity. ASIC 2006, Montpellier, France.

Berthaud, J and Charrier, A.. 1988. Genetic resources of Coffea. In: R.J. Clarke and R. Macrae (eds), Coffee: Volume 4 – Agronomy. London, Elsevier Applied Sciences, PP. 1-42.

Branson, J. 2008. deforestation of African rainforests. http://www.ccds.charlotte.nc.us/history/Africa/03/bransom/branson.html.

Dulloo, M.E., L.Guarino, F.Engleman, N.Maxted, J.H.Newbury, F.Attere, and B.. V.ford-Lioyd .1998. Complementary conservation strategies for the genus coffea: a case study of Mascarene *Coffea* species. Genetic Resources and Crop Evolution, 45:565-579.

FAO. 2000. Biological diversity in food and agriculture: convention on biological diversity. http://www. fao. org/ biodiversity/cbd_en.asp

FAO. 2004. The international treaty on plant genetic resources for food and agriculture. http://www.fao.org/ag/cgrfa/itpgr.htm

Getachew mengistie. 2008. Capturing intangible value of coffee – the Ethiopian fine coffee designation of trade marking and licensing initiatives. ICO council meeting, 19-23 May 2008.

Hennig R.C. 2008. Forests and deforestation in Africa – the wasting of an immense resource. Afrol News, http://www.afrol.com/features/10278.

ICO. 2007. Renovation of CATIE's international coffee collection (project proposal), WP Board 1036/07. International coffee council, 27 and 28 September 2007, London, England.

Labouisse J.P., S. Kotecha. 2008. production systems and genetic resources of Arabica coffee in Ethiopia. SCAA 20th Annual conference, mineapolis, 2-5 May 2008.

Medina-Filho, H.P., M.P. Maluf, R. Bordignon, O. Guerreiro-Filho, L.C. Fazuoli. ?. Traditional breeding and modern genomics: a summary of tools and developments to exploit biodiversity for the benefit of coffee agroindustrial chain. Acta Horticulturae, http://www.actahort.org/members/showpdf?booknrarnr

Missionaries of Africa. 2008. A few facts about Africa. http://www.missionariesofafrica.org/africa/

Richerzhagen, C. and Virchow D. 2002. sustainable utilization of crop genetic diversity through property right mechanisms?: the case of coffee genetic resources in Ethiopia. BioEcon workshop, May 30-31, 2002.

Sondahl M.R, and H.A.M. van der Vossen. 2005. The plant: origin, production and botany. In: A. Illy and R.Viani (eds), Expresso Coffee – the Science of Quality (second edition). Elsevier Academic Press, Amsterdam.

Surendra K. 2008. Arabicas from the garden of Eden – Coffea Aethiopica. http://www.scae.com/news/214/arabicas.14pp

Wikipedia 2008. Ethiopian deforestation. Wikipedia, the free encyclopedia http://www.wikipedia.org/wiki/Ethiopia_deforestation.

Chemometric Discrimination of Coffee (*Coffea arabica* L.) Genotypes and Growing Origins

D. VILLARREAL[1], B. BERTRAND[2], A. LAFFARGUE[3], H. POSADA[1], P. LASHERMES[4], S. DUSSERT[3]

[1]Centro Nacional de Investigaciones de Café (Cenicafé), A.A.2427 Manizales, Caldas, Colombia
[2]CIRAD, UMR RPB, 911 Av. Agropolis, BP64501, 34394 Montpellier Cedex 5, France
[3]IRD, UMR DIAPC, 911 Av. Agropolis, BP64501, 34394 Montpellier Cedex 5, France
[4]IRD, UMR RPB, 911 Av. Agropolis, BP64501, 34394 Montpellier Cedex 5, France

SUMMARY

The objective of this work was to compare the effectiveness of three chemical families – namely elements, chlorogenic acids (CGA) and fatty acids (FA) – for the discrimination of Arabica genotypes (traditional *versus* modern introgressed lines) and potential *terroirs* within a given coffee growing area. The experimental design included three Colombian locations (Location 1, Location 2, and Location 3) in full combination with five (one traditional and four introgressed) Arabica genotypes and two field replications. Elements, chlorogenic acids and fatty acids were analyzed in coffee bean samples by ICP-AES, HPLC and GC, respectively. Analysis of variance (ANOVA), principal component analysis (PCA) and discriminant analysis (DA) were carried out to compare the three methods. A significant effect of the location was observed for almost all compounds measured, as inferred by two-way ANOVA, revealing the potential of the three chemical classes studied for discriminating coffee *terroirs* within a given country. The effect of the genotype was highly significant with most of the chlorogenic and fatty acids measured. By contrast, most of the elements analysed showed no significant differences among genotypes. Though elements provided an excellent classification of the three locations studied, as estimated by combined PCA-DA approach, this chemical class was useless for genotype discrimination. Chlorogenic acids gave satisfactory results, but fatty acids clearly offered the best results for the determination of both genotypes and environments, with very high percentage of correct classification (79 and 90%, respectively). In order to take advantage of both climatic and soil diversity, one major practical recommendation which can be drawn from the present work would thus to undertake the simultaneous analysis of FA and elements for coffee origin authentication.

RESUMEN

El objetivo de este trabajo fue el de comparar la eficacia de tres familias químicas a saber, los elementos, los ácidos clorogénicos (CGA) y los ácidos grasos (FA) para la discriminación de genotipos de Arabica (tradicionales *versus* genotipos introgresados) y su posible relación con las condiciones de origen "*terroirs*" dentro de una área determinada de cultivo de café. El diseño experimental incluyó una combinación de tres localidades Colombianas (Localidades 1, 2 y 3) y cinco genotipos de Arabica (uno tradicional y cuatro introgresados) con dos réplicas de campo. Los elementos, los ácidos clorogénicos y los ácidos grasos fueron analizados en muestras de granos de café por ICP-AES, HPLC y GC, respectivamente. Los análisis de varianza (ANOVA), de componentes principales (PCA) y discriminante (DA) fueron realizados para comparar los tres métodos. Un efecto significativo de la localidad fue observado para la mayoría de los compuestos analizados, revelando así el potencial de las tres

clases de compuestos químicos estudiados para la discriminación de *terroirs* para una región específica dentro un país determinado. El efecto del genotipo fue altamente significativo con la mayoría de los ácidos clorogénicos y de los ácidos grasos medidos. Por el contrario, la mayoría de los elementos analizados no mostraron diferencias significativas entre genotipos. Aunque los elementos proporcionaron una excelente clasificación de las tres localidades estudiadas, esta variable química fue menos útil para la discriminación de los genotipos. Los ácidos clorogénicos dieron resultados satisfactorios, pero los ácidos grasos ofrecieron claramente los mejores resultados para la determinación tanto de los genotipos como de los ambientes, con porcentajes muy altos de clasificación correcta (79 y 90%, respectivamente). Con el fin de aprovechar tanto de la diversidad del clima como de la diversidad de suelo, una importante recomendación práctica que puede extraerse de este trabajo sería la de llevar a cabo análisis simultáneos de ácidos grasos y elementos para la autenticación del origen del café.

INTRODUCTION

Coffee breeding programs have focused their efforts on transfer the resistance genes to main pest and diseases from *C. canephora* to *C. arabica*. Since 50 years ago breeding programs have transferred resistance to rust, root-knot nematodes and coffee berry disease from the Timor hybrid to traditional cultivars of *C. arabica*. Nevertheless, during this process the amount of alien genetic material (from the *C. canephora* genome thought the Timor hybrid) is substantial, ranging from 8% to 27%. Although some introgressed varieties of Arabica show a beverage quality similar to that of traditional varieties, most coffee buyers claim that they frequently possess a poorer beverage quality. Thus it seems likely that introgression process has not been restricted to resistance traits but could also involve undesirable genes. Therefore, development of effective methods for adequate identification of traditional and introgressed materials would provide an important tool for future selection strategies focusing cup quality.

Together with the discrimination of traditional and modern introgressed genotypes, there is an increasing demand for the determination of coffee origin (Tzouros et al., 2001). This includes not only the authentication of the country of origin, as carried out by Anderson and Smith (2002) using bean element composition, but also the differentiation of small *terroirs*, the emergence of which on the market is expanding rapidly. The environmental factors most frequently mentioned in *terroir* effects are altitude and, to a certain extent, rainfall (Avelino et al., 2005). Accordingly, within national markets of most mountainous countries, coffee originating from high-elevation areas fetches a higher price than coffees from low-altitude localities. Therefore, it seems also timely to develop appropriate methods for the determination of genetic and geographic bean origin within a country area.

The chemometric discrimination of the two coffee species *C. arabica* and *C. canephora* has been an active area of research during the last years. In addition to near-infrared (NIR) spectrometric approaches (Esteban-Díez et al., 2007), various chemical families such as amino acids, chlorogenic acids (CGA), lipids, elements, purinealkaloids, and sugars have been tested for this purpose (Carrera et al., 1998; Martín et al., 1998; Casal et al., 2000; González et al., 2001; Guerrero et al., 2001; Ky et al., 2001; Martín et al., 2001; Casal et al., 2003; Rui et al., 2003; Rubayiza and Meurens, 2005; Speer and Kölling-Speer, 2006). By contrast, very little has been done for the authentication of Arabica genotypes and geographic growing origins. To our knowledge the present study is the first to carry out a chemometric analysis of traditional (Caturra as the standard) and new introgressed Arabica lines based on the chemical profile of beans harvested in different environments, chosen to mimic potential *terroirs* within a given country. The objective of this work was not only to test the possibility to discriminate Arabica genotypes and their growing areas on the basis of their chemical

profile but also to compare, for the first time, the effectiveness of different chemical families (elements, chlorogenic acids, and fatty acids) for this purpose.

MATERIAL AND METHODS

Materials

The experimental design included three Colombian locations (Location 1, Location 2, and Location 3) in full combination with five *C. arabica* L. genotypes (four introgressed lines and Caturra) and two field replications (total of 30 coffee bean samples). The variety Caturra was selected for representing high-quality traditional varieties. The four introgressed lines (BGB.1033, BGB.1044, BGB.1076, and BGB.1040) are advanced lines (at least generation F5) derived from crosses between Caturra and the Timor hybrid accession CIFC-1343. The three locations studied represent the main coffee growing regions in Colombia. Samples were collected during the harvest peak, using healthy ripe cherries.

Storage and preparation of coffee samples

For each sample, green coffee beans were dried and stored over silica gel in a hermetic plastic box placed at room temperature in the dark. Then the coffee beans were reduced to a fine powder using an analytical grinder (IKA A15). The water content of powders was estimated after complete drying of 0.2 g aliquots in an oven at 105 °C overnight. The water content was measured in triplicate using a totally random experimental design.

Element determination

Nitrogen was determined according to the Dumas method (Ebling, 1968). The other elements (P, K, Ca, Mg, Fe, Cu, Zn, and B) were determined by inductively coupled argon plasma atomic emission spectrometry (ICP-AES) after dry mineralization. Samples were then analyzed by ICP-AES using a Varian-Vista MPX (Varian Inc., Palo Alto, CA), equipped with a CCD detector. All elements were determined in triplicate and expressed in milligrams per gram or micrograms per gram or ppm on a dry weight basis.

Chlorogenic acid measurement

Chlorogenic acids, namely, caffeoylquinic (3-CQA, 4-CQA, 5-CQA), feruloylquinic (3-FQA, 4-FQA, 5-FQA), dicaffeoylquinic (3,4-diCQA, 3,5-diCQA, 4,5-diCQA), caffeoylferuloylquinic (CFQA), and caffeic acids (CA), were determined by HPLC. CGA-like components, caffeoyl-tyrosine (CT) and caffeoyltryptophane (CTR), where the caffeic unit is coupled with an amino acid, were also measured in the same chromatogram. CGA were extracted at 20 °C in 70% aqueous methanol as described in Bertrand et al., 2008. Chromatographic separation was carried out on an Uptisphere ODB 5 μ column (250 mm × 4.6 mm) from Interchim. The LC equipment comprised a Varian 9010 pump, a Rheodyne valve with a 20 μL loop, and a UV detector (Shimadzu SPD 10AV). CGA isomers, as well as CGA-like components, were identified by comparison of their retention time at 327 nm with those of commercial standards (Sigma). All samples were analyzed in triplicate (from three different extractions) and CGA were expressed in milligrams per gram.

Fatty acid determination

Total lipids were extracted from 2 g samples of dried powder using a modified Folch method (Folch et al., 1957) with methylene chloride replacing chloroform. Extracted lipids were dried under nitrogen at 40 °C, then dissolved in 1 mL of methylene chloride/methanol (2:1), and stored at -20 °C until further analysis. Fatty acid methyl esters (FAMEs) were prepared according to the ISO-5509 standard. GC analyses were performed using an HP 6890 system with flame ionization detection (FID). FAMEs were identified by comparing their retention times with those of the fatty acid methyl ester standards (Supelco) and were quantified as percentages over total FA (w/w). For each genotype-environment combination studied, the fatty acid composition was analyzed in triplicate (from three different lipid extracts).

Data analysis

Data analyses were carried out using SAS (SAS, Cary, NC) for two-way ANOVAs and Statistica (Statsoft, Tulsa, OK) for PCA and discriminant analysis. For each chemical class studied, discriminant analysis was performed using principal components having eigenvalues > 1. All samples were used to establish the classification rule.

RESULTS AND DISCUSSION

Chemical composition of beans: Effects of location and genotype

The overall bean chemical composition obtained in the present study is in full agreement with previous reports dealing with the composition in elements (Martín et al., 1998; Etienne and Bertrand, 2001; Anderson and Smith, 2002), CGA (Guerrero et al., 2001; Ky et al., 2001), and FA (Dussert et al., 2001; Martín et al., 2001; Rui et al., 2003) of *C. arabica* and *C. canephora* beans. Because many reports have presented in detail the overall coffee bean composition for these three chemical families, they are not further described in the present study. A significant effect of the location was observed for almost all compounds measured, as inferred from two-way ANOVA, revealing the potential of the three chemical classes studied for discriminating coffee *terroirs* within a given country (Table 1).

Compounds exhibiting no differences among locations were potassium and iron, 5-CQA, caffeic acid (CA), and caffeoyltyrosine (CT), and oleic (18:1n-9) and lignoceric (24:0) acids for elements, chlorogenic acids, and fatty acids, respectively. All other constituents showed significant variations, ranging quantitatively from slight [e.g., 3–5% for nitrogen, 5-FQA, or palmitic (16:0) acid] to moderate [e.g., about 10% for 4,5-di CQA or linoleic (18:2) acid] to very high variations (up to 142% for Zn) (Table 1). By contrast with the effect of the location, most of the minerals and metals analyzed showed no significant differences among genotypes (Table 1, elements), suggesting that these elements offer a poor discriminating capacity of genotypes.

Conversely, the effect of the genotype was highly significant with most of the chlorogenic and fatty acids measured, with the exception of 3-CQA, 3-FQA, caffeic acid (CA), and caffeoyl-tyrosine (CT) and vaccenic (18:1n-7), behenic (22:0), and lignoceric acids (24:0) (Table 1, CGA and FA). Again, the degree of variations over genotypes differed considerably among the various constituents studied, ranging from low (e.g., palmitic acid, 16:0) to intermediate (e.g., oleic acid, 18:1n-9) to high (e.g., 3,4-diCQA) variability. It is worth mentioning that for various fatty and chlorogenic acids, the effect of introgression was monodirectional; that is, the value of Caturra beans was significantly lower- or higher- than those measured in the four introgressed genotypes, as assessed by post hoc Newman and Keuls' tests (Table 1).

For instance, introgression was always associated with a decrease in 3,5-diCQA or stearic acid (18:0) and an increase in oleic acid (18:1n-9). In some cases, the direction of the effect coincided with the difference reported in previous works between *C. arabica* and *C. canephora*. For example, the bean content in oleic acid of *C. canephora* is generally higher than that of *C. arabica* (Dussert et al., 2001; Martín et al., 2001; Rui et al., 2003). However, these occurrences were not observed with all FA and CGA, emphasizing that introgression of *C. canephora* genes in *C. arabica* may lead to complex regulations of metabolic pathways.

PCA of element, chlorogenic acid, and fatty acid data

For each of the three chemical families studied, the same statistical approach was performed. PCA was employed to set up noncorrelated -a prerequisite for discriminant analysis achievement- variables that contain the maximum of the initial variance. For all chemicals studied, PCA provided a similar pattern for the cumulative percentage of variance explained by the first principal components. In all cases, the first, the first two, and first three factors explained about 35, 60, and 75% of the total variance, respectively. This homogeneity in PC-explained variance offers here the opportunity to compare these three chemical classes for coffee chemometrics without any speculation about the impact of PCA results.

Discriminant analysis of genotypes

For the three chemical classes studied, the same approach was carried out. Factorial scores of PCs showing an eigenvalue >1 were used to calculate the discriminant function models. When the genotype was employed as the criterion, significant classifications were obtained with FA and CGA, as estimated by the *P* value ($P = 0.00$) associated with the Wilk's lambda coefficient (0.049 and 0.095, respectively), whereas genotypes could not be significantly discriminated ($P = 0.77$) according to their bean elemental composition (Wilk's $\lambda = 0.712$). The non significance of the discriminant analysis performed with elements is congruent with the non significance of the effect of the genotype on these traits, as analyzed by ANOVA (Table 1). The percentage of correct genotype classifications was satisfactory with both FA and CGA (ca. 70–80%), revealing for the first time the high potential of these two chemical families for the chemometric discrimination of *C. arabica* genotypes.

However, the proportion of well-classified samples was slightly higher with FA and, moreover, some Caturra (the quality standard employed here) samples were not appropriately classified with CGA, suggesting that the bean FA composition could be the best candidate among the three families tested for Caturra authentication. It is worth mentioning that the overall proportion of correct classification obtained with FA (79%) is similar to that (76%) obtained by Bertrand et al. (2005) on the basis of the NIR spectra of traditional and new introgressed varieties.

Two significant canonical functions were obtained using the discriminant model obtained with FA ($P = 0.001$), whereas with CGA only the first canonical function was significant ($P = 0.001$).

Table 1. Effects of the genotype and the environment on the Element, Chlorogenic acid (CGA, mg/g) and Fatty acid (FA, percent of total fatty acids) composition of green coffee beans: Means and probability of significance (*P*) as determined by two-way analysis de variance over five different genotypes and three different locations.*

Chemical composition		Genotypes						Locations			
		Caturra	BGB.1033	BGB.1044	BGB.1076	BGB.1040	*F* probability	Location 1	Location 2	Location 3	*F* probability
Elements	N (mg/g)	23.01a	22.58a	21.51b	22.61a	21.99ab	0.00	21.65b	22.81a	22.52a	0.00
	P (mg/g)	1.52bc	1.62a	1.45c	1.56ab	1.46c	0.00	1.47b	1.53a	1.57a	0.00
	K (mg/g)	14.53	15.14	14.68	14.12	14.39	0.68	14.27	15.20	14.24	0.17
	Ca (mg/g)	1.22	1.21	1.24	1.29	1.26	0.76	1.07b	1.36a	1.30a	0.00
	Mg (mg/g)	0.180a	0.165b	0.166b	0.172ab	0.169ab	0.00	0.148c	0.168b	0.196a	0.00
	Cu (ppm)	16.07	17.12	16.62	20.47	17.58	0.13	18.55a	19.75a	14.41b	0.00
	Fe (ppm)	45.60	42.70	41.95	51.42	53.73	0.81	45.46	44.07	51.71	0.72
	Zn (ppm)	11.88	10.07	13.02	19.12	13.18	0.58	21.83a	9.52b	9.01b	0.01
	B (ppm)	10.68	11.72	11.60	12.05	11.65	0.56	9.76a	12.75b	12.11b	0.00
CGA	3-CQA	3.70	3.83	3.54	3.65	3.70	0.76	3.45b	4.09a	3.49b	0.00
(mg/g)	4-CQA	5.37a	5.71a	5.78a	5.54a	5.79a	0.03	5.37b	6.05a	5.50b	0.00
	5-CQA	39.15b	38.72b	44.94a	42.21ab	46.51a	0.00	41.60	43.04	42.62	0.54
	3-FQA	0.06a	0.01ab	0.00b	0.03ab	0.01ab	0.05	0.01b	0.03ab	0.05a	0.03
	4-FQA	0.46a	0.32bc	0.35b	0.30c	0.34ab	0.00	0.32b	0.42a	0.30b	0.00
	5-FQA	3.52b	3.42b	3.83a	3.22c	3.50b	0.00	3.61a	3.43b	3.45b	0.02
	CFQA	0.19a	0.14b	0.10d	0.12c	0.10d	0.00	0.13b	0.14a	0.10c	0.00
	3,4-diCQA	1.16b	1.83a	0.99b	1.28b	0.94b	0.01	1.19b	1.62a	0.89b	0.00
	3,5-diCQA	5.52a	4.83b	4.47b	4.98b	4.42b	0.00	5.18a	3.74b	5.63a	0.00
	4,5-diCQA	2.96b	3.34a	2.75b	2.89b	2.56c	0.00	3.10a	2.78b	2.80b	0.00
	CA	0.17	0.19	0.12	0.32	0.25	0.59	0.16	0.13	0.35	0.17
	CTR	0.31b	0.52a	0.55a	0.49a	0.54a	0.00	0.47b	0.42b	0.58a	0.00
	CT	0.11	0.13	0.11	0.11	0.10	0.20	0.11	0.12	0.11	0.39
FA	16:0	33.31b	32.66c	33.91a	34.04a	33.32b	0.00	33.92a	32.77b	33.69a	0.00
(% TFA)	17:0	0.11a	0.11a	0.10b	0.11a	0.10b	0.00	0.10b	0.11a	0.11a	0.00
	18:0	7.90a	6.88c	7.26b	7.25b	7.42b	0.00	7.24b	7.74a	6.94c	0.00
	18:1n-9	8.94c	9.32b	9.83a	10.15a	9.96a	0.00	9.65	9.74	9.61	0.36
	18:1n-7	0.47	0.48	0.46	0.48	0.47	0.46	0.47b	0.46b	0.49a	0.00
	18:2	43.60b	45.20a	43.01e	42.39d	43.12c	0.00	43.20b	43.38b	43.84a	0.00
	18:3	1.48c	1.56a	1.52b	1.45d	1.56a	0.00	1.43c	1.58a	1.55b	0.00
	20:0	2.80a	2.52b	2.64ab	2.74a	2.73a	0.03	2.67ab	2.80a	2.55b	0.00
	20:1	0.33c	0.35ab	0.34bc	0.35a	0.34bc	0.00	0.35ab	0.33a	0.34b	0.00
	22:0	0.76	0.63	0.62	0.72	0.66	0.20	0.68ab	0.76a	0.59b	0.01
	24:0	0.25	0.25	0.25	0.28	0.25	0.49	0.25ab	0.28a	0.24b	0.06

*Means followed by the same letter are not significantly different at *P* = 0.05 as determined by the Newman and Keuls' test.

Scatterplots presented in Figure 1, based on canonical scores of the 30 samples analyzed, illustrate that the bean FA composition allowed a better discrimination of Caturra and BGB.1033 bean samples than the CGA composition. Interestingly, the two introgressed lines, which are derived from the same plant at generations F1, F2, and F3, BGB.1044 and BGB.1040, could not be satisfactorily discriminated on the basis of either their FA or CGA profiles.

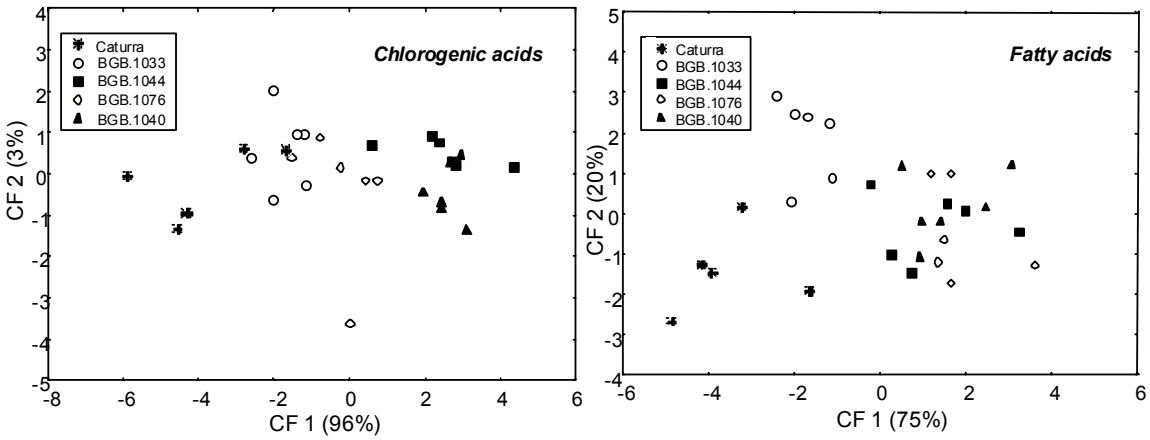


Figure 1. Scatterplot of canonical scores for the first two canonical functions resulting from the discriminant analysis of the 5 genotypes studied (Caturra, BGB.1033, BGB.1044, BGB.1076, and BGB.1040) based on bean chlorogenic acid and fatty acid composition.

Discriminant analysis of locations

The bean elemental composition provided an excellent classification of the 30 samples studied, because 100% of samples analyzed were correctly classified through discriminant analysis (Wilk's $\lambda = 0.080$, $P = 0.00$). The proportion of well-classified samples was also very satisfactory with FA (ca. 90%) and acceptable with CGA (ca. 83%). With elements, the first canonical function allowed an excellent separation of Location 1 samples, on one side, and Location 2 and Location 3 samples, on the other side (Figure 2). By contrast, with FA and CGA, the first axis offered a very good partition of Location 2 samples from samples collected in the two other locations. In all cases, the second canonical function separated the two locations that could not be discriminated by the first canonical function. However, although this partition on the second axis was satisfactory with elements and FA, an overlapping of Location 1 and Location 3 samples was observed with CGA.

These results reveal for the first time the potential value of elements and FA and, to a lower extent, CGA for coffee terroirs determination. The excellent location classification obtained here with elements confirms the very high proportion of coffee samples correctly classified according to their environment using this chemical class (Anderson and Smith, 2002).

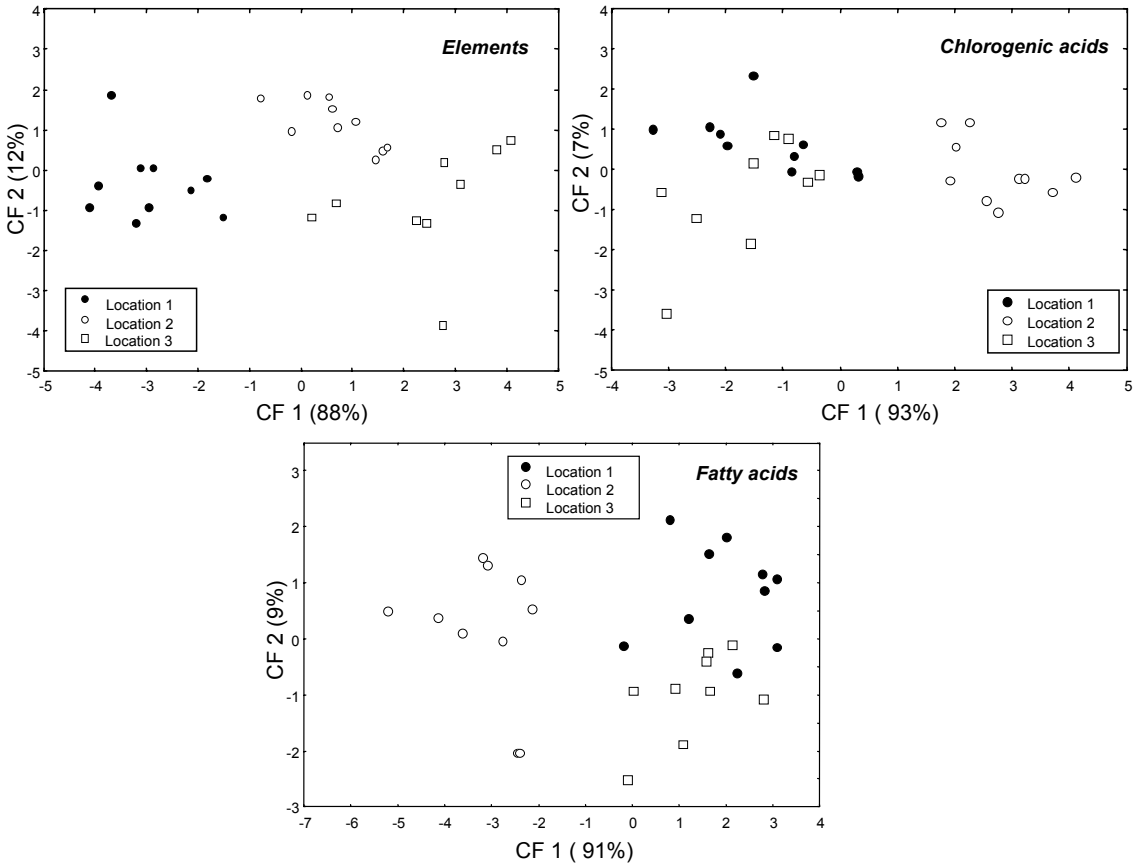


Figure 2. Scatterplot of canonical scores for the first two canonical functions resulting from the discriminant analysis of the 3 locations studied (Location 1, Location 2, And Location 3) based on bean element, chlorogenic acid, and fatty acid composition.

The effectiveness of elements for origin determination is very likely associated with the fact that the mineral and trace metal composition of beans reflects the composition of the soil in which the plants grow, which certainly varies significantly among locations. Whereas the utility of FA has been unambiguously shown for the discrimination of Robusta and Arabica green coffees (Martín et al., 2001; Rui et al., 2003), to our knowledge, the present study establishes for the first time their value for determining coffee genotypes as well as environments.

ACKNOWLEDGEMENTS

The authors greatly acknowledge the financial support of the National Federation of Coffee Growers of Colombia and the Colombian Ministry of the Agriculture.

REFERENCES

Anderson K. A., Smith B. W. Chemical profiling to differentiate geographic growing origins of coffee. J. Agric. Food Chem. 2002, 50, 2068-2075.

Avelino J., Barboza B., Araya J.C., Fonseca C., Davrieux F., Guyot B., Cilas C. Effects of slope exposure, altitude and yield on coffee quality in two altitude terroirs of Costa Rica, Orosi and Santa María de Dota. J. Sci. Food Agric. 2005, 85, 1869-1876.

Bertrand B., Etienne H., Lashermes P., Guyot B., Davrieux F. Can near infrared reflectance of green coffee be used to detect introgression in *Coffee arabica* cultivars. J. Sci. Food Agric. 2005, 85, 955-962.

Bertrand B., Villarreal D., Laffargue A., Posada H., Lashermes P., Dussert S. Comparison of the effectiveness of fatty acids, chlorogenic acids and elements for the chemometric discrimination of coffee (Coffea arabica L.) varieties and growing origins. J. Agric. Food Chem. 2008, 56, 2273-2280.

Casal S., Oliveira M.B., Alves M.R., Ferreira M.A. Discriminate analysis of roasted coffee varieties for trigonelline, nicotinic acid, and caffeine content. J. Agric. Food Chem. 2000, 48, 3420-3424.

Casal S., Rui Alves M., Mendes E., Oliveira M.B.P.P., Ferreira, M. A. Discrimination between Arabica and Robusta coffee species on the basis of their amino acid enantiomers. J. Agric. Food Chem. 2003, 51, 6495-6501.

Carrera F., Leon-Camacho M., Pablos F., Gonzales A.G. Authentication of green coffee varieties according to their sterolic profile. Anal. Chim. Acta 1998, *370*, 131-139.

Dussert S., Chabrillange N., Rocquelin G., Engelmann F., Lopez M., Hamon S. Tolerance of coffee (*Coffea* spp.) seeds to ultra-low temperature exposure in relation to calorimetric properties of tissue water, lipid composition and cooling procedure. Physiol. Plant. 2001, 112, 495-504.

Ebling M.E. The Dumas method for nitrogen in feeds. J. Assoc. Off. Anal. Chem. 1968, 51, 766-770.

Esteban-Díez I., González-Sáiz J.M., Sáenz-González C., Pizzaro C. Coffee varietal differentiation based on near infrared spectroscopy. Talanta 2007, 71, 221-229.

Etienne H., Bertrand B. Trueness-to-type and agronomic characteristics of *Coffea arabica* trees micropropagated by the embryonic cell suspension technique. Tree Physiol. 2001, 21, 1031-1038.

Folch J., Lees M., Sloane Stanley G.H. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 1957, 226, 497-509.

González A.G., Pablos F., Martín M.J., León-Camacho M., Valdenebro M.S. HPLC analysis of tocopherols and triglycerides in coffee and their use as authentication parameters. Food Chem. 2001, *73*, 93-101.

Guerrero G., Suarez M., Moreno G. Chlorogenic acids as a potential criterion in coffee genotype selections. J. Agric. Food Chem. 2001, 49, 2454-2458.

ISO-5509. Animal and Vegetable Fats and Oils Preparation of Methyl Esters of Fatty Acids; AFNOR: Paris, France, 2000; pp 36.

Ky C.L., Louarn J., Dussert S., Guyot B., Hamon S., Noirot M. Caffeine, trigonelline, chlorogenic acids and sucrose diversity in wild *Coffea arabica* L. and *C. canephora* P. accessions. Food Chem. 2001, *75*, 223-230.

Martín M.J., Pablos F., González A.G. Characterization of green coffee varieties according to their metal content. Anal. Chim. *Acta* 1998, *358*, 177–183.

Martín M.J., Pablos F., González A.G., Valdenebro M.S., León-Camacho M. Fatty acid profiles as discriminant parameters for coffee varieties differentiation. Talanta, 2001, *54*, 291-297.

Rubayiza A.B., Meurens M. Chemical discrimination of Arabica and Robusta coffees by Fourier transform raman spectrometry. J. Agric. Food Chem. 2005, 53, 4654-4659.

Rui Alves M., Casal S., Oliveira M.B.P.P., Ferreira M.A. Contribution of FA profile obtained by high-resolution GC/ chemometric techniques to the authenticity of green and roasted coffee varieties. JAOCS 2003, 80, 511-517.

Speer K., Kölling-Speer I. The lipid fraction of the coffee bean. Braz. J. Plant Physiol. 2006, 18, 201-216.

Tzouros N.E. Arvanitoyannis I.S. Agricultural produces: synopsis of employed quality control methods for the authentication of foods and application of chemometrics for the classification of foods according to their variety or geographical origin. Crit. Rev. Food Sci. Nutr. 2001, 41, 287-319.

Yield Stability Over Several Years in *Coffea canephora*: Definition of Synthetic Traits and Longitudinal Data Analyses

C. CILAS, C. MONTAGNON

CIRAD, TA A31/02, 34398, Montpellier, France

SUMMARY

The effectiveness of the perennial plants breeding is restricted by particular constraints linked to the crops lifespan. Moreover, acting of a production of fruits or seeds as to the coffee-tree, how many years of observation are necessary to define the trait of "production"? And how to integrate the variations of the trait in time with a breeding purpose in which stability and durability are often mentioned? To try to answer these questions, a trial comparing 20 clones of *Coffea canephora*, monitored over 9 years of production, was analyzed according to various methods. Two main methods were proposed: i) the first one proposed indexes definition – the indexes described precocity, alternation and intensity of inter-annual variations; ii) the second one proposed a modelization of the data by a longitudinal data analysis approach. It was found that the first cycle (first 4 years) are not always sufficient for predicting the yield capacity of the clones. The intensity of the relative differences between yields in successive years was a trait that was only heritable in the second production cycle. Low intensities for those differences were favorable to cumulative production. Strong alternations were not propitious to optimizing cumulative production. Several models were tested for longitudinal data analyses and *Compound Symetric's* one with heterogeneous variances (CSH model) allowed to better describe data structure. Thus, correlations between yields of successive years were pretty stable that reveals an important trees' effect among this clonal population. Variability was found for yield distribution over time. It is therefore possible to define "stability" traits and to select cultivars with lower annual yield variations, thereby ensuring regular income for coffee farmers.

INTRODUCTION

Cumulative production over several years of observations is often one of the only agronomic selection criteria used in fruit tree cultivation. However, different strategies exist that can culminate in identical cumulative yields, some varieties being early yielders, some being more stable over time; but that distribution of yields over time is only rarely taken into account in breeding. Yet, annual yield stability is of interest to growers, who count on regular income from their crops. Annual yield stability is therefore a trait that ought to be taken into account more, to enable genetic control of fruit load distribution over time.

For coffee producers, cumulative yields over a large number of years is the priority target trait for selection, but the "ways" of achieving the same accumulation are not equivalent: early-yielding cultivars that are stable over time are generally preferred. The first annual yields – when the bushes are young – have often been used to test their ability to predict cumulative yields over several years (Cilas et al., 1998; Montagnon, 2000), but few studies have been made of production stability over time with a view to improving yield distribution over time (Cilas et al., 2003). It is very important to study this stability trait because alternation phenomena are often reported (Coste, 1989; Amoah et al., 1997). Such alternation phenomena are a major drawback for growers. Apart from the applied aspect of such a study, it also

proves important to gain a clearer understanding of production strategies in perennial fruit species, particularly with a view to finding out whether several strategies might exist within the same species. If genetic variability existed for those temporal characteristics, it could be used to select varieties that derive the most from certain cultivated ecosystems, depending on the cultural techniques adopted and farmers' cropping strategies – notably the pruning system.

Production stability between successive years was examined in a clonal trial involving the species *Coffea canephora* Pierre, monitored over 9 years of production: during the first 4-year cycle, then, after cutting back, for a cycle of 5 years after a year of vegetative growth without yields. The species *C. canephora* produces robusta coffee, which has less appreciated sensory qualities than arabica coffee (Coste, 1989), hence it is traded at a lower price. It nonetheless remains an essential product for the hot, humid tropics at low elevations, and some robusta varieties can achieve the qualities of certain arabica coffees when post-harvest processing is carried out properly. The coffee trees of this species are generally less susceptible to diseases and pests, and are often more productive, though sometimes with considerable irregularities.

MATERIAL AND METHODS

Planting material and experimental design

The planting material comprised 20 clones, selected on research stations from controlled crosses, or surveyed on smallholdings (Table 1). The genetic origin of clones was determined in compliance with the known genetic diversity of *C. canephora,* composed of two large genetic groups: Guinean and Congolese, whose hybrids display group heterosis (Berthaud et al., 1984; Leroy, 1993).

Table 1. Genetic origin of the 20 clones used.

Clones	Origin	Genetic group
503	Open pollination of Clone A1	Congolese
512, 513	410 x 411	Guinean x Congolese
526, 529	410 x 464	Guinean x Congolese
539	178 x A03	Congolese x Congolese
586, 587, 588, 594	181 x A03	Inter-group hybrid x Congolese
589	181 x 182	Inter-group hybrid x Congolese
609	A01 x 200	Congolese x Congolese
619, 621	392 x 200	Congolese x Congolese
119, 126, 305, 461	Selected in plantations	Inter-group hybrids
202, 392	Selected at INEAC (ex Zaire)	Congolese

The trial was set up at the Divo research station belonging to *Centre National de Recherches Agronomiques* (CNRA) in Ivory Coast, in a totally randomized single-tree plot design. There were 30 replicates per clone. The planting density was 1667 plants per hectare, i.e. a spacing of 3 m x 2 m. The coffee trees were grown from horticultural cuttings and were planted in 1987; they were grown freely on three stems. Mineral fertilization corresponded to the usual recommendations.

Yields were estimated annually and cumulative production was estimated over the first cycle (4 production years: from 1989 to 1992), over the second cycle (5 production years: from 1994 to 1998) and for the nine years as a whole. The yields were expressed in kilograms of green coffee per hectare (y_i being the yields in year No. i).

Construction of indexes, and statistical analyses

With tree crops, how can we process the information provided by several years of observations? When fruit or seed production is involved, the first trait that is generally used is the accumulation over all the years for which harvests are available. Annual yields may also be analysed. However, with *n* years of production available, one might imagine constructing *n* quantitative traits that possess a biological sense. The first analysis carried out was a Principal Components Analysis (PCA), which was used to summarize the information and recover the main components. The principal components were then interpreted and the heritability values for the newly constructed traits were estimated. The other avenue investigated was constructing synthetic traits linked to yield distribution over time. That approach was proposed by Monselise and Goldschmidt (1982) and has been used on mango (Reddy *et al.*, 2002), but it has never been used to define selection criteria. In addition, such indexes making it possible to determine yield distribution over time had never been used on coffee. It was therefore a matter of constructing indexes, or rather composite traits with a biological sense.

The first composite trait we used was an earliness index (EI):

$$EI = \frac{ny_1 + (n-1)y_2 + ... + 2y_{n-1} + y_n}{nY}$$

In our case, we constructed one earliness index for the first cycle, one for the second cycle, and an overall earliness index:

$$EI_1 = \frac{4y_1 + 3y_2 + 2y_3 + y_4}{4Y_{1-4}} \;\; ; \;\; EI_2 = \frac{5y_6 + 4y_7 + 3y_8 + 2y_9 + y_{10}}{5Y_{6-10}} \;\; ;$$

$$EI_g = \frac{9y_1 + 8y_2 + 7y_3 + 6y_4 + 5y_6 + 4y_7 + 3y_8 + 2y_9 + y_{10}}{9Y_{1-10}}$$

The overall index calculated over 10 years took 9 years of production into account, given that year 5 was a year without yields, as the trees were cut back after the harvest in year 4.

We then defined different two-yearly indexes. The first was an intensity index for the differences between successive years, *I,* for the first two cycles, and for both cycles together:

$$I_1 = \frac{1}{3}\left(\frac{|y_2 - y_1|}{y_2 + y_1} + \frac{|y_3 - y_2|}{y_3 + y_2} + \frac{|y_4 - y_3|}{y_4 + y_3}\right) \;\; ; \;\; I_2 = \frac{1}{4}\left(\frac{|y_7 - y_6|}{y_7 + y_6} + \frac{|y_8 - y_7|}{y_8 + y_7} + \frac{|y_9 - y_8|}{y_9 + y_8} + \frac{|y_{10} - y_9|}{y_{10} + y_9}\right)$$

$$I_g = \frac{1}{7}\left(\frac{|y_2 - y_1|}{y_2 + y_1} + \frac{|y_3 - y_2|}{y_3 + y_2} + \frac{|y_4 - y_3|}{y_4 + y_3} + \frac{|y_7 - y_6|}{y_7 + y_6} + \frac{|y_8 - y_7|}{y_8 + y_7} + \frac{|y_9 - y_8|}{y_9 + y_8} + \frac{|y_{10} - y_9|}{y_{10} + y_9}\right)$$

An alternation index was then defined. This was a matter of characterizing the property of certain coffee trees in which a year of high yields was followed by a year of low yields, and a year of low yields was followed by a year of high yields. These yields in peaks and troughs were characterized by differences between successive production years that changed sign between successive differences. Alternation index *A* was therefore the sum of an indicative variable *B* (0,1).

If $y_{i+1} - y_i$ was of the same sign as $y_{i+2} - y_{i+1}$, or if one of the two differences was nil then B_i=0;

If $y_{i+1} - y_i$ was of the opposite sign to $y_{i+2} - y_{i+1}$, and if none of the differences was nil then B_i=1;

We therefore defined an alternation index for the first cycle, an index for the second cycle and an index for both cycles together:

$$A_1 = \sum_{i=1}^{4} B_i \;\; ; \;\; A_2 = \sum_{i=6}^{10} B_i \;\; ; \;\; A_g = A_1 + A_2$$

We also used an index of maximum intensity for the differences between successive years:

$$MI = \frac{\max(|y_{i+1} - y_i|)}{Y_{1-10}}$$

The broad sense heritability for these different traits was estimated, along with the associated confidence intervals. The confidence intervals were estimated by the Wald method (Agresti and Coull, 1998). We also estimated the genetic and environmental correlations between some of the traits.

Another avenue investigated was to describe relationships between successive years by longitudinal data analyses with mixed models (Verbeke and Molenberghs, 2000). Repeated data analyses allowed to precise autocorrelation structures of individual data on time. Several models were investigated: a first-order autoregressive model, with homogenous variances (AR), with heterogeneous variances (ARH), a *Compound Symetric* model (i.e. with constant correlation between years) with homogenous variances (CS), with heterogeneous variances (CSH) and the unstructured model (UN) where correlations between different years are independent as well as variances of the different years. Models can be written as:

$$Y_{ijkl} = \mu + C_i + A_j + (CA)_{ij} + b_k + E_{ijkl}$$

with: μ : mean
C_i : clone i effect
A_j : year j effect
$(CA)_{ij}$: clone x year interaction
b_k : bloc k effect
E_{ijkl} : residual of tree l belonging to clone i bloc k for the year j
Y_{ijkl}: yield of tree l belonging to clone i bloc k for the year j

and :

1- in the first-order autoregressive model:

$V(E_{ijk}) = \sigma^2$ if the variances are homogenous between years (AR1)
$= \sigma_j^2$ otherwise (ARH1)
and $\text{Corr}(E_{ijkl}, E_{i'j'k'l'}) = \rho^{|j-j'|}$ if i=i' , k=k', l=l'
0 otherwise

2- in the Compound Symetry model:

$V(E_{ijkl}) = \sigma^2$ if the variances are homogenous between years (CS)

$= \sigma_j^2$ otherwise (CSH)

and $Corr(E_{ijkl}, E_{i'j'k'l'}) = \rho$ if $i=i'$, $k=k'$, $l=l'$

0 otherwise

3- in the unstructured model:

$V(E_{ijkl}) = \sigma_j^2$

and $Corr(E_{ijkl}, E_{i'j'k'l'}) = \rho_{jj'}$ if $i=i'$, $k=k'$, $l=l'$

0 otherwise

To choose the most suitable model, several criteria exist: log of likelyhood, Akaike criteria (AIC) or Schwartz criteria (BIC); lower these criteria are better the model is. When two models are nested, the comparison of their likelyhood allows to choose the best one without ambiguity (Verbeke and Molenberg, 2000).

Table 2. Means, broad sense heritabilities and confidence intervals for the broad sense heritabilities relative to yield traits.

Trait	Mean	h^2	Confidence interval at 95%
y1	25.33	0.286	[0.140, 0.432]
y2	5.49	0.001	[0, 0.007]
y3	95.21	0.450	[0.278, 0.622]
y4	7.15	0.383	[0.223, 0.543]
y1-4	133.18	0.452	[0.282, 0.623]
y6	11.86	0.466	[0.298, 0.633]
y7	23.27	0.405	[0.241, 0.569]
y8	43.77	0.523	[0.358, 0.688]
y9	84.14	0.482	[0.313, 0.650]
y10	30.95	0.464	[0.297, 0.630]
y6-10	193.99	0.615	[0.459, 0.771]
y1-10	327.17	0.607	[0.449, 0.765]
PC1		0.629	[0.475, 0.783]
PC2		-0.002	[-0.025, 0.021]
PC3		0.266	[0.129, 0.403]

Where PC1, PC2 and PC3 are the first 3 principal components of the PCA.

RESULTS

Annual yields and cumulative yields

The broad sense heritabilities for the annual and cumulative yield traits are shown, as are the associated confidence intervals (Table 2); the means for those traits for the trial as a whole are also shown. Alternation was found for mean yields for the first cycle: over 4 years, a year of high yields was followed by a year of low yields. That was no longer the case in the second cycle, during which average yields increased over the first four years and decreased in the 5th year. After initial cutting back, a more developed root system probably limited competition between fruit production and vegetative growth. Cumulative yields over several years had higher heritabilities than annual yields. It was thus necessary to take several years into account for the productive ability of the genotypes to be expressed. The lowest heritability, which was not significantly different from 0, concerned the lowest annual yields for the trial

as a whole. Unfavourable environmental conditions may therefore have masked the genetic potential of the coffee trees.

Principal components analysis

The Principal Components Analysis was used to represent linkages between yields in the different years (Figure 1). For instance, annual yields were closely linked, except for the yields in 1990, which were very low and atypical. The first principal component was linked to cumulative production, whereas the second was linked to the yields in 1990. These first two axes accounted for 55.5% of total variability (44.1% for the first component and 11.4% for the second). The 3rd component accounted for no more than 8.7% of total variability and could be used to separate yields in the first cycle from those in the second cycle. The heritabilities for the components were estimated at the same time as those for annual and cumulative yields (Table 2).

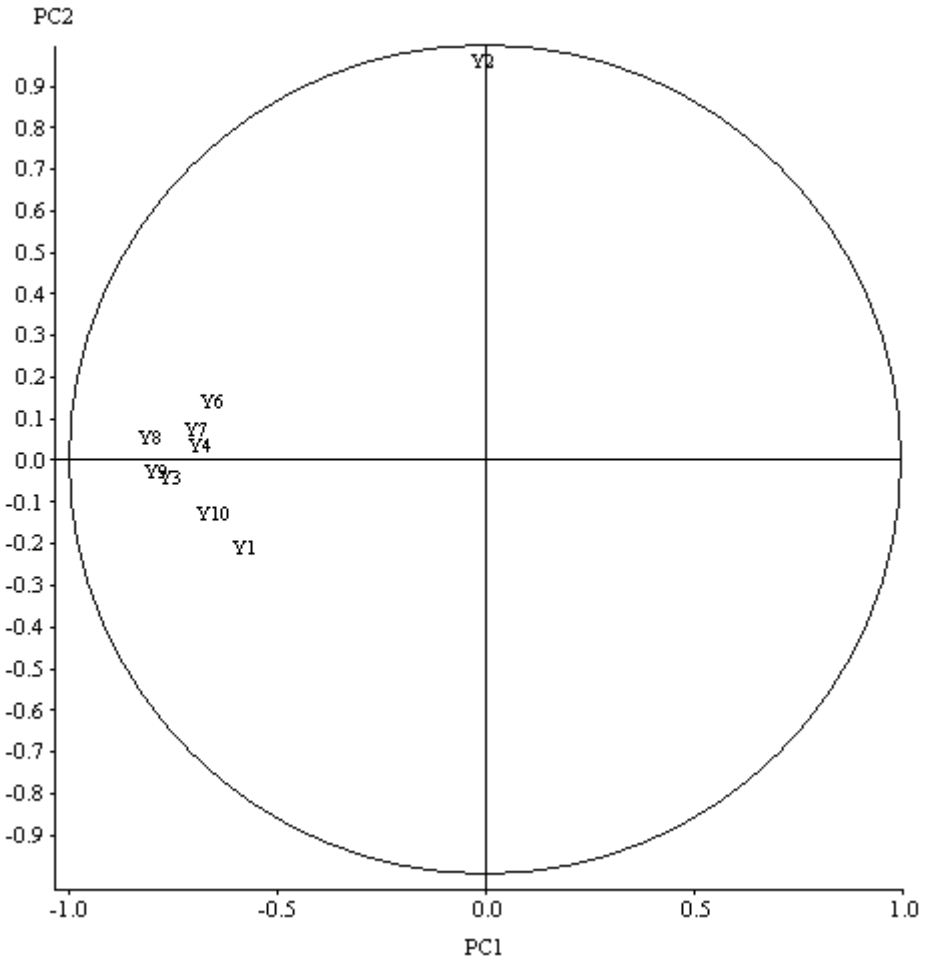


Figure 1. Principal Components Analysis. Circle of correlations between annual yields.

Synthetic traits

The same parameters – heritabilities and associated confidence intervals – were estimated for the synthetic traits described earlier (Table 3). The earliness traits were heritable irrespective of the cycle considered. The intensity of differences between yields in successive years was only heritable for the second cycle and for the 2 cycles together. The alternation traits had low, but significant, heritability.

Table 3. Means, broad sense heritabilities and confidence intervals for the broad sense heritabilities relative to the synthetic traits defined.

Trait	Mean	h^2	Confidence interval at 95%
EI_1	0.596	0.307	[0.150, 0.464]
EI_2	0.497	0.258	[0.122, 0.395]
EI_g	0.501	0.211	[0.089, 0.333]
I_1	0.867	0.026	[0, 0.068]
I_2	0.448	0.378	[0.218, 0.538]
I_g	0.628	0.267	[0.128, 0.406]
A_1	1.83	0.088	[0.006, 0.169]
A_2	0.516	0.096	[0.020, 0.172]
A_g	0.675	0.061	[0.001, 0.121]
MI	0.334	0.286	[0.143, 0.428]

Clone classification and correlations

In order to gain a clearer picture of the linkages between these traits, we classed the clones for the most heritable traits (Tables 4 and 5). Genetic correlations between the traits were then estimated (Table 6).

The classification for annual yields evolved over time (Table 4). The most productive clones appeared to be those which had lower alternation intensities (Table 5), with lower I_g and *MI* values. The earliness of the first cycle did not seem to be a trait that was favourable for cumulative yields (Table 5).

The genetic and environmental correlations between yields in successive years provided a clearer understanding of production trends (Table 6). The genetic correlations were relatively stable, between 0.418 and 0.916, and the correlations were not higher for years close to each other. The environmental correlations were more variable and tended to decrease the further the years were apart.
The correlations between cumulative production and the synthetic traits defined earlier were used to identify those characteristics that were conducive to good overall production (table 7). First cycle earliness was not a favourable criterion for cumulative production. In addition, the more intense the differences between successive years, the lower were the cumulative yields (*Ig* and *MI* correlated negatively with *Y1-10*). The most stable clones were therefore also the most productive over the 2 production cycles.

Table 4. Comparison of the clones for yield traits: Newman-Keuls multiple comparison of means test at 5%.

Clones	*y1-10*	*y1-4*	*y6-10*	*Y1*	*y2*	*y3*	*y4*	*y6*	*y 7*	y*8*	*y 9*	*y10*
503	558 **a**	215 **a**	343 **a**	38 **ab**	6	151 **ab**	21 **a**	26 **a**	49 **a**	107 **a**	122 **ab**	38 **bcde**
526	514 **ab**	178 **ab**	337 **a**	37 **ab**	5	121 **bcd**	15 **b**	21 **ab**	35 **bc**	82 **b**	134 **a**	66 **a**
119	480 **abc**	188 **ab**	292 **a**	35 **ab**	5	126 **bc**	22 **a**	19 **bc**	36 **bc**	49 **cd**	139 **a**	49 **b**
588	449 **bcd**	215 **a**	234 **b**	35 **ab**	5	167 **a**	8 **bcde**	13 **cde**	23 **cde**	46 **cde**	90 **bcde**	62 **a**
461	414 **cde**	179 **ab**	235 **b**	48 **a**	8	110 **cde**	13 **bc**	9 **ef**	23 **cde**	52 **cd**	104 **bc**	47 **bc**
594	382 **def**	151 **bc**	231 **b**	29 **bc**	4	114 **cde**	5 **cde**	23 **ab**	24 **cd**	49 **cd**	99 **bcd**	35 **bcdef**
539	360 **defg**	139 **bcd**	221 **bc**	27 **bcd**	5	101 **cdef**	6 **cde**	18 **bcd**	29 **bc**	53 **cd**	90 **bcde**	31 **cdefg**
126	350 **efg**	120 **cde**	230 **b**	20 **bcd**	6	89 **cdef**	5 **cde**	6 **ef**	41 **ab**	56 **c**	98 **bcd**	29 **defgh**
529	331 **efg**	114 **cde**	217 **bc**	22 **bcd**	8	81 **def**	3 **de**	7 **ef**	24 **cd**	44 **cde**	98 **bcd**	44 **bcd**
512	317 **efg**	124 **cde**	192 **bcd**	19 **bcd**	1	93 **cdef**	11 **bcd**	7 **ef**	24 **cde**	39 **cde**	95 **bcd**	27 **defgh**
305	308 **gh**	121 **cde**	186 **bcd**	30 **abc**	6	82 **def**	3 **de**	9 **ef**	35 **bc**	33 **def**	99 **bcd**	11 **h**
619	266 **gh**	128 **cde**	138 **def**	36 **ab**	6	84 **cdef**	2 **e**	8 **ef**	23 **cde**	27 **ef**	66 **defg**	14 **gh**
609	266 **gh**	126 **cde**	140 **def**	12 **cd**	6	99 **cdef**	9 **bcde**	17 **bcd**	11 **def**	33 **def**	59 **efg**	21 **efgh**
513	259 **gh**	95 **cdef**	165 **cde**	11 **cd**	8	70 **efg**	5 **cde**	5 **ef**	23 **cde**	38 **cde**	81 **cdef**	18 **fgh**
621	202 **hi**	82 **def**	120 **efg**	35 **ab**	5	41 **gh**	1 **e**	2 **f**	12 **def**	23 **ef**	61 **efg**	22 **efgh**
589	201 **hi**	98 **cdef**	103 **efg**	25 **bcd**	4	70 **efg**	0.1 **e**	1 **f**	8 **ef**	26 **ef**	41 **gh**	27 **defgh**
587	201 **hi**	114 **cde**	87 **fg**	12 **cd**	5	97 **cd**	0.2 **e**	12 **de**	2 **f**	26 **ef**	34 **gh**	13 **gh**
202	179 **hi**	76 **efg**	103 **efg**	11 **cd**	5	59 **fg**	0.3 **e**	3 **f**	8 **def**	24 **ef**	56 **fg**	12 **gh**
586	122 **i**	52 **fg**	70 **g**	7 **d**	5	39 **gh**	1 **e**	11 **ed**	6 **f**	12 **f**	24 **h**	15 **gh**
392	110 **i**	35 **g**	76 **fg**	7 **d**	10	16 **h**	1 **e**	2 **f**	9 **def**	12 **f**	40 **gh**	12 **gh**

Table 5. Comparison of the clones for synthetic traits: Newman-Keuls multiple comparison of means test at 5%.

Clones	EI_1	EI_2	I_2	I_g	A_g	MI
503	0.572 **ef**	0.541 **ab**	0.377 **ef**	0.576 **de**	0.653 **ab**	0.266 **def**
526	0.587 **def**	0.490 **bcdef**	0.351 **f**	0.555 **e**	0.680 **ab**	0.242 **e**
119	0.567 **ef**	0.487 **bcdef**	0.391 **ef**	0.586 **cde**	0.690 **ab**	0.289 **cdef**
588	0.574 **ef**	0.459 **def**	0.325 **f**	0.566 **e**	0.648 **ab**	0.372 **bcd**
461	0.626 **cde**	0.466 **cdef**	0.414 **def**	0.600 **bcde**	0.593 **ab**	0.258 **ef**
594	0.594 **def**	0.518 **abcd**	0.354 **f**	0.592 **bcde**	0.759 **a**	0.308 **cdef**
539	0.593 **def**	0.521 **abcd**	0.374 **ef**	0.581 **bcde**	0.693 **ab**	0.281 **cdef**
126	0.587 **def**	0.508 **bcde**	0.454 **cdef**	0.619 **bcde**	0.690 **ab**	0.277 **def**
529	0.609 **cdef**	0.463 **cdef**	0.410 **ef**	0.602 **cde**	0.640 **ab**	0.275 **def**
512	0.550 **ef**	0.470 **cdef**	0.452 **cdef**	0.645 **bcde**	0.680 **ab**	0.355 **bcde**
305	0.630 **cd**	0.520 **abcd**	0.560 **bc**	0.701 **b**	0.752 **a**	0.384 **bc**
619	0.672 **bc**	0.529 **abc**	0.493 **bcde**	0.660 **bcde**	0.717 **ab**	0.362 **bcde**
609	0.542 **f**	0.522 **abcd**	0.401 **ef**	0.598 **bcde**	0.733 **a**	0.370 **bcd**
513	0.576 **ef**	0.491 **bcdef**	0.492 **bcde**	0.646 **bcde**	0.607 **ab**	0.348 **bcde**
621	0.735 **a**	0.446 **ef**	0.540 **bcd**	0.688 **bcd**	0.655 **ab**	0.324 **cdef**
589	0.628 **cde**	0.428 **f**	0.443 **cdef**	0.658 **bcde**	0.593 **ab**	0.388 **bc**
587	0.565 **ef**	0.569 **a**	0.729 **a**	0.817 **a**	0.752 **a**	0.493 **a**
202	0.692 **def**	0.467 **cdef**	0.565 **bc**	0.693 **bc**	0.621 **ab**	0.384 **bc**
586	0.577 **ef**	0.539 **ab**	0.449 **cdef**	0.604 **bcde**	0.683 **ab**	0.354 **bcde**
392	0.713 **ab**	0.452 **ef**	0.602 **b**	0.657 **bcde**	0.580 **b**	0.440 **ab**

Table 6. Correlations between annual yields and cumulative yields (genetic, lower triangle; environmental, upper triangle) .

	y1	*y3*	*y4*	*y6*	*y7*	*y8*	*y9*	*y10*	*y1-10*
y1	-	0.347	0.207	0.157	0.196	0.261	0.158	0.109	0.481
y3	0.672	-	0.248	0.325	0.266	0.333	0.401	0.256	0.778
y4	0.594	0.753	-	0.158	0.225	0.302	0.213	0.140	0.410
y6	0.418	0.767	0.712	-	0.295	0.265	0.374	0.213	0.505
y7	0.623	0.642	0.717	0.565	-	0.360	0.286	0.110	0.514
y8	0.614	0.773	0.790	0.733	0.846	-	0.341	0.261	0.633
y9	0.737	0.739	0.844	0.618	0.916	0.831	-	0.354	0.729
y10	0.688	0.769	0.695	0.541	0.529	0.688	0.747	-	0.494
y1-10	0.783	0.904	0.876	0.757	0.855	0.915	0.938	0.838	-

Table 7. Correlations between synthetic traits and cumulative yields (genetic, lower triangle; environmental, upper triangle).

	EI_1	EI_2	I_2	I_g	A_g	*MI*	*y1-10*
EI_1	-	0.028	-0.051	-0.059	-0.127	-0.136	-0.068
EI_2	-0.461	-	-0.189	-0.157	0.059	-0.071	0.065
I_2	0.376	0.204	-	0.726	0.122	0.247	-0.136
I_g	0.307	0.212	0.988	-	0.303	0.228	-0.111
A_g	-0.378	0.963	0.110	0.162	-	-0.029	0.068
MI	0.147	0.119	0.792	0.844	0.226	-	0.262
y1-10	-0.433	0.129	-0.719	-0.690	0.148	-0.747	-

Longitudinal data analyses

Among the tested models, the ARH1 model allowed a better adjustment than the AR1 model and the CSH model allowed a better adjustment than the CS model (table 8). The CSH model gave an AIC and a BIC similar to those of the UN model. With the UN model, we have to estimate 45 parameters, but only 10 with the CSH model. The UN model took into account the irregularities of the correlations' values between the yields of the different years; these correlations, estimated with the UN model, are the environmental correlations between the yields of the different years (Table 6). With the ARH1 model, the correlation between the yields of two successive years was r = 0.22 and between the yields of two years (y and y + i) the correlation was $r_i = (0.22)^i$. With the CS model, the correlations between two years (y and y + i) was, for all i, r = 0.178 whereas with the CSH model r = 0.204. The CS model, like the AR1 model, needed only 2 estimated parameters; however these two models were removed on the basis of the adjustment's criteria (AIC, BIC) values (table 8). The best adjustment of the CSH model indicated that a tree effect existed within each clone. This effect means that either strong micro-environmental effects on yield expression existed, or individual tree effects, linked to tree life or tree origin, existed within clonal effects; and this effect persists on time. With this autcorrelations' control in the model, a significant interaction "clone x year" remained ($F_{152, 4104} = 12.70$, with $p < 0.0001$).

Table 8. Choice criteria of longitudinal data analysis model for annual yield.

Model	**-2 Log Vrais.**	**AIC**	**BIC**
AR1	42309	42313	42322
ARH1	39772	39792	39835
CS	42078	42082	42091
CSH	39540	39560	39603
UN	39321	39411	39603

DISCUSSION AND CONCLUSION

Different methods were thus used to judge the production stability of clones over successive production years. The Principal Components Analysis did not bring out any traits indicating yield distribution over time. That analysis indicated that annual yields per tree were generally positively correlated. When we considered mean plot yields, we found alternating production in the first cycle, but that was no longer the case in the second cycle, during which average

yields rose in the first 4 years, then decreased in the 5th year. Alternation phenomena, which have often been reported in coffee, appeared primarily to exist in the first production cycle, when the root systems of the trees had not developed much. The heritabilities for annual yields were generally higher for second cycle yields than for first cycle yields, and the highest heritabilities were for cumulative yields.

The intensity of relative differences between yields in successive years was a trait that was only heritable in the second production cycle. The low intensities found for those differences were favourable for cumulative yields. First cycle earliness was genetically and negatively correlated with cumulative yields. The productive clones were therefore more regular over time and high annual yields penalized their production capacity less in the following years.

Longitudinal data analyses supplied complementary points of view. These analyses allowed to define the correlations' structure between the individual tree yields of the different years. A first-order autoregressive model with heterogeneous variances fitted well data. However, the *Compound Symetric* model with heterogeneous variances (CSH) was the best one. This model indicated that correlations were constant between individual yields of the different years. This good adjustment revealed an important tree effect within the tested clones. A significant interaction "clone x year" remained and this interaction was mainly due to the clones with extreme yields – high or low. The « clone x environment » interaction was a *contrario* lower for the high yields clones (Montagnon *et al*, 2000). Thus, different years cannot be considered as different sites probably because of the positive autocorrelations at tree scale.

Annual yields are therefore not a stable trait in the *C. canephora* coffee tree. There is greater alternation in the first cycle when trees have yet to be subject to pruning. It is likely that competition between vegetative growth and fruit production is less once the root systems are more developed. Variability was found for yield distribution over time within the planting material studied. It is therefore possible to select cultivars with lower annual yield variations, thereby ensuring regular income for coffee farmers.

REFERENCES

Agresti A., Coull B.A., 1998. Approximate is better than "Exact" for interval estimation of binomial proportions. The American Statistician 52: 119-126.

Amoah F.M., Osei-Bonsu K., Oppong F.K., 1997. The effect of shade, stem number and spacing on robusta coffee yield in Ghana. In: 17th International conference on coffee science, Nairobi, Kenya, 20-25 July 1997. ASIC, Paris, p. 528-534.

Berthaud J., Charrier A., Guillaumet J.L., Lourd M., 1984. Les caféiers. *In*: Gestion des ressources génétiques des plantes. Tome 1: Monographies/ Pernes J, pp. 45-104.

Cilas C., Bouharmont P., Boccara M., Eskes A.B., Baradat Ph., 1998. Prediction of genetic value for coffee production in *Coffea arabica* from a half-diallel with lines and hybrids. *Euphytica* 104: 49-59.

Cilas C., Bouharmont P., Bar-Hen A., 2003. Yield stability in *Coffea canephora* from diallel mating designs monitored for 14 years. Heredity, 91, 528-532.

Coste R., 1989. Caféiers et cafés. Ed. Maisonneuve et Larose. Paris, France, 373 p.

Leroy T., 1993. Diversité, paramètres génétiques et amélioration par sélectionrécurrente réciproque du caféier *Coffea canephora* P. ENSA doctorate thesis, Rennes, 147 p.

Monselise S.P., Goldschmidt E.E., 1982. Alternate bearing in fruit trees. In Janick J (Ed.) *Hort. Rev.* 4, AV Publishing Co. Inc., Connecticut, pp. 128-173.

Montagnon C., 2000. Optimisation des gains génétiques dans le schéma de sélection récurrente réciproque de *Coffea canephora* Pierre. ENSA doctorate thesis, Montpellier, 133 p.

Montagnon C., Cilas C, Leroy T, Yapo A, Charmetant P, 2000. Genotype-location interactions for *Coffea canephora* yield in the Ivory Coast. *Agronomie*, 20, 101-109.

Reddy Y.T.N., Kurian R.M., Ramachander P.R., Singh G., Kohli R.R., 2002. Long-term effects of rootstocks on growth and fruit yielding patterns of "Alphonso" mango (*Mangifera indica* L.). *Scientia Horticulturae* 97, 95-108.

Verbeke G., Molenberghs G., 2000. Linear mixed models for longitudinal data. Springer Verlag, New York.

Assessment of the Genetic Diversity of Wild Coffee Populations from Southeastern Ethiopia Using ISSR Marker

K. TESFAYE[1,2], S. BALEMI[1], E. BEKELE[1], K. GOVERS[3], T. BORSCH[3]

[1]Department of Biology, Addis Ababa University, Addis Ababa, Ethiopia
[2]Ethiopian Coffee Forest Forum
[3]Botanical Garden und Botanical Museum of Berlin-Dahlem & Institute for Biology/Botany, Free University of Berlin, Berlin, Germany

SUMMARY

Coffea arabica L. in its natural habitat occurs as an undergrowth in the Afromontane rainforests of southwest and southeast Ethiopia. Genetic diversity analysis of wild Arabica coffee populations using Inter Simple Sequence Repeats (ISSR) markers have shown higher diversity than landraces and cultivars. The present study was conducted in the Harenna Forest of southeastern Ethiopia. Hundred individuals of wild coffee trees were selected representing four populations: two from semi-disturbed (Harenna population-1 and Harenna population -3) and two from undisturbed (Harenna population -4 and Harenna population-6). The Inter Simple Sequence Repeats marker system with nine primers (seven di- and two tetranucleotide) were employed to evaluate the genetic diversity of the wild coffee populations. A total of 137 bands were detected and the number of bands per ISSR primer ranges from 10 to 21 with an average of 15.2. Out of the total bands produced, 61 (44.5%) were polymorphic and the number of polymorphic bands per ISSR primer ranges from 1 to 19, with an averages of 6.8. The similarities between individual genotypes were estimated using UPGMA (unweighted pair group method with arithmetic mean) and NJ (neighbor-joining) analysis. The populations were found to be clustered based on their respective origin. The UPGMA cluster analysis showed that the four populations form two major clusters according to locations from which they were collected. The two major clusters further divided into two based on populations' origin. Analysis of molecular variance (AMOVA) indicated that genetic diversity within population level was relatively high (56.8%). Such partioning of the total genetic diversity could be attributed to gene flow via insect pollinators, seed flow by wild animals, birds and human. Generally, ISSR marker was able to reveal moderate genetic diversity within and among wild coffee populations located in the southeastern parts of the country.

INTRODUCTION

Coffees are member of the tribe Coffeeae of the large family Rubiaceae and genus *Coffea* (Davis et al., 2007). All *Coffea* species are native to tropical forest of Africa, Madagascar and islands of the Indian Ocean (Mascarene islands). All the species are diploid (2n = 2x = 22), except *Coffea arabica* (2n = 4x = 44) which is autogamous (self-fertile) and considered as allotetraploid. The genus *Coffea* comprises approximately 103 species currently accepted (Davis et al., 2007).

Coffee is currently being produced in more than 50 countries, and for many developing countries it is the most important source of foreign currency. Out of the total 103 *Coffea* species of the genus, the commercial coffee production relies only on two species of coffee (*C. arabica* and *C. canephora*) (Coste, 1992). Of the two commercially important species

better quality coffee is associated with *Coffea arabica* which contributes over 70 percent of the world's coffee production (Raina et al., 1998).

Arabica coffee (*Coffea arabica*) originates from the Ethiopian highlands where it also has its primary centers of diversity. Wild populations of *C. arabica* grow naturally in the undergrowth of the montane rain forests in southwest and southeast Ethiopia at altitudes between 1,400 and 1,900 m. (Nigatu and Tadesse,1989; Gole et al., 2003). However, Senbeta (2006) described the altitudinal range between 1,300-1,600 m a.s.l. as critical limit for the occurrence wild coffee.

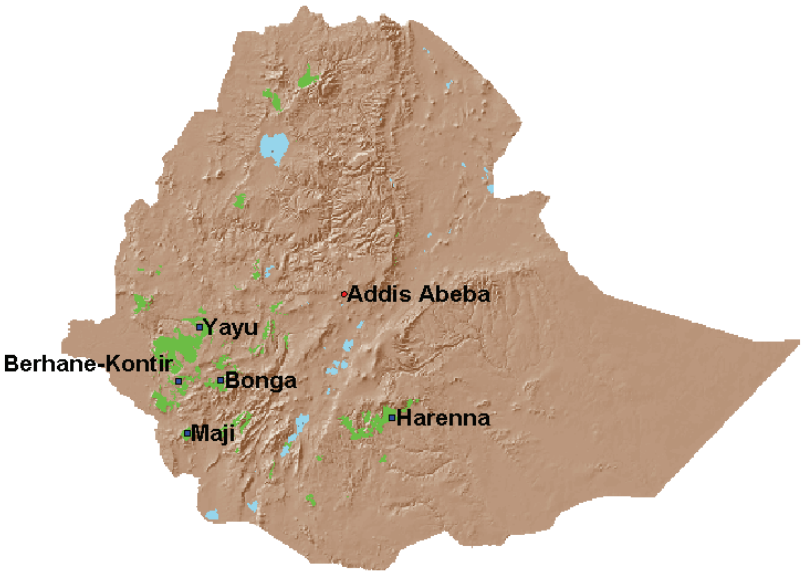
Arabica coffee is the only coffee species that occurs naturally in Ethiopia.The populations of wild coffee in SW and SE Ethiopia is observed to be very diverse in terms of phytopathological and ecophysiological traits (Adugna and Hindorf, 2001; Kufa, 2006). More importantly, the genetic diversity analysis of wild populations collected from all over the country showed the presence of enormous genetic diversity (Anthony et al., 2001; Aga, 2005; Tesfaye, 2006). The comparative study of wild population with landraces collected from different parts of the country further illustrated the existence of distinct true wild coffee in the Afromontane rainforests of Ethiopia with diversity varies from region to regions. Furthermore, the intraregional diversity analysis of coffee from west of the Great Rift Valley (Berhane Kontir and Yayu) with dense sampling of individuals demonstrates considerable genetic diversity within both regions. Moreover, it shows a predominant fine scale spatial patterning of genotypes and adjacent individuals are more likely to be genetically similar than distant individuals (Tesfaye, 2006).

It was reported that the wild *Coffea arabica* from South west part of Ethiopia showed a relatively high genetic diversity as compared to the only wild coffee found in Southeastern Ethiopia (Anthony et al., 2001; Chaparro et al., 2004). However, in most of these studies, wild *Coffea arabica* from Bale either were not included at all or not well represented. Recent study by Aga (2005) and Tesfaye (2006) indicate the presence of diverse genotypes, though they used limited samples. ISSR marker showed high level of genetic diversity within and among the wild C*offea arabica*, and this led to their selection to study the intraregional diversity of wild coffee occurs in Harrena forest, Bale Mountain (Tesfaye, 2006; Aga, 2005).

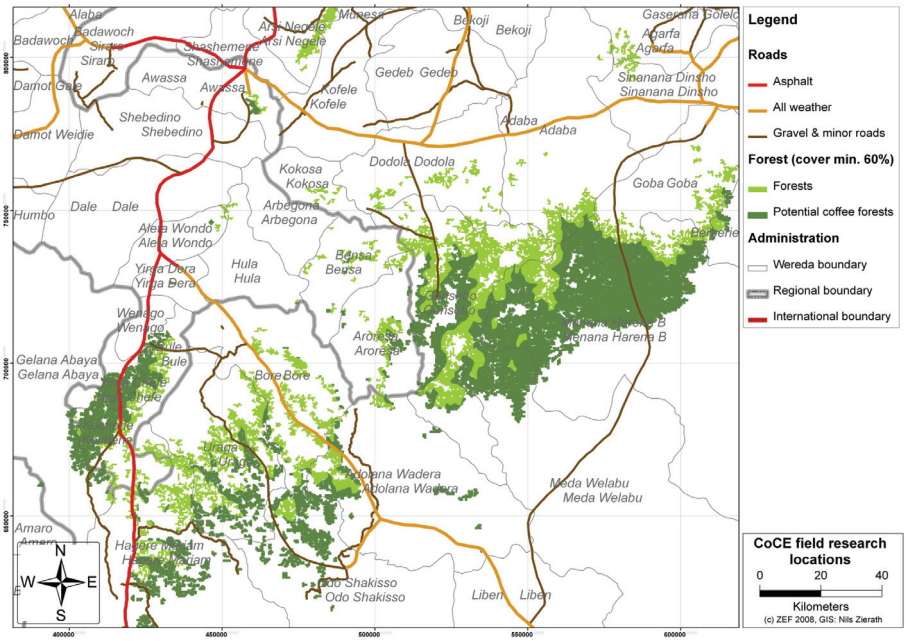
Generally, except very few studies made by Tesfaye (2006) and Aga (2005) on the standing population of wild coffee in Harenna, the genetic diversity within the forest *Coffea arabica* gene pool in Ethiopia has not been extensively studied using molecular markers. This shows that little attention is given to Harenna wild coffee that is considered as drought tolerant as compared to other wild coffee regions (Kufa, 2006). With the current climate change and increment of global heat, the genotypes in Harenna can play a key role in breeding for moisture stress environments (Kufa, 2006; Tesfaye, 2006). Hence, the present study is to understand the extent of genetic diversity and its patterns of distribution wild *Coffea arabica* in Harenna forest in order to generate information for conservation and use plan.

MATERIALS AND METHODS

The study was carried out in Harenna forest of Bale Mountain located in the South Eastern part of Ethiopia. (Figure 1). The Harenna forest is located in the Bale Zone of the Oromia State. It is the most eastern Afromontane rainforest and constitutes the largest subsection of the Bale Mountains National Park. Bale mountains are peaked by the afro-alpine Sanetti Plateau which reaches up to 4,300 meter asl. The Harenna forest lies between 6^{o} and 7^{o} N and 39^{o} and 40^{o} E with altitudinal range of 1300 and 3000 m a.s.l. However, forest coffee only occurs in the lower lying areas of the forest between 1300 and 1850 m a.s.l.



A



B

Figure 1. A) Map of Ethiopia showing the forest where wild coffee occurs. B) The detailed map of the study area forest.

Plant Materials and Sampling strategies

Individual arabica coffee trees from two years to very old were randomly sampled from four plots with a size of 50 X 50 m. The fifty by fifty meter plots selected for this analysis were considered as a population. Young leaves were collected from individual sample and dried in silica gel. Overall 25 to 27 individuals were collected from each population/plot and in total 105 individuals were sampled.

DNA Extraction

Total genomic DNA was extracted from a total of 105 silica gel dried young leaves of *C. arabica* following a modified version of triple CTAB extraction (Borsch et al., 2003). Only one of the three extractions was selected based on the amount and purity of the DNA after running on agarose gel (0.98%).

ISSR primers and ISSR-PCR amplification

ISSR (Inter Simple Sequence Repeat) marker was used to carry out the intraregional diversity assessment of *Coffea arabica* in Harenna forest. This research project execute as the follow up of CoCE-I with in the framework of the CoCE-II project (Conservation and Use of Wild Populations of *Coffea arabica* in the Montane Rainforests of Ethiopia; www.coffee.uni-bonn.de).

The PCR protocol described in Tesfaye (2006) were used for this intraregional analysis. Therefore, a total of nine primers, seven di-nucleotide (810, 812, 813, 814, 818, 834, and 844) and two tetra-nucleotide (CoIS001, CoIS002) primers were chosen and used for ISSR amplification in this analysis.

ISSR bands were scored as present '1' or absent '0'. The resulting binary matrix was employed to calculate UPGMA (unweighted pair group methods on arithmetic average) and PCO (principal coordinate analysis) diagrams based on Jaccard's similarity coefficient.

RESULT AND DISCUSSION

ISSR primers and level of polymorphism

ISSR markers are observed to be highly variable within species and reveal more polymorphisms since they use longer primers that allow more stringent annealing temperatures (Zietkiewicz et al., 1994; Wolf and Liston, 1998). In this study overall 137 scorable bands were generated by the seven di-nucleotide and two tetra-nucleotide ISSR primers for 105 individuals of *Coffea arabica* representing four populations of Harenna forest, Bale Mountain. The size of the band generated ranged from 400 to 3000bp. The number of bands produced by each primer varied from 10 bands for 814 to 21 for CoISOO1 primers and with an average of 6.8 fragments (Table 1). Out of the total 137 scorable bands produced with nine ISSR primers only 61 bands were observed to be polymorphic.

In terms of level of polymorphism per classes of primer, tetranucleotides were found to be superior. While the seven di-nucleotide primers generated 99 bands, the two tetra-nucleotide primers alone generated 38 bands, of which 26 and 35 were polymorphic loci respectively (Table 1).The same patterns were reported by Tesfaye (2006) on arabica coffee collected from Southwestern Ethiopia. Moreover, Raus et al. (2003) obtained 100% polymorphism with five tetra primers including CoIS001 and CoIS002 in the study of genetic relationship in *Coffea* species and parentage determination of inter-specific hybrid crosses.

Table 1. Lists of all primers used in the analysis, primer sequence, number of scorable bands, number of polymorphic loci, percentages of polymorphic loci for *Coffea arabica* individuals collected from Harenna wild populations (Balemi et al., submitted).

Primers	Sequence	No of Scorable bands	No of PL loci	%PL
810	GAGAGAGAGAGAGAGAT	16	5	31.3
812	GAGAGAGAGAGAGAGAA	18	4	22.2
813	CTCTCTCTCTCTCTCTT	16	3	18.8
814	CTCTCTCTCTCTCTCTA	10	1	10.0
818	CACACACACACACACAG	11	3	27.3
834	AGAGAGAGAGAGAGAGYT	12	4	33.3
844	CTCTCTCTCTCTCTCTRC	16	6	37.5
CoIS001	CCTACCTACCTACCTA	21	19	90.5
CoIS002	GGTAGGTAGGTAGGTA	17	16	94.2
Total		137	61	43.5

Intraregional genetic diversity

The overall analysis with both dinucleotide and tetranucleotide primers, the number and percentage of polymorphic bands within populations varied from 40 (29.2%) for population 1 to 50 (36.5%) for population 3. However, the analysis with only di-nucleotides only, ranged from 9 (9.1%) to 19 (19.2%) and with tetra nucleotides 25 (65.8%) for population 4 to 31 (81.6%) for population 1 and 3. This also shows the higher diversity revealed by the two tetra-nucleotide primers (Table 1 and 2).

Table 2. Genetic variation of *Coffea arabica* population interims of number of polymorphic locus (PL) and percent polymorphism (%PL) based on 137 ISSR bands with all primers together (di- and tetra-nucleotides) (Balemi et al., submitted).

Populations	No of plant Examined	Total No PL			% PL		
		Di-+ tetra	Di-nucl.	Tetra-nucl.	Di-+ tetra-nucl	Di-nucl	Tetra-nucl.
Population-1	22	40	9	31	29.2	9.1	81.6
Population-3	27	50	19	31	36.5	19.2	81.6
Population-4	26	42	17	25	30.7	17.2	65.8
Population-6	25	42	15	27	30.7	15.2	71.1
Total	100	61	26	35	44.5	26.3	92.1
Mean	25	43.5	15	28.5	31.7	15.2	75.0

Among the four populations analyzed in this study, Population-1 is the least diverse while Population -3 is more variable for all primers. Generally, out of the total primers employed in the analysis, tetra- nucleotide showed a significantly maximum polymorphism while the smallest amount of polymorphism resulted from di-nucleotide primers both in terms of the number and percent polymorphisms.

Using the over all nine ISSR primers: seven di- (810-H, 812-H, 813-H, 814-H, 818-H, 834-H and 844-H) and two tetra-nucleotides (CoIS001 and CoIS002) we found out the existence of

divers populations of arabica coffee in Harenna forest, Bale Mountain. The results of the genetic diversity study indicated the presence of moderate levels of genetic diversity within and among sub-divided populations of Harenna forest.

The genetic diversity with in population ranges from P = 29.2% for population Population-1 to P = 56.56% for population-3 and both of them are from semi-disturbed region. However, the genetic diversity for Population-4 and -6, both of which from undisturbed regions was found to be equal with P = 30.66% slightly larger than population-1 (Table 2). This means that the genetic diversity was high for Population-3 and lower for Population-1, both of which from semi-disturbed region, indicating that higher and lower variability were obtained from semi-disturbed regions of the forest. This could be attributed to the levels of management implemented in the two populations. In this case Population-1 has been intensively managed than population-3 since population-1 is very close to Magnette village. The closer the plot to the village, the more chance to replace the standing original populations with more uniform high yielding one. However, Tesfaye (2006) observed general trends of a slightly higher diversity of semi-disturbed plots than undisturbed one in south western Ethiopia.

On the basis of percent polymorphisms, the total genetic diversity for the whole populations was 44.5%. A relatively larger genetic variability was observed in this study which is comparable with what Tesfaye (2006) found for wild coffee in South western Ethiopia. Shannon's diversity index ranged from 0.24 for population-1 to 0.30 for population-3 and followed the same patterns of genetic diversity as in the case of percentages of PL (Table 2 and 3). The interregional diversity assessment by Tesfaye (2006) and Aga (2005) showed low level of diversity for coffee samples collected from Bale Mountain. This could be because of the absence of other forest pockets accommodating wild coffee in eastern part of the Great Rift Valley. The other possible reason could be the fire incident in Bale Mountain which is common as compared to South West. Moreover, extreme environment in both sides of the Harenna forest (Chilling temperature on the tope of Bale Mountain and extremely dry and higher temperature from the low land of Bale) could also be other reasons.

Clustering and principal coordinate analysis

In UPGMA clustering of an over all analysis, all the individuals clustered according to their respective origin of the populations in the region in to two major groups representing semi-disturbed (Population -1and Population-3) and undisturbed (Population-4 and Population-6) groups (Figure 2). Clear patterns of grouping were observed except in few intermixing of individuals from other population. Similar patterns of grouping were also observed in the case of NJ (tree not shown, see for detail Balemi et al. submitted).

The data also subjected to PCO analysis using Jaccard's similarity coefficient. The plot of the first three principal coordinate analysis of the data with eigenvalues ranging from 1.5 to 4.8 accounts for 14.8%, 5.3% and 4.5% of the total variance (24.6% cumulative; Figure 3). PCO revealed that population-4 and -6 were clearly separated from the rest, while the population -1 and -3 observed to occupy parallel position on the 3D spaces of the PCO and tend to spread and intermix.

The UPGMA dendrogram has two main cluster or group in which individuals from semi disturbed and undisturbed regions are separated. These could be because of human selection process in the forest for particular traits. The semi-disturbed populations tend to be more scattered and inter-mixed with individuals from the other populations as compared to the undisturbed populations. The grouping pattern observed was similar to those of Oljira (2006) and Tesfaye (2006) in the intraregional analysis of Yayu and Berhan Kontir regions.

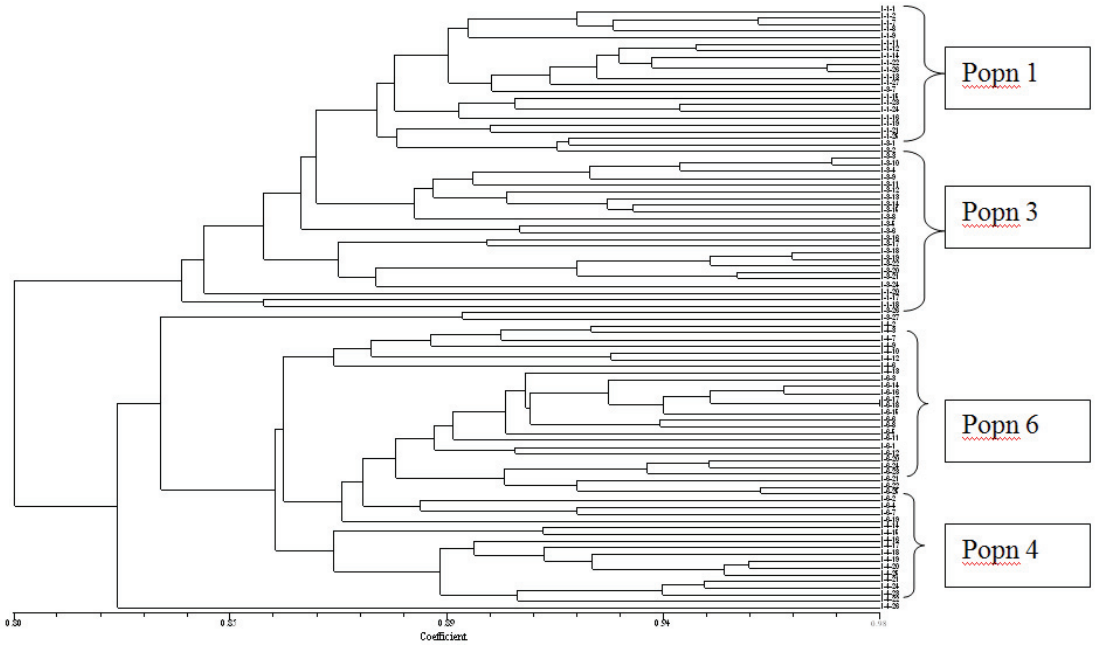


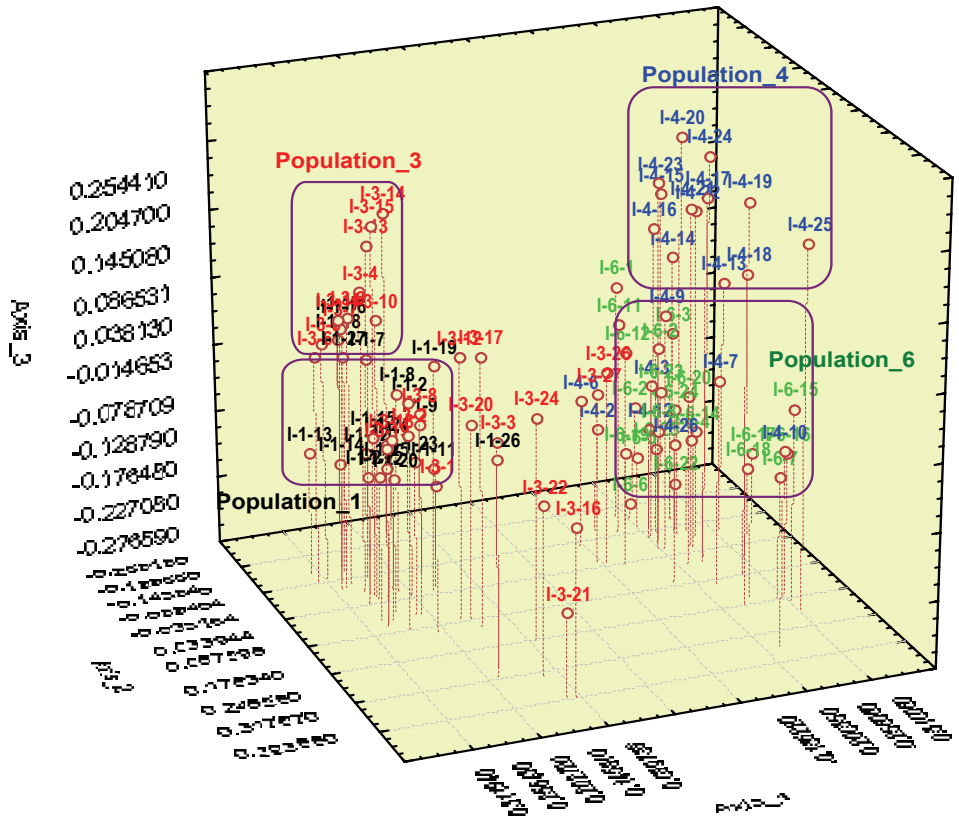
Figure 2. Dendrogram generated based on UPGMA analysis demonstrating the genetic similarity between one hundred individuals of forest *Coffea arabica* L population using seven di (810-H, 812-H, 813-H, 814-H, 818-H, 834-H and 844-H) and two tetra-nucleotides (CoISoo1 and CoISOO2) data. The diagram was base on the Jaccard's coefficients of similarity from 137 ISSR fragments (modified from Balemi et al., submitted).

Partitions of genetic variation

Shannon's diversity index was used to partition the existing genetic variation in to different components (Table 3). Accordingly, higher within population diversity (84%) were observed than among populations (16%).Moreover, the analysis of molecular variance (AMOVA) indicated that genetic diversity within population level was relatively high (56.8%, data not shown, for detail see Balemi et al., submitted) and showed the same pattern like that of Shannon's index.

Life form and reproductive biology are the main factor to determine the structure of populations (Hamrick and Godt, 1996). It is also observed that the selfing plant species tend to retain 51% the genetic variation among populations, while outcrossing species present on average 10% of the variation between populations (Hamrick and Godt, 1989).

In arabica coffee population, higher among population diversity was expected than within population diversity as the plant is predominantly self-pollinated species (Carvalho et al., 1969; Charrier and Berthaud, 1985). However, Mayer (1965) observed considerable amount of out crossing rate (40-60%) among wild and semi-wild *Coffea arabica* populations' from montane rain forests of Ethiopia. The result generally indicated more genetic diversity within populations (84%) rather than among populations (16%) in an over all analysis by Shannon's diversity index analysis. Oljira (2006) observed the general trends of the larger within population (57%) than among population (43%) in his study conducted at Yayu forest of Ethiopia.



III)

Figure 3. The two (I and II) and three dimensional (III) representation of PCO analysis of the genetic relation ship among 100 individuals of wild *Coffae arabica* L. obtained from Harenna on the basis of Jaccard's similarity matrix of 137 ISSR markers using nine primers: seven di (810-H, 812-H, 813-H, 814-H, 818-H, 834-H and 844-H) and two tetra-nucleotides (CoIS001 and CoIS002) (Modified from Balemi et al., submitted).

Although the levels of variations are different, the patterns of this result were similar to the result of Tesfaye (2006) obtained from studies on samples from south west Ethiopia (Brhane Kontir and Yayu). The result of Tesfaye (2006) study also indicated high genetic diversity within populations (63%) than between population (37%) in Brhane Kontir wild coffee populations. Similar patterns of high genetic diversity within populations (53%) than between population (47%) were obtained in Yayu.

The higher within populations' genetic diversity rather than among population diversity might be accounted to two reasons. It could be speculated from the result that wild *Coffea arabica* might have mixed mating system (partial out-crossing by pollen and partial selfing) for which some extent of gene flow is expected as reported by Loveless and Hamrik, (1984) for other species which could result in high within genetic diversity. Secondly, insects, birds and monkeys could also play a role in disseminating pollen and seed of *C. arabica* and facilitates gene flow among different populations in Harenna forest.

Table3. Summary of Shannon's genetic diversity index for each population and all individuals of wild *Coffae arabica* L. from Harenna forest and partitioning of the genetic variation in to within and among populations (Balemi et al., submitted).

Parameters	Di-nucleotide primers	Tetra-nucleotide primers	Over all
Population-1	0.24	0.08	0.65
Population-3	0.30	0.14	0.73
Popuplation-4	0.26	0.15	0.56
Population-6	0.26	0.12	0.64
H-popn	0.12	0.64	0.27
H-species	0.21	0.73	0.35
H-popn/Hspp	0.83	0.86	0.84
1-Hpopn/Hspp	0.17	0.14	0.16

Hpop=mean genetic variation for the populations; Hsp = mean genetic variation for the entire data set; Hpop/Hsp = proportion of the genetic variation within the population; [(Hsp-Hpop)/Hsp] = proportion of the variation among the populations.

CONCLUSIONS

The present study showed that ISSR was able to reveal intraregional diversity exists in Harenna wild coffee population. The tetra-nucleotide primers surprisingly showed high levels of genetic diversity as compared to the di- nucleotides. Generally, moderate levels of diversity observed in Harenna forest. Even though the diversity observed in Harenna forest is moderate, because of the availability of moisture stress tolerant trait; it needs to be conserved at *in situ* level to be used for breeding program that targets drought prone area. Moreover, the coffee genetic resources in the Harenna forest has been significantly vanished by deforestation cause by migrant coming from lowland areas of Bale and Harar it needs due attention before completely changed to farm land.

This study provided information on the genetic variation at the intra-population levels of the region in Harenna and helps to identify the effect of intensive management of wild coffee. This study suggested that the programs of *in-situ* conservation should be implemented to create strategies for maintaining wild coffee populations' genetic diversity by appropriate forest management. This *in-situ* conservation strategies is considered to be the most important that allows the dynamic evolutionary events to continue in the original sites. However, care should be taken during forest management not to disrupt other biodiversity components in the forest to avoid loss of natural forest to the point of no return.

ACKNOWLEDGEMENTS

This was carried out in the framework of the CoCE project phase II (Conservation and use of wild populations of *Coffea arabica* L. in the montane rainforests of Ethiopia) in the BIOTEAM program, sponsored by the German Federal Ministry of Education and Research (BMBF).

REFERENCES

Adugna G. and Hindorf H. 2001. Recent investigation on coffee Tracheomycosis, *Gibberella xylarioides* (*Fusarium xylarioides*) in Ethiopia. 19th International Conference on Coffee Science, Trieste, May 14th-18th, 2001.

Aga E. 2005. Molecular genetic diversity study of forest coffee tree (*Coffee arabica* L.) populations in Ethiopia: Implications for conservation and breeding. Doctoral Thesis, Faculty of Landscape planning, Horticulture and Agricultural Science, Swedish University of Agricultural Sciences (SLU)

Anthony F., Bertrand B., Quiros O., Wilches A., Lashermes P., Berthaud J. and Charrier A. 2001. Genetic Diversity of wild coffee (*Coffea arabica* L.) using molecular markers. Euphytica 118:53-65.

Balemi S., Tesfaye K., Bekele E., Govers K. and Borsch T. (submitted). Genetic diversity analysis of the wild *Coffea arabic*a L. populations from Harenna forest, Bale Mountains of Ethiopia, using Inter Simple Sequence Repeats (ISSR) marker.

Borsch T., Hilu K.W., Quandt D., Wilde V., Neinhuis C. and Barthlott W. 2003. Noncoding plastid *trn*T-*trn*F sequences reveal a well resolved phylogeny of basal angiosperms. J. Evol. Biol 16: 558-576.

Carvalho A., Ferwerda F.P., Frahm-Leliveld J.A., Medina D.M., Mendes A.J.T. and Monaco L.C. 1969. Coffee (*Coffea arabica* L. and *C. canephora* Pierre ex Froehner). In: Ferwerda F.P. and Wit F. (eds.), Outlines of perennial crop breeding in the tropics. Wageningen, The Netherlands, pp. 189-241.

Chaparro A.P., Cristancho M.A., Cortina H.A. and Gaitan A.L. 2004. Genetic variability of *Coffea arabica* L. accessions from Ethiopia evaluated with RAPDs. Genet. Resour. Crop. Evol. 51: 291-297.

Charrier A. and Berthaud J. 1985. Botanical classification of coffee. In: Clifford M.N. and Wilson K.C. (eds.), Coffee Botany, Biochemistry and Production of Beans and Beverage, Croom Helm, London, pp. 13-47.

Coste R. 1992. Coffee; The plant and the product. CTA Waginingen, The Netherlands. Davis A.P., Chester M, Maurin O, and Fay M. F., 2007. Searching for the relatives of *Coffea* (Rubiaceae, Ixoroideae): the circumscription and phylogeny of coffeeae based on plastid sequence data and morphology. American Journal of Botany 94: 313-329.

Gole T.W. 2003. Vegetation of the Yayu forest in SW Ethiopia: impacts of human use and implications for *in situ* conservation of wild *Coffea arabica* L. populations. Ecoloyg and Development Series, No.10, Zentrum fur Entwicklungsforschung, Center for Development Research, University of Bonn.

Hamrick J.L. and Godt M.J.W. 1996. Effects of life history traits on genetic diversity in plant species. Phil. Trans. Roy. Soc. L. B 351: 1291-1298.

Kufa T. 2006. Ecophysiological diversity of wild Arabica coffee populations in Ethiopia: Growth, water relation and hydraulic characteristics along a climatic gradient. Doctoral Thesis. Center for Development Reseach (ZEF). University of Bonn. Germany

Lovelles M.D. and Hamirik J.L. (1984). Ecological determinants of genetic structure in plant Populations. Ann. Rev. Ecol. Syst. 15: 65-95.

Meyer F. 1965. Notes on wild *Coffea arabica* from southwestern Ethiopia, with some historical considerations. Economic Botany 19: 136-151.

Nigatu L. and Tadesse M. 1989. An ecological study of the vegetation of the Harenna forest, Bale, Ethiopia. *SINET:* Ethiop. J. Sci. 12(1): 63-93.

Oljira, T. (2006). Genetic diversity of The Wild Populations of *Coffea arabica* L.in Yayu Forest of Ethiopia Using Inter Simple Sequence repeat (ISSR) marker. M. Sc Thesis Submitted to Addis Ababa University, Addis Ababa, Ethiopia.

Raina S.N., Mukai Y. and Yamanoto M. 1998. *In situ* hybridization identifies the diploid progenitor species of *Coffea arabica* (Rubiaceae). Theor. Appl. Genet. 97: 1204-1209.

Ruas P.M., Ruas C.F., Rampim L., Carvalho V.P., Ruas E.A. and Sera T., 2003. Genetic relationship in Coffea species and parentage determination of interspecific hybrids using ISSR (Inter- Simple Sequence Repeat) markers. Genet. Mol. Biol. 26: 319-327.

Senbeta W.F. 2006. Biodiversity and ecology of afromontane rainforests with wild *Coffea arabica* L. populations in Ethiopia. Ecology and Development Series No. 38, Zentrum fur Entwicklungsforschung, Center for Development Research. University of Bonn. Germany.

Tesfaye, K. (2006). Genetic Diversity of wild *Cofea arabica* Populations in Ethiopia as a contribution to conservation and Use planning. Ecology and Development series. University of Bonn,Germany. No.44.

Wolf A.D. and Liston A. (1998). Contributions of PCR-based methods to plant systematic and evolutionary biology. In: Soltis, D.E. Soltis, P.S. and Doyle, J.J. (Eds). Plant molecular systematics II. Kluwer academic publisher, Boston, MA, Pp 43-86.

Zietkiewicz E., Rafalski A., Labuda D. (1994) Genome fingerprinting by simple sequence repeats (SSR)-anchored PCR amplification. Genomics, 20, 176-183.

Heterosis and Combining Ability for Yield and Yield Related Traits in Arabica Coffee (*Coffea arabica* L.)

M. WASSU[1], B. BAYETTA[2] AND H. SINGH[1]

[1]Haramaya University, P.O.Box 219
[2]Jima Agricultural Research Center, Ethiopia

SUMMARY

Five distinct *Coffea arabica* L. lines in origin and 10 hybrids made among them in half diallel fashion planted in 1997, following randomized complete block design were evaluated for fresh cherry and clean coffee yield for four consecutive years (2001 to 2004) and yield related traits. Five hybrids exhibited positive and significant heterosis relative to mid parent and better parent up to 116.4% and 115.9% for fresh cherry yield, and 121.7% and 98.2% for clean coffee yield, respectively, of which three were the between region crosses. All except one the within region hybrids that exhibited highest level of heterosis also gave highest yield better than best yielding parent. The study indicated the importance of both additive and non-additive gene actions in determining the expression of 9 out of 12 traits but variance component ratio being less than one indicated relative importance of non-additive gene actions. The study suggested wide genetic variability within Ethiopian *Coffea arabica* L. populations originated in different coffee growing regions and the importance of continuing coffee hybrid programs considering parents diverse geographical origin for high yield and exploitable heterosis.

INTRODUCTION

Ethiopia is the primary center of origin and diversification for arabica coffee, (*Coffea arabica* L.) (IBPGR, 1980). Moreover, a wide genetic variation within the arabica coffee population was reported (Sylvain, 1955). Despite the presence of wide genetic variation for improvement, the average national coffee yield of Ethiopia is low (less than 600 kg/ha). Among the major factors responsible for low yield, lack of improved cultivars for different ecological zones of the country is important (Bayetta, 2001). To overcome this constraint, coffee hybridization was started in 1978 to develop high yielding hybrids and subsequent crossings among indigenous cultivars showed the presence of considerable magnitude of heterosis (Mesfin and Bayetta, 1983; Bayetta, 2001). The studies in the past, however, were conducted by making crosses among selections from South Western Ethiopia (Kaffa type) only. Due to this, information was lacking on combining ability and heterosis generated from crosses among selections from Southern Ethiopia (Sidamo type) and South Western region (Kaffa type).

Keeping this in view, the present study was conducted to investigate the extent of heterosis and combining ability of parents in crosses among and between lines from South Western region (Kaffa type) and from Southern region (Sidamo type) of Ethiopia. It was aimed at identifying good combiner parents' vis-à-vis to determine the type of gene actions involved in the inheritance of yield and yield related traits.

MATERIALS AND METHODS

Five distinct lines, three from South Western Ethiopia (Kaffa type) namely, 744, 7440 and 75227 and two from Southern region (Sidamo type), 1377 and 1681 and their 10 hybrids made among them in half-diallel fashion planted in August 1997 at Wonago research sub station (6'3'N and 38'3'E) following randomized block design (RCBD) in four replications, five trees per plot with 2 m x 2 m spacing. These materials were evaluated for four consecutive years (2001 to 2004) for yield. For other yield related traits, viz., stem girth (SG), plant height (PH), average internodes length on main stem (AINS), number of primary branches (NBP), average length of primary branches (ALPB), average internodes length on primary branches (AINLPB), canopy diameter (CD), single berry weight (SBW), percent pulp (PP) and out turn ratio (OR), the data were recorded in 2004 on tree basis.

Fresh cherry (YMF00-04) and clean coffee yield (YMC00-04) were recorded on tree basis and average of the five trees for four years computed for each experimental unit that were used for statistical analysis. Yield of clean coffee was obtained as the product of fresh cherry weight and out turn ratio of each genotype. Mean percent pulp was obtained as the difference between the weights of fresh cherries took from each experimental tree before and after pulping and expressed as the percentage weight of fresh cherries. Out turn ratio was calculated as percent of clean coffee weight obtained from fresh weight of cherries.

Data analysis was conducted using MSTAT-C 1986 Michigan University statistical soft ware (MSTAT, 1986). The data from the F_1 crosses and parents were subjected to simple analysis of variance for randomized complete block design. Further genetic analysis was performed for those characters in which statistically significant differences existed among genotypes. To study hybrid performance for each cross combination (P1 x P2), the mid parent value (MP), absolute mid parent heterosis (MPH) and relative mid-parent heterosis (MPH %) and better parent heterosis (BPH %) were calculated as follows: MP = (P1 + P2)/2; MPH = HYB - MP; MPH (%) = (MPH/MP) x 100 and BPH (%) = (HYB-BP)/BP x 100 where MP and BP is mean of two parents and better parent values of the hybrid, respectively. The significance of heterosis effect of hybrids was tested by comparing mean differences between hybrid performances to LSD values derived from genotypes (hybrid and parents) analysis of variance. The genetic analysis was performed according to Griffing's method 2- Model I (Griffing, 1956).

RESULTS AND DISCUSSION

Highly significant differences among genotypes were detected for all the 12 traits. Therefore, further genetic analysis was carried out for all the traits. The overall mean of the hybrids considerably exceeded that of the parents for 10 out of 12 traits (Table 1). On the average, hybrids yielded 46% higher fresh cherry yield and 40% more clean coffee yield over the average of their respective parents. In this study for 9 out of 12 traits, equal or greater than 70% of the hybrids had mean values higher than their mid parent while equal or greater than 60% of the hybrids had mean values higher than their better parent values.

Heterosis relative to mid parent (MPH %) ranged from -22.6 to 116.4 and for better parent (BPH %) from -41.1 to 115.9 (Table 3). All the lowest and highest level of heterotic effects were for clean coffee and fresh cherry yield, respectively. At least one hybrid exhibited statistically significant heterosis relative to mid parent (MPH %) and/or better parent heterosis (BPH %) either in positive or negative direction for 10 of 12 traits (Table 3). None of the hybrids exhibited significant heterotic effect for canopy diameter and percent pulp.

Table 1. Mean values, ANOVA, GCA and SCA mean squares for yield and yield related traits in 5 x 5 half diallel of *Coffea arabica* L. crosses.

Trait	Mean Values			Mean Squares					Variance Ratio
	Parent	Hybrid	Parents plus hybrids	Replication (3) @	Genotype (14)	Error (42)	GCA (4)	SCA (10)	
SG(cm)	5.51	5.86	5.74	0.06	0.32**	0.07	0.34**	0.29**	0.27
PH(cm)	236	263	254.16	1141.24*	2069.83**	379.3	4010.63**	1293.51**	0.57
AINLS(cm)	5.55	6.06	5.89	0.91*	0.93**	0.26	1.484**	0.713**	0.38
NPB	79	82	81	34.2	142.29**	29.74	228.72**	107.7**	0.37
ALPB(cm)	87.14	92.96	91.02	22.67	106.19**	36.81	177.98**	77.47*	0.29
AINLPB(cm)	3.7	3.87	3.81	0.77**	0.21**	0.04	0.50**	0.087**	1.38
CD (cm)	165.5	171.13	171.13	183.42*	299.14**	60.20	827.05**	87.94	----
SBW(g/fruit)	1.88	2.02	1.97	0.19	0.139**	0.018	0.371*	2.47*	0.20
PP (%)	39.17	39.16	39.17	14.23**	13.940**	7.043	41.55**	2.904	----
OR (%)	17.36	16.85	17.02	2.98	8.09*	3.21	4.05	4.289*	----
YMF(kg/tree)	2.87	4.09	3.69	1.27	4.670**	0.72	2.15*	5.673*	0.04
YMC(kg/tree)	0.50	0.70	0.63	0.04	0.15**	0.024	0.08*	0.173**	0.05

**, **, Significant at 5% and 1% probability levels, respectively.*

@, Numbers in parenthesis indicates degree of freedom.

Table 2. Estimates of general combining ability (GCA) effect for yield and yield related traits of five coffee parental lines in half diallel cross of *Coffea arabica* L. grown at Wonago.

Traits	Parent GCA Effect					
	P1	P2	P3	P4	P5	SE (g_i)
SG (cm)	-0.077	-0.099	0.081	-0.057	0.153	0.089
PH (cm)	-13.917*	-3.690	0.617	19.006**	-2.015	6.584
AINLS (cm)	-0.204	-0.172	-0.102	0.146	0.331	0.172
NPB	-0.476	-1.994	2.199	3.571	-3.300	1.843
ALPB (cm)	2.022	-3.827	0.546	2.304	-1.044	2.051
AINLPB (cm)	0.012	-0.153*	-0.083	0.022	0.202**	0.068
CD (cm)	5.089	-8.55**	1.786	-1.861	3.536	2.619
SBW (g/fruit)	0.196**	-0.018	-0.076	-0.090*	-0.012	0.045
PP (%)	0.719	0.651	-0.620	-1.846*	1.097	0.897
YMF (Kg/tree)	-0.222	-0.042	0.437	0.071	-0.244	0.287
YMC(Kg/tree)	-0.045	-0.012	0.073	0.033	-0.049	0.052

**, **, Significant at 5% and 1% probability levels, respectively.*
Note: P1=744, P2=7440, P3=75227, P4=1377 and P5=1681

Five hybrids exhibited positive and significant heterosis for both fresh cherry yield and clean coffee yield in terms of relative mid-parent heterosis (MPH %) and better parent heterosis (BPH %) (Table 3). All these hybrids except one hybrid also gave highest yield which was better than the best parent in the experiment. Three out of five hybrids that registered positive and significant BPH (%) for clean coffee yield were the between region crosses. In addition, all the lowest and highest heterotic effects in all traits registered for the within and between region coffee hybrids, respectively. The observed higher mean yield of hybrids than the average of parents for clean coffee and fresh cherry yield over years and the better performance of hybrids with respect to majority of yield related traits clearly indicated the possibility of improvement coffee yield by developing hybrids among and between lines from different coffee growing regions of Ethiopia.

Variation due to both general (GCA) and specific (SCA) combining ability effects was significant for all the traits except canopy diameter, percent pulp and out turn ratio (Table 2). For canopy diameter and percent pulp the variation was significant for only GCA while in case of out turn ratio there was significant variation for SCA only. Calculated variance component ratio did not exceed unity for all the characters except average internodes length on primary branches.

In this study 75227 and 1377 parents possessed positive GCA effects for 7 out of 11 traits and negative GCA effect for percent pulp (which was in desired direction) and both parents recorded negative GCA effects for three but different traits. In fact, 1377 was the highest yielding parent with respect to both fresh cherry yield (3.64 Kg/tree) and clean coffee yield (0.69 kg/tree). Each of two other parents, 744 and 1681, exhibited positive GCA effects for five different traits and negative GCA effects for the remaining six characters. One of the parents, 7440, recorded positive GCA effects only for one trait i.e. for percent pulp (which is in undesired direction). According to the results of this study the two parents; 75227 and 1377 could be considered as good general combiners, which could be considered to use as parents in other hybrid experiments. Moreover, 1377 could be released as improved variety in the region.

All hybrids, except 744 x 75227 and 1377 x 1681, exhibited positive SCA effects for at least six and at the most for all 10 traits. Among the hybrids, 75227 x 1377 for five; 744 x 1377, 744 x 1681 and 7440 x 1681 for four; and 1377 x 1681 for two traits exhibited positive and significant SCA effects. The hybrid 1377 x 1681 had negative and significant SCA effects for three traits and the hybrids 7440 x 1377 and 744 x 377 recorded negative and significant SCA effect for one trait each. Five hybrids, namely, 744 x 1377, 744 x 1681, 7440 x 75227, 75227 x 1377 and 75227 x 1681 had significant positive SCA effect for mean fresh cherry yield. Except the hybrid 744 x 1377, these hybrids had significant positive SCA effect for mean clean coffee yield as well (Table 3). The hybrid 1377 x 1681 had significant and negative SCA effect for both the yield traits, which was obtained by crossing two Sidamo coffee parents.

The significant variation due to both general (GCA) and specific (SCA) combining ability effects for majority of traits (9 out of 12 traits) including yield traits indicated the importance of both additive and non additive gene actions in determining the expression of these characters. However, calculated variance component ratio being less than one for these characters, except average internodes length on primary branches, clearly indicated the relative importance of non-additive gene actions and thus the need to promote hybrid programs to improve genetic potential of arabica coffee to obtain higher coffee yields. The results of present study were in agreement with the previous investigators (Mesfin A. and Bayetta, 1983; Cilas et al., 1998; Walayro, 1983; Bayetta, 2001), who reported the importance of both the gene actions for the expression of these traits. However, in case of canopy diameter, in contrast to Walayro (1983) and Bayetta (2001), who reported the importance of both additive and non-additive gene actions for the expression of this trait, but additive gene action, was detected to be important in the present investigation.

Hybrids 744 x 1681 and 75227 x 1377 were good specific combinations as evident from their high and significant SCA effects and percent heterosis for majority of the traits. The other two high yielding hybrids, 75227 x 1681 and 7440 x 75227 exhibited high and significant SCA effects for the two important yield traits only. These hybrids could be recommended for further verification to be released soon since all the four hybrids out smarted the best yielding parent and the three released coffee berry disease resistance (CBD) cultivars that were used as elite parents in this experiment.

Table 3. Better parent, mid parent, F_1 mean values, percent heterosis and specific combining ability for yield and yield related traits in 5x5 arabica coffee crosses experiment conducted at Wonago (6'3'N and 38'3'E).

Traits	Mid (MP) , Better Parent (BP)Heterosis (%) and SCA Effect of Hybrids															LSD 5%	SE (Sij)
	744 x 7440			744 x 75227			744 x 1377			744 x 1681			7440 x 75227				
	MPH	BPH	SCA	MPH	BPH	SCA	MPH	BPH	SCA	MPH	BPH	SCA	MPH	BPH	SCA		
SG(cm)	6	5.6	0.066	8.7**	5.4	0.192	9.4*	8.2*	0.247*	5.4	0	0.083	4.7	2	0.019	0.396	0.115
PH(cm)	13.8	9.8	5.55	-0.9	-4.1	-0.41	3.5	-0.5	19**	8.1	3	19.52**	4.6	-2.2	11.41	27.79	8.5
AILS(cm)	2.7	-3.2	0.36	-0.9	-2.8	-0.1	5.9	4.7	-0.06	8.7	3	0.6	5	-1.5	-0.23	0.722	0.22
NPB	-0.1	-2.5	-1.69	6.4	3.8	-0.6	9.1	1.1	5.27*	9.9*	9.2*	5.34*	6.9	6.3	3.31	7.78	2.38
ALP(cm)	2.7	-3.2	0.89	-0.9	-2.8	-3.22	5.9	4.7	2.17	8.7	3	4.04	5	-1.5	0.5	8.68	2.65
AINLP(cm)	5	-2.2	0.07	-4	-5.7	-0.14	5.1	3	0.09	2.9	-0.1	0.02	5.6	0.2	0.11	0.28	0.09
SBW	11.1*	3.7	0.11	9.4	1	0.07	7.1	-3.2	0.04	15.5*	5.2	0.14*	0.8	-2.5	-0.03	0.194	0.059
OR (%)	-7.8	13*	-0.6	-3.2	-10.1	-0.19	-18*	-18.4*	-2.31**	-4.1	-9.8	0.13	5.1	3.3	0.49	2.04	0.782
YMF(kg/tree)	8.7	-12.8	-0.33	**59.5***	**41.3***	0.08	52.8*	22.1	0.91*	**116.4****	**115.9****	1.48**	**52****	**36.4****	0.76*	1.214	0.37
YMC(kg/tree)	1.2	-14.4	-0.07	**55.7****	**48.1***	-0.01	25.2	-0.3	0.07	**107.3****	**94.6****	0.26**	**59.2***	**40.9****	0.14**	0.22	0.068

Traits	Mid (MP) , Better Parent (BP)Heterosis (%) and SCA Effect of Hybrids														
	7440 x 1377			7440 x 1681			75227 x 1377			7227 x 1681			1377 x 1681		
	MPH	BPH	SCA	MPH	BPH	SCA	MPH	BPH	SCA	MPH	BPH	SCA	MPH	BPH	SCA
SG(cm)	5.2	4.4	0.065	8	3	0.217*	9*	6.9	0.254*	4.9	2.5	0.075	0.8	-3.4	-0.137
PH(cm)	15.3*	7.1	4.81	19.7*	10.3	18.39*	1.2	0.5	15.84	8.5	6.8	2.49	18.7**	17.7**	-5.89
AILS(cm)	8.9	1.4	0.35	13.5*	10	0.46*	10.1*	9.3	-1.11	7	3.4	0.19	4.4	0.1	0.58**
NPB	-8.3	-13.5**	-5.39*	0	-2.5	0.86	11.2*	5.6	6.8*	5.8	2.5	1.42	-11*	-18**	-8.18*
ALP(cm)	8.9	1.4	2.78	13.5*	10	5.68*	10.1*	9.3	5.51*	7	3.4	2.16	4.4	0.1	-1.2
AINLP(cm)	9.1*	3.7	0.12	8.4*	-1.7	0.12	1.7	1.5	-0.02	1.5	-3.3	-0.01	9.5*	4.2	0.19*
SBW	-0.3	-3.7	-0.04	7.5	4.7	0.04	2.2	1.9	-0.01	9.5	8.2	0.07	9	7.5	0.07
OR (%)	0	-6	0.38	-3.4	-3.7	-0.43	5.7	-2.3	0.95	3.5	2	0.28	-4.6	-10.7	-0.38
YMF(kg/tree)	-13.9	-14.2	-0.6	28.4	3.3	0.27	**66.7****	**48***	1.19**	**114****	**89****	1.45**	-17.4	-34.2*	-1.12**
YMC(kg/tree)	-13.1	-19.3	0.1	34.4	8	0.07	**74.3****	**44.6****	0.26**	**121.7****	**98.2****	0.24**	-22.6*	-41.1*	-0.21**

**, **Ssignificant at 5% and 1% probability level, respectively, ALP= average length of primary branches, AINLPB= average internodes length on primary branches, SBW=single berry weight, PP= percent pulp, OR= outturn ratio, YMF= four years mean fresh cherry yield, YMC= four years mean clean coffee yield, MPH (%) = percent heterosis relative to mid parent, BPH (%) = better parent percent heterosis, SCA= specific combining.*

REFERENCES

Bayetta B. 2001. Arabica coffee breeding for yield and resistance to coffee berry disease (*Collectotrichum kahawae* sp. nov.). Doctoral thesis, Imperial College at Wye, University of London, London.

Cilas C., Bouharmont P., Boccara M., Eskes A.B. and Baradat Ph.1 998. Production of genetic value for coffee production in *Coffea arabica* L. from half diallel with lines and hybrids. Euphytica 104, 49-59.

Griffing B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Austr. J. Biol. Sci. 9, 463-493.

IBPGR. 1980. Coffee Genetic Resources – International Board for Plant Genetic Resources Working Group (AGP: IBPGR/80/3). Rome, 11-13 December 1979. IBPGR, Rome, Italy.

Mesfin A. and Bayetta B. 1983. Heterosis in crosses of indigenous coffee selected for yield and resistance to coffee berry disease: II. First three years. Eth. J. Agri. Sci. 5, 13-21.

MSTAT Development Team. 1986. MSTAT – A Microcomputer Program for the Design, Management and Analysis of Agronomic Research Experiments. 4th ed. Dep. of Crop and Soil Science, Michigan State Univ., East Lansing.

Sylvain P.G. 1955. Ethiopian coffee its significance to the world coffee problems. Econ. Bot. 12, 11-139.

Walayro D.J.A. 1983. Consideration in breeding for improved yield and quality in arabica coffee (*Coffea arabica* L.). Doctoral thesis, Agricultural University, Wageningen, the Netherlands.

Evaluation of Conilons for Genetic Diversity, Cup Quality and Biochemical Composition

C. LAMBOT[1], D. CROUZILLAT[1], A.F.A. DA FONSECA[2], V. LELOUP[3], A. HAIDUC[3], P. BROUN[1], V. PETIARD[1]; M.A.G. FERRÃO[4], R.G. FERRÃO[5]

[1]Centre de Recherche et Développement Nestlé, Tours, France
[2]Embrapa Café, Brasília, DF, Brazil
[3]Product Technology Center Nestlé, Orbe, Switzerland
[4]Embrapa Café/ Incaper, Vitória, DF, Brazil
[5]Incaper, Vitória, ES, Brazil

SUMMARY

A Collaborative Research started in May 2005 between Nestlé and INCAPER for the evaluation and possibly the improvement of cup quality of Conilon varieties developed by the Brazilian Institute. A total number of 54 clones constituting 6 different varieties were evaluated for the genetic diversity (DNA fingerprinting), biochemical composition and cup quality aspects. The clones of INCAPER were compared for different characteristics to a genetically more diverse coffee collection maintained in Ecuador (reference collection). The 54 Conilon clones have a quite narrow genetic base, well differentiated from the other *Coffea canephora* types and are also characterized by a lower level of heterozygosity in comparison with Congolese and Guinean genetic groups. The clones are mainly characterized by a low sucrose and lipids content and higher ash content compared to the concentration observed in the reference collection. The diversity encountered allows to select for cup quality although some of the clones are characterized by a lack of aroma and a strong bitterness. Generally speaking, the diversity in the biochemical composition is lower compared to the one observed in the reference collection, probably in relation to the narrow genetic base. Nevertheless, despite the relative lack of diversity it is possible to propose a selection of clones with the best cup quality performances. This positive selection could lead to the development of new varieties. Considering the narrow genetic base of the Conilon group, it is recommended to introduce some clones to broaden the genetic base of the collection maintained at INCAPER. These clones should be selected with the purpose to introduce genetic source of cup quality into the breeding materials under selection at INCAPER.

INTRODUCTION

Coffea canephora Pierre originated in the humid lowland forests of tropical Africa, which stretch from Guinea to Uganda and Angola. It is customary to assign cultivated coffee trees to one of the two groups of *C. canephora*, Robusta and Kouillou which have different morphology. Robusta type has two geographic groups which can be differentiated by isozymes, the Congolese group originated from central African countries and the Guinean group originated from Ivory Coast and Guinea. Robusta type has an erect habit, with strong primaries that are little branched, large leaves and fruits, and late ripening of fruits. The Kouillou type has a bush like habit, with primaries that are very branched and that tend to droop, elongated leaves and small fruits, early flowering and good drought tolerance. Kouillou coffees are found in the Congo as well as in the Ivory Coast, in Guinea and in Uganda (Charrier and Berthaud, 1988).

The cultivation of *Coffea canephora* Pierre was initiated in the 19th century due to the damage of rust (*Hemileia vastatrix*) on *Coffea arabica* plantations. The beginnings of *C. canephora* cultivation were characterized by many inter-regional genetic material exchanges (Montagnon et al., 1998). In Brazil, a single selection of Kouillou coffee is cultivated under the local name Conilon, orthographic distortion of the original name (Charrier and Berthaud, 1988). A genetic improvement program for Conilon has been conducted since 1985 at the Instituto Capixaba de Pesquisa, Assistencia Tecnica e Extensao Rural (INCAPER) (Fonseca et al., 2004). The production of *Coffea canephora* in Brazil is mainly located in the State of Espirito Santo representing 72 % of the national production. Brazil is the second world producer of *Coffea canephora* after Vietnam with more than 10 million bags per year (Table 1). Close to 7 millions bags are used on the National market (Ferrão et al., 2007) and Nestlé is the main industrial processor of this coffee.

Table 1. World production of "Robusta" coffee in 2006 (million bags).

Countries	Production	%
Vietnam	16.1	34.7
Brazil	10.7	23.1
Indonesia	6.3	13.6
India	3.2	7.0
Ivory Coast	2.2	4.7
Uganda	1.9	4.2
Other	5.9	12.7
Total	46.4	100

USDA (2007) cited by Ferrão et al. (2007).

Considering the importance of Conilon as a raw material for Nestlé instant coffee products and the recent release of new varieties to the producers, it was decided to elaborate a collaborative research between INCAPER and Nestlé to evaluate different aspects of the clones under selection.

MATERIALS AND METHODS

Up to the release of the first INCAPER clonal varieties in 1993, Conilon was empirically propagated by seeds. INCAPER has selected and release 6 new Conilon varieties, 5 of them are multi-clonal varieties and 1 is propagated by seeds. The Institute has developed and released in Espírito Santo Brazilian State varieties having clear advantages for field performances but also for maturation date and uniformity (Fonseca et al., 2004).

Green coffee samples of 6 Conilon varieties were produced by INCAPER at Experimental Station of Marilândia, ES, Brazil. A total of 53 clones and one population variety produced by INCAPER were analyzed for DNA diversity, biochemical composition and cup quality.

The main characteristics of the six varieties are:

- **Variety 1** – "Emcapa 8111" – Early fruit ripening: this variety has an early harvest (May) and is represented by 8 clones,
- **Variety 2** – "Emcapa 8121" – Intermediate fruit ripening: this variety has an intermediate harvest (June) and is constituted by 14 clones,
- **Variety 3** – "Emcapa 8131" – Late fruit ripening: this variety has a late harvest (July) and is constituted by 9 clones,
- **Variety 4** – "Emcapa 8141 – Robustão Capixaba: this variety is resistant to drought and is constituted by 10 clones,
- **Variety 5** – "Vitória - Incaper 8142": this variety is resistant to drought and to rust. It is represented by 12 clones,
- **Variety 6** – "Emcaper 8151 – Robusta Tropical": is a population variety propagated by seeds, formed from the recombination of 53 clones.

For DNA and biochemical composition, the 54 samples were compared with samples produced in the coffee collection maintained by Nestlé in Ecuador including genotypes from other *Coffea canephora* groups (Congolese, Guinean and hybrids) (Pétiard et al., 2004).

DNA was extracted by a CTAB method from green leaves. A set of 13 SSRs (microsatellite markers) have been selected according to their polymorphism level, genome location and the quality of the DNA profiles obtained. For each coffee genotypes evaluated, the DNA fingerprinting is recorded from the 13 SSRs used with the allele sizes in base pairs. Each allele is coded as: absence (0), heterozygous (1) and homozygous 2) status resulting of the determination of the allele copy number on the locus studied for a given genotype. The database obtained is analyzed for the determination of several genetic parameters as allele frequency for each SSR locus in the different genetic groups (Conilon, Congolese, Guinean and hybrids). All statistical analyses were performed using NCSS software Multivariate analysis were done using principal component analysis (PCA) on the 140 coffee genotypes studied and a dendrogram showing the genetic diversity on Conilon coffee was established with Ward's minimum variance as clustering method and Euclidian distance.

The biochemical composition was predicted by Near InfraRed (NIR) spectroscopy using calibrations previously developed for green coffee. NIR reflectance spectra were collected using a scanning monochromator FOSS NIRsystems spectrophotopeter (model 6500, Gerber Instruments). The analyses were performed on green coffee beans in a full size rectangular cell. For each sample 16 scans were recorded in reflectance mode in the 1100-2500 nm range.

Sensory evaluation was performed by profiling of the samples using 5 main attributes (aroma, flavor, body, bitterness, woody-rubbery), and the identification of undesirable flavors and off-flavors. Scoring was on a scale of 0 to 5, in which a score of 0 correspond to the total absence of the criterion and 5 to the maximum. The cup quality was assessed by 4 experts.

RESULTS

A total of 140 genotypes were studied for DNA diversity. The thirteen SSR markers yielded 137 alleles with an average alleles number per locus of 10.5, minimum 6 and maximum 16. The heterozygosity observed for the different genetic groups is compared in Table 2.

Table 2. Genetic characteristics of the four genetic groups analysed among *Coffea canephora* accessions using 13 SSRs.

Group	Number of clones	Total number of alleles detected	Heterozygosity (%)	Number of specific alleles
Conilon	74	63	40.6	5
Congolese	41	99	48.6	5
Guinean	17	85	71.9	**8**
Hybrids	8	64	70.2	5

The Guinean group has a reduced number of clones (17) representing only 12 % of the total. Nevertheless, this genetic group has the highest number of specific alleles (8) and 85 (62%) of the 137 alleles detected in the study. These results indicate the high level of genetic diversity among the Guinean group. The heterozygosity level ranks from 40.6% for Conilon to 71.9% for Guinean group showing that Conilon group is more homozygous than the other genetic groups. The Conilon group has also the lowest number of alleles (63) representing only 46% of the total alleles detected. All these results demonstrate the narrow genetic diversity of the Conilon group, possibly related to the limited number of trees at the historical origin of this specific group (Ferrão et al., 2007). The heterozygosity level of the five Conilon varieties (V1 to V5) is, respectively, 41%, 35.7%, 38.5%, 46.2% and 39.9%. The genetic diversity analysis based on multivariate statistical analysis using SSRs clearly shows the characterization of the four genetic groups (Guinean, Congolese, Conilon and hybrids) among the 140 *Coffea canephora* clones (Figure 1). Most of the genotypes owning to one of the four genetic groups are clustered according to their genetic origin. Hybrid group is located between the three other genetic groups suggesting a contribution of each of these three groups even if some specific hybrid alleles have been found. A hierarchical clustering analysis indicates that the constituting clones of the 5 varieties are well distributed along the diversity. Only the clones of variety 5 (Vitória - Incaper 8142) are more grouped, suggesting that they are less diverse than the other varieties.

The chemical composition of the six varieties is compared to a Conilon commercial sample and to the average value observed in the coffee collection maintained in Ecuador (Table 3). The composition of each Conilon variety is evaluated as the average of all the constituting clones.

- The six varieties are very close in their biochemical composition. The main differences are observed for the variety 6 (Robusta Tropical) having a lower content in proteins and total chlorogenic acids but a higher content in sucrose.

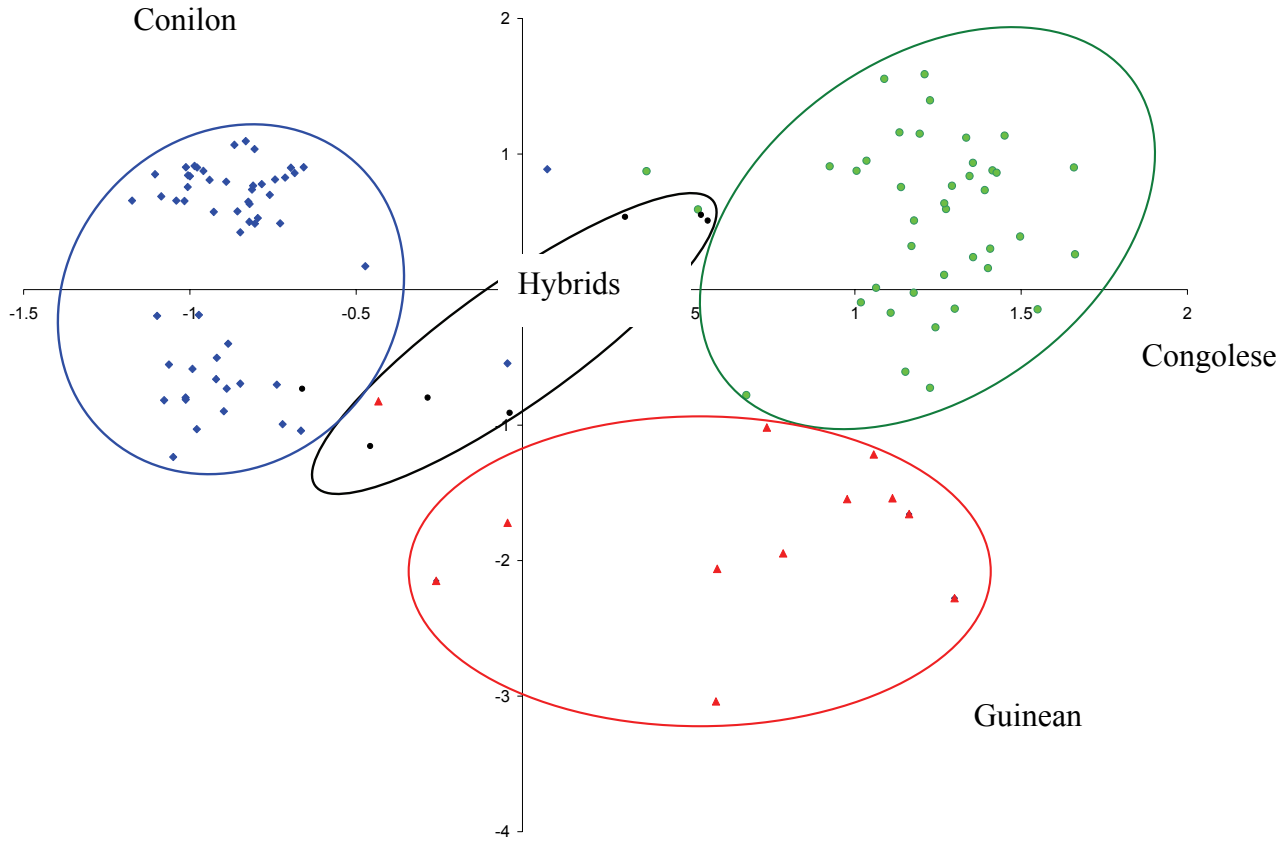


Figure 1. Principal component analysis (PCA) showing the genetic diversity using 13 SSRs on the 140 coffee accessions. The two axes 1 and 2 represents, respectively, 29.9% and 7.3% of the total variance.

Table 3. Average values for main compounds (% of dry matter).

Varieties	Lipids	Proteins	Sucrose	Total Chloro. acids	Total organic acids	Caffeine	Trigo.
Emcapa 8111 (Early fruit ripening)	6.7	13.4	4.7	10.9	1.7	2.6	0.5
Emcapa 8121 (Intermediate fruit ripening)	6.5	13.4	**4.3**	11.1	1.8	2.5	0.5
Emcapa 8131 (Late fruit ripening)	6.6	13.1	4.9	10.8	1.8	2.4	0.5
Emcapa 8141 - Robustâo Capixaba	**7.1**	12.6	4.9	10.9	1.8	2.5	0.5
Vitoria Incaper 8142	6.7	12.9	4.8	10.7	1.8	2.4	0.5
Emcaper 8151 - Robusta Tropical	6.4	**12.4**	**5.7**	**9.8**	1.9	2.4	0.5
Commercial sample	7.7	11.9	4.8	9.5	1.9	2.0	0.7
Coffee collection	9.8	13.1	6.9	11.2	1.7	2.4	0.5

- The variety of intermediate fruit ripening, Emcapa 8121, is lower in sucrose and Emcapa 8141 - Robustão Capixaba, higher in lipids.
- Comparing the 6 varieties with the commercial Conilon, it appears that they are lower in lipids and trigonelline but higher in proteins, total chlorogenic acids and caffeine.

The comparison with the Coffee collection is discussed in Table 4. Generally speaking, the range of values is larger in the Coffee collection compared to the one observed with Conilon clones. Average values can be higher or lower depends the biochemical compounds, but averages are influenced by the genetic and the environmental effects.

Table 4. Comparison between Conilon and Coffee collection (CC) for biochemical compounds.

Compounds	Diversity *	Average values
Lipids	Similar	Conilon < CC
Proteins	Conilon < CC	Similar
Sucrose	Conilon < CC	Conilon < CC
Total Chlorogenic acids	Conilon < CC	Conilon < CC
Total Organic acids	Conilon < CC	Conilon > CC
Caffeine	Similar	Similar
Trigonelline	Similar	Similar

**Diversity is evaluated by the difference between maximum and minimum values.*

The sensory performance of the six Conilon varieties is illustrated in Figure 2. Variety Emcaper 8151 – Robusta Tropical (variety 6) has the highest value for positive attributes (sum of aroma and body), its bitterness and woody attributes are also lower compared to other varieties. The variety Emcapa 8141 Robustão Capixaba (variety 4) has the lowest value for positive attributes. Except for Emcaper 8151 – Robusta Tropical (6) and Emcapa 8141 - Robustão Capixaba (4), the 4 other varieties are quite similar on an average base for the sensory quality. Nevertheless, when looking at the individual value of the constituting clones, it is possible to observe some differences inside the varieties regarding the number of clones evaluated with the highest cup quality.

The results indicate that among the 54 samples, 46 samples (85 %) fitted into the categories of green coffee accepted by Nestlé, and 10 of them can be considered of highest quality standard. These data are of fundamental importance for Incaper as they show the presence of genetic diversity in the clones used for the coffee breeding program related to product quality. They should therefore be included as selection criteria to develop new varieties.

The data show that it is also possible to form new varieties composed only with genetic material evaluated in the best categories, obviously combined with basic principle for the formation of clonal varieties.

Finally, considering that these results relate to samples of only one season, it is recommended to repeat the evaluation on new samples.

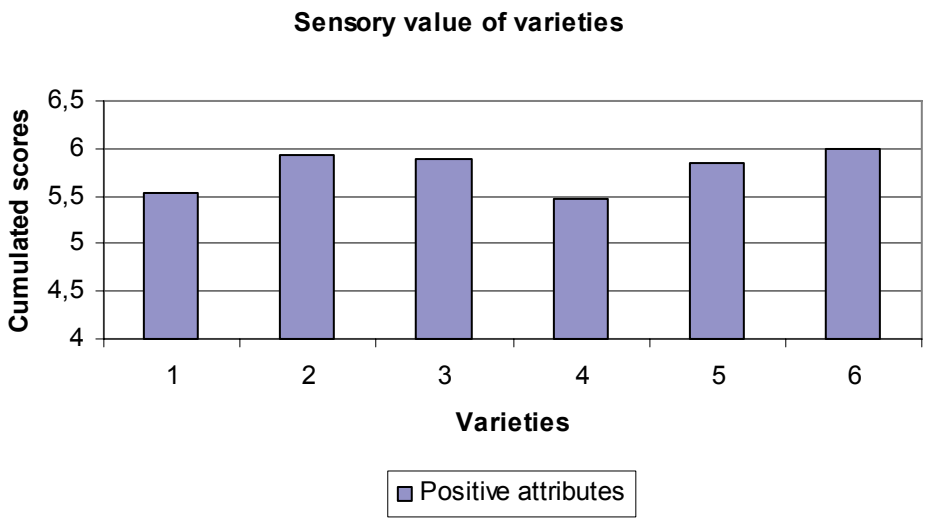


Figure 2. Positive sensory attributes of the 6 Conilon varieties.

CONCLUSIONS

The diversity encountered in the 54 samples of Conilons was evaluated for the genetic but also for the biochemical composition and cup quality aspects.

Results indicated that Conilon group carries a narrow genetic base and a low heterozygosity level compared to the existing diversity of *Coffea canephora*.

The genetic diversity based on biochemical composition is lower compared to the one observed in the Coffee collection maintained by Nestlé in Ecuador but nevertheless significant. In relation to the variety Emcaper 8151 Robusta Tropical, the biochemical composition is also slightly different from the others with a lower content in proteins and chlorogenic acids but with more sucrose.

The cup quality of the 5 clonal varieties is very similar. It is noticeable that the variety Emcaper 8151 Robusta Tropical propagated by seeds is slightly better than the others in terms of cup quality. Results will be confirmed with new samples.

REFERENCES

Charrier A. and Berthaud J. (1988). Principles and methods in coffee plant breeding : *Coffea canephora* Pierre. In: Coffee Vol 4 Agronomy (eds R.J. Clarke and R. Macrae), pp 167-97. Elsevier Applied Science, London and New York.

Ferrão R.G., Fonseca A.F.A. da, Bragança S.M., Ferrão M.AG., De Munner L.H. (Ed.) 2007. Café Conilon. Vitória, ES: INCAPER.702 p.

Fonseca A.F.A. da, Ferrão M.A.G. F., Ferrão R.G., Verdin Filho A.C., Volpi P.S., Zucatelli F. 2004. 'Conilon Vitoria – Incaper 8142': improved *Coffea canephora* var. Kouillou clone cultivar for the state of Espirito Santo. Crop Breeding and Applied Biotechnology 4:503-505. Brazilian Society of Plant Breeding.

Montagnon C., Leroy T., Eskes A.B. 1998. Amélioration variétale de *Coffea canephora*. I. Critères et méthodes de sélection. Plant. Rech. Dévelop., 5, 18-33.

Pétiard V., Lepage B., Lambot C., Crouzillat D., Florin B., Brulard E., Alvarez M., Von Rutte S., Leloup V., Gancel C., Liardon R., Jung M.L., Rytz A. and Labbe D. 2004. Establishement and preliminary agronomic and qualitative evaluation of collections of *Coffea Arabica* and *Coffea canephora* cultivated varieties. P.834-841. In: ASIC (ed.), 20th International Scientific Colloquium on Coffee, Bangalore.

Analysis of Organic and Traditional Coffee Farming in Londrina

R. MIGLIORANZA, J. MASSARUTTI, E. MIGLIORANZA

Center of Applied Social Sciences, Dep. of Business and Administration, Universidade Estadual de Londrina, Rodovia Celso Garcia Cid PR 445, Km 380, Londrina, Brazil

SUMMARY

An organic coffee farm has as objective the sustainability by conscious management. A result of this is the low cost of inputs to farming, because organic coffee growing does not use synthetic inputs. In spite of that, the monetary return regards the productivity and the sales price as well. Studies demonstrate that it is necessary constant vigilance to pest and disease of the coffee farm to obtain better profitability. In this study, it was possible to evaluate the financial gains involved in organic and traditional coffee farming in Londrina. Organic coffee farmer showed to have lower costs but also lower gross profit than traditional coffee farmers. Even though the price paid for organic coffee in Londrina is higher than traditional coffee, the productivity was unable to generate better margins.

INTRODUCTION

The objective of organic production is sustainable agriculture. Sustainable agriculture concerns present and future resources that guarantee economic, ecological, and social return to producer. The most important term to consider is the ecosystem that involves the property. The organic production implies vigilance over environment; pollution reduced to lowest possible; renewable energy source; fair labor conditions; and absence of synthetic chemicals (COFFEE CONTACT, 2005).

In addition to the The Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods (adopted by the 23rd Session of the Codex Alimentarius Commission from Food and Agriculture Organization – FAO, 2005); in Brazil there is also specific legislation on the matter (Instrução Normativa Nº 007, de 17 de maio de 1999, do Ministro do Estado da Agricultura e do Abastecimento).

An organic production is the result of a balanced environment and nutrition. Frequently, an organic coffee farm has a diversified production system, which helps to improve biological control, control of diseases and pests. It is important to note that organic production can have insects and pests, but they should not cause economical damage. The soil is considered a biological environment, so fertilization is done by recycling, rock grinding, and organic substances.

Prevention is the main factor in organic agriculture. Caution begins before sowing, using varieties adapted to climate and region. Organic farmers can use natural insecticide, derived from plants or minerals that do not harm man or the environment.

This study aims at considering the responsible use of natural resources, against the financial aspects in coffee farming in Londrina, to quantify the capacity of local development using different production systems.

MATERIALS AND METHODS

The object of this study is coffee farmers from Londrina, Paraná. This region is 590 meters over sea level and has rainy winters. The study happened between March and June of 2006, and considered a sample from the 400 coffee producers, 58 being organic coffee growers in the second year of conversion.

A structured questionnaire was used to obtain data, regarding cost and sales prices. Social and demographical characteristics, cost and financing methods, commercialization channels, post-harvest handling information was also collected. The sample was non-probabilistic stratified, where the population of coffee growers was divided in two groups: organic and traditional. The criteria used for the selection was accessibility. The error margin considered for this study was 5%.

LITERATURE REVIEW

Cost analysis is complex, especially in perennial agriculture, as coffee. Meanwhile, it is essential that the producer knows the annual production costs, because it is the only way to determine the profitability of the production and, if observed any deficiency, it is the only way to question an involved management (Coste, 1975). The more and better cost information the producer holds, the better is the decision making process over the production methods or products. Matz, 1974, suggests that efficient use of capital is a primary function of management, which justifies cost analysis.

Cost can be calculated through different conceptions, therefore represents different values. Costs can be total expenditure with production factors, monetary spending in a period, among others. Although for the effective analysis and control, it necessarily separates and groups to calculate cost of each product, tendencies and cost progress.

Martins' (2002) cost concepts defines that expense is delivery or promise to deliver an asset. Nevertheless, expenses do not represent the whole value of sacrifices made, because it does not account the value of opportunity cost and interest over shareholders' equity, not identified by delivery of assets. Expenses are classified as investments, cost, general and administrative expenses or loss. Cost, consequently, is a type of expense.

Investments are expenses that bring benefits for various future periods, consumed by amortization or devaluation of the asset. Investment, opportunity cost and interest over shareholders' equity do not differ between organic and traditional coffee farming, because preliminary requirement is equal. Investment in coffee renewal can improve productivity or quality and should be carried by both types of producers. Investments in coffee farming are storages, patios, aquapulp, and dryer, among others.

Costs are expenses used in the production of products or services. Costs can be directly or indirectly associated to production. Direct costs in coffee growing are the costs of inputs used and labor during a period to obtain the production. Direct costs are easily allocated to coffee sold, because it is possible to calculate the unit per tree, or area, having a usage unit. Direct labor costs are salaries paid for activities related to picking or producing (Martins, 2002; Santos, 2002).

Indirect costs can be indirect materials, indirect labor and other expenses. Indirect materials are used to produce, but the consumption is considered little or complex, not having an objective measurement. As examples of indirect materials in coffee farms are: oil used in

machines to maintain operating conditions; cost to maintain infrastructure; transportation cost to dry (Matz et al., 1974).

General and administrative expenses are goods or services used directly or indirectly in the production, which reduces the owners' equity. General and administrative expenses constitute the expenses to generate income not easily allocated to a product, and does not refer only to production sold during the period. Examples of general and administrative expenses can represent cost of advertising, research or development (Martins, 2002; Santos, 2002).

Loss is the result of involuntary or abnormal consumption and do not represent intent in revenue. In coffee production, loss can be associated with drought, excessive rain during picking, or freeze that has negative impact on production. These expenses are unpredictable.

As observed, systematic information gathering of not only values expend, as well as physical volume consumed and produced, increases considerably the producer's administrative control. Through adequate domain of costs, the producer is able to define if the coffee farm is generating profit or loss, and which resources can be optimized (Martins, 2002).

Coffee production costs can be divided by their nature in four categories: maintenance, phytossanitary protection, harvest and coffee preparation to sell. The maintenance costs refer to the costs to maintain farm's production. The phytosanitary costs are those to control pests and diseases. The harvest costs are the expenses with extra work force and with transportation inside the farm. Finally, the preparation costs are those to prepare the coffee for commercialization. The annual costs of coffee plantation are established in accordance with the following table:

Table 1. Coffee production costs.

Cost nature	**Labor**	**Products**	**Total**
Maintenance: Stairs Replant Pruning Fertilization Etc.			
Phytosanitary protection: Pests Diseases			
Harvest: Harvest Internal transportation			
Coffee Preparation: Labor Bags Grain dryers Transport to expedition			
TOTAL			

Source: Coste, 1975

The production goals must address productivity as well as profitability. The productivity was considered as being the amount of coffee per planted area. The profitability is the maximization of the revenue and the minimization of the costs. The profitability is the difference between the revenue and the costs. These two factors are important in any investment's analysis (Barbosa, 1983).

ANALYSIS OF ORGANIC AND TRADITIONAL COFFEE FARMING IN LONDRINA

Table 2 indicates that each property produces an average of 135.89 bags of benefited coffee (60 kg). The productivity in organic coffee farms is on average 10.38 bags ha, which is equivalent to 35% of the productivity in traditional farms that is of 29.5 bags ha. It is important to notice, however, that the fall in organic production in Londrina was due to bad rust control.

In a specific case, production was by 12.5% of the average production prior to the conversion. The main factor was the lack of investment in coffee renewal on the part of the farmers who had initiated the conversion process. With old varietys, not resistant to rust and without conditions to apply properly the natural defenses (allowed), the producers had two depressed harvests.

For comparison, Brazilian productivity index was considered. As it is possible to analyze, the productivity of organic farms is 36.34% less than Brazilian productivity, and the productivity of the traditional coffee farms is 81% superior to the national average.

Table 2. Production of graded coffee per farm and productivity in 60 kg per ha.

Harvest period	Total production of graded coffee (60 kg)		Productivity in 60 kg benefited per ha			
	organic	traditional	organic	Traditional	Paraná*	Brazil*
2005/2006	40.82	194.38	8.05	27.13	13.49	14.86
2004/2005	71.5	236.84	12.71	31.88	21.57	17.75
Average	56.16	215.61	10.38	29.5	17.53	16.3
Index			63.66	181	107.5	100

**Source: ABIC.*

As Coste's (1975) model, Table 3 describes the nature of the main costs of the coffee culture. As it is possible to observe, the cost per hectare of the organic culture is 79% less than traditional system. The main expense of traditional farming is with fertilization, representing approximately 30% of the total expenses. After that, the most significant expenses are harvest labor (25%), followed by coffee berry borer control (24%).

In organic farms the coffee berry borer control is via baits of easy production and low cost (annual US$ 43.66), being considered very efficient. The labor cost in the organic system is low (annual US$ 358.04); for two main reasons: the property is smaller; and labor is most often supplied by the farmer and his family. The largest costs in organic production are: fertilization (47%), and the preparation of the coffee for the commercialization (23%).

Table 3. Nature of the costs related to coffee production in Londrina.

Cost nature	Organic	Vert. Analysis	Traditional	Vert. Analysis
Maintenance:	599.24	47%	2864.62	30%
Fertilization	594.52	47%	2856.00	30%
Others	4.72	0%	8.62	0%
Phytosanitary protection:	118.11	9%	3253.00	34%
Rust	74.45	6%	522.64	6%
Weed	0	0%	146.61	2%
Coffee berry borer	43.66	3%	2309.08	24%
Coffee leaf miner	0	0%	274.67	3%
Harvest:	255.10	20%	2390.43	25%
Labor	255.10	20%	2390.43	25%
Coffee Preparation:	294.35	23%	941.45	10%
Post-harvest treatment	102.94	8%	508.65	5%
Bags	88.47	7%	256.16	3%
Labor	102.94	8%	66.35	1%
Others	0	0%	110.29	1%
Total	1266.80	100%	9449.50	100%
Cost per ha	**52.37**		**390.64**	

As demonstrated in Table 4, the organic coffee producers on average receive 18% more per bag of benefited coffee commercialized.

Table 4. Prices obtained by coffee farmers in Londrina.

Price of commertialized coffee		Organic	Traditional
2005/2006	averege	120.79	89.22
	green coffee (40 kg)	27.57	36.19
	graded coffee (60 kg)	130.11	106.20
2004/2005	averege	110.53	82.20
	green coffee (40 kg)	36.76	34.32
	graded coffee (60 kg)	117.90	97.52

From Tables 4 and 2, it was possible to identify an average of revenue, represented in Table 5. Between 2004 and 2005, annual average revenue of organic coffee producers was of US$ 6495.13, while traditional production was of US$ 18480.85. However, the annual gross profit per hectare in traditional farm was US$ 2528.76 and annual gross profit in organic coffee farm was US$ 1200.30.

Table 5. Annual revenue of coffee farms in Londrina.

Harvest period	Organic		Traditional	
	Total	Per ha	Total	Per ha
2005/2006	4930.24	972.05	17344.05	2420.44
2004/2005	7902.64	1404.64	19468.54	2620.39
Averege	6495.13	1200.30	18480.85	2528.76

Gross profit per ha of coffee farming was calculated using data from Tables 3 and 5, and results are demonstrated in Table 6 and Figure 1. The revenue is the product of the average production by the average sales price. The average gross profit in organic farms per hectare represents 96% of the value of the revenue, while the profit for traditional producers is 85% of the revenue. Nevertheless, the gross profit per hectare of organic system is 46% less than gross profit of traditional farming.

Table 6. Gross profit per ha of coffee farms in Londrina.

Nature	Organic	Vert. Analysis	Traditional	Vert. Analysis
Revenue	1200.30	100%	2528.76	100%
Cost of sold coffee	52.37	4%	390.64	15%
Gross profit	1147.93	96%	2138.12	85%

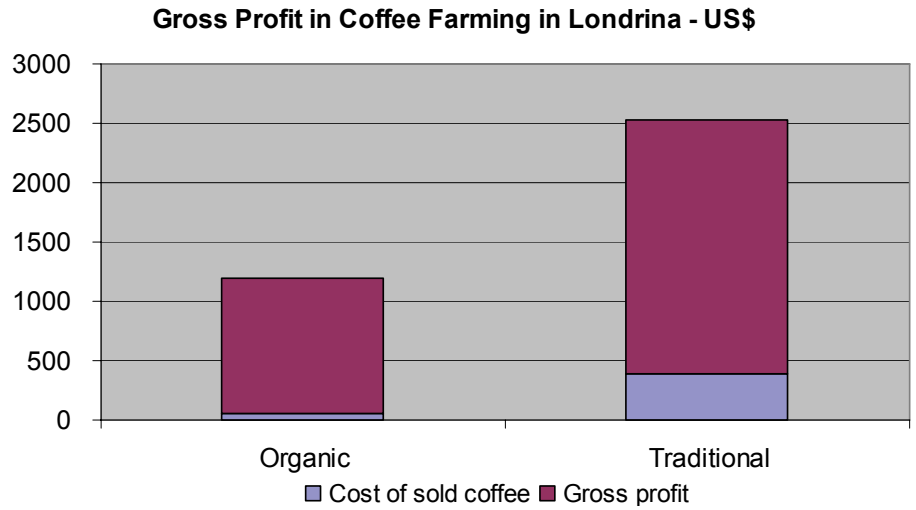


Figure 1. Gross profit, cost and revenue of coffee farms in Londrina.

CONCLUSION

Coffee is not a demanding culture in terms of investments. Therefore, it is possible to find inexperienced coffee producers, experienced sellers, speculators, environmentalists, among others. Each producer has its personal criteria of evaluation their farm, based on his or hers experiences and world awareness, being the administration of the production usually informal. In the majority of the properties costs, annual budget, formal planning, or quantitative information are not maintained. Generally, the producer does not know how much he spends, only knowing the value of the revenue.

The costs of a productive coffee farm are of four natures: maintenance, phytossanitary protection, harvest and coffee preparation to sell. The maintenance of farming is made through the control of the fertility of the ground. The phytosanitary refers to protection and control of pests, diseases and weeds. For this, a farmer can apply insecticides, herbicides, fungicides, weeding, among others. Coffee harvest is laborious and long, therefore the expenses of this period are representative. Finally, there is the necessary post-harvest treatment steps, namely separation of grains, drying, and grading. Both cultures have these costs, although the products used are not always the same and production costs tend to be smaller in organic farms.

The organic coffee producers in Londrina on average produce 56.16 bags of benefited coffee annually (60 kg), presenting the productivity of 10.38 bags per hectare. The traditional production, however, presents average production of 215.61 annual bags and productivity of 29.5 bags per hectare, above average national (16.30 bags per hectare).

The costs of the traditional production are high, totalizing on average US$ 390.64 per hectare. These costs are divided in: maintenance 30%; phytosanitary control 34%, harvest 25%; and preparation 10%. Costs in organic farming are on average US$ 52.37: maintenance 47%; phytossanitary control 9%; harvest 20%; and preparation: 23%. Thus, it is important to notice that annual costs of organic farming are significantly smaller than the traditional production.

The gross profit of organic producers was US$ 1147.93 per hectare, on the other hand the gross profit of traditional farmers was in US$ 2138.12 per hectare. Because of the productivity difference, the profit of organic coffee was much smaller than the traditional coffee. In consequence, from costs analysis perspective, the traditional production is more profitable.

It is remarkable, however, that both systems possess strong and weak points. The organic system would be preferable, for less significant environmental impact, the low costs and the perspective of growth in consumption in developed countries, where the population chooses products with appeals to preservation values and ecology. The consumers are also more opened to direct commercialization with organic producers, increasing the revenue by eliminating intermediates. Considering that the majority of the properties are small and use family labor, the main obstacle for implementing organic coffee is productivity.

REFERENCE

Associação Brasileira Da Indústria De Café (ABIC). Available in: <www.abic.com.br>. Access in: February 2006.

Barbosa Jairo S. Administração rural a nível de fazendeiro. 3rd edition. São Paulo: Nobel, 1983.

COFFEE CONTACT. Café Orgânico. Available in: http://www.geocities.com/rainforest/ canopy/1290/basics.html. Access in: November, 2005.

Coste R. (1975) El Café: Técnicas agrícolas y producciones tropicales. Barcelona, Editorial Blume.

FAO – CODEX Alimentarius Commission, 2005. Available in: http://www.fao.org/ag/portal/ home/ en/. Access in July 2008.

INSTRUÇÃO NORMATIVA Nº 007, de 17 de maio de 1999. Available in: http://www. agricultura. gov.br/. Access in July 2008.

Martins P.G., Laugeni F.P. Administração da Produção. São Paulo: Saraiva, 2002.

Matz A. et al. Contabilidade de Custos. Volume 1. São Paulo: Atlas, 1974.

Santos G.J., Marion J.C., Segatti S. Administração de Custos na Agropecuária. 3rd edition. São Paulo: Atlas, 2002.

Morpho-Agronomic Characterization of Seedlings of Wild *Coffea arabica* Accessions Under Controlled Conditions in Southwestern Ethiopia

T. KUFA[1*], J. BURKHARDT[2]

[1]Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Center, P. O. Box 192, Jimma, Ethiopia. [*]E-mail: kufataye@yahoo.com
[2]University of Bonn, Institute of Crop Science and Resource Conservation-Plant Nutrition, Karlrobert-Kreiten-Str.13, D-53115 Bonn, Germany

SUMMARY

The study was conducted with the primary objective to characterize accessions of wild *Coffea arabica* germplasm accessions collected from the montane rainforests in the south eastern and south western Ethiopia and *ex-situ* established at the Jimma Research Center, southwest Ethiopia. In this regard, morpho-agronomic descriptors were measured on one-year-old coffee seedlings managed under two contrasting irradiance (full sun and moderate shade) and irrigation (well watered and drought stressed) conditions. The results show highly significant differences among the seedlings in morphological and agronomic attributes under controlled nursery environments. The Harenna accessions were also noted to have higher values for such variables as average leaf area and main stem and these accessions were better adjusted to drought stress through such features as high root to shoot ratio, deep root system and thick leaves. This underlines the variability for most heritable attributes in Arabica coffee, demonstrating immense genetic diversity among wild *Coffea arabica* populations for breeding works in its birth place, Ethiopia.

INTRODUCTION

Ethiopia is the cradle land for the highland coffee (*Coffea arabica* L.*),* which has its origin in the montane rainforests of the southwest Ethiopia. It is a shade-adapted plant occurring in the undergrowth of natural rainforests of Ethiopia, where it has its center of origin and diversity. However, the remnant rainforests with the occurrence of wild coffee populations are under continuous threat largely due to a high deforestation rate and recurrent drought, and demand urgent actions for their conservation. Arabica coffee shows some remarkable features, which distinguish it from the other coffee species. The characteristics controlled by major genes from those under polygenic determinations include, among others, branching habit, young leaf color and measured plant morphological parameters. Such responses, however, depends upon plant species and cultivar and have been used as criteria to characterize coffee cultivars (Wintgens, 2004). Moreover, this information is necessary as a selection criterion for coffee cultivars as well as for determining optimum management inputs.

Therefore, knowledge of the effects of irradiance levels on growth characteristics of coffee plant is important to examine the mechanism involved in the shade-adapted Arabica coffees. Therefore, an *ex-situ* study was conducted for early morphological and agronomic characterizations of seedlings of the wild *Coffea arabica* accessions in full sunlight and moderate shade conditions at the Jimma Research Center nursery site, southwestern Ethiopia.

MATERIALS AND METHODS

The study area

The experiment was conducted at the Jimma Agricultural Research Center (JARC) (7° 46' N and 36° E) nursery site, southwest Ethiopia. Here, several national and international coffee collections are maintained with which various breeding, agronomy-soil-related studies are undertaken. JARC is situated within the temperate to cool humid highland agro-ecological zone at an altitude of 1753 m a.s.l. The area receives a high amount of rainfall with a mean total of 1556.9 mm per annum. The average maximum and minimum air temperatures are 26.7 and 12.8 °C, respectively (EARO, 2002).

Plant materials and seedling management

Fully ripe red cherries were collected from selected mother trees at three sub-sites within four wild *Coffea arabica* populations, namely, at Harenna, Bonga, Berhane-Kontir and Yayu. Consequently, a total of 12 coffee accessions three from each: Harenna (I-1, I-2, I-3), Bonga (II-1, II-2, II-3), Berhane Kontir (III-1, III-2, III-3) and Yayu (IV-1, IV-2 and IV-3) were established and examined under controlled nursery settings at JARC for early stage phenotypic variations.

At the same time, an ideal nursery site was selected and all pre-sowing activities were accomplished in January 2004. The media ingredients were blended at the recommended ratio of 3:1 topsoil and decomposed coffee husk compost, respectively (Taye et al., 2002, 2003). The black plastic pots were perforated at the bottom, firmly filled with the soil medium and arranged on a nursery seedbed.

All conventional post-sowing nursery operations (mulching, watering, shading, weeding, disease and insect control) were uniformly and timely applied according to the recommendations of the center (IAR, 1996). These practices were adhered to until the seedlings had reached the desired growth stage to start the treatments. They were uniformly managed under partial shade conditions and irrigation was applied at a 4-day interval during the dry months.

A split-split plot design with three replications was used to arrange the shade, irrigation and accessions as main-, sub- and sub sub-plot treatments, respectively. The blocks (terraces) and the shade treatment were oriented in east to west direction. The treatments included two shade levels (moderate shade and full sun), two irrigation regimes (well-watered and drought-stressed) and 12 coffee accessions. Each accession consisted of 25 seedlings per plot. Moderate (50% light interception) overhead shade (2 m height) and side shades were constructed from bamboo slants. Maximum care was taken to avoid side-shading effects between the treatments. In this case, the shade plots were far apart from each other (12 m), while the distances between irrigation and accession plots were 2 m and 1 m, respectively. The spacing between coffee seedlings was also changed with increased extension growth of the seedlings. Accordingly, at the beginning, it was 10 cm x 10 cm and later this increased to 20 cm x 20 cm.

DATA MEASUREMENTS

Leaf and branch orientation

Leaf and branch angles were examined on one-year-old seedlings of the coffee accessions in the moderate shade condition. Five seedlings per accession and two primary branches per seedling were sampled for measurement. The angle between the main trunk and lateral branch as well as leaf angle at the base of the petiole (bottom and upper nodes) were measured using a protractor compass (0-180°). In addition, visual scores on the frequency of young leaf color (deep bronze, light bronze, deep green and light green) were recorded for the same seedlings. A plot consisted of 25 seedlings per accession. The well-watered seedlings were placed in the light and moderate shade conditions using an elephant grass and the bamboo slants, respectively.

Shoot and root parameters

These were measured on coffee seedlings grown under optimal nursery environments with moderate shade and irrigation applied. Five central seedlings per plot were used to record intact and destructive morphological growth parameters. The non-destructive vegetative data were recorded on one-year-old seedlings. Intact leaf area, crown area and leaf area index were derived from the collected primary data on the coffee seedlings. The shade treatment was applied between March and May 2005. Dry weight of leaves, main stems, primary branches and root were recorded for the sample seedlings. The roots were immersed and washed in water to remove adhering soil. Measurements on root parameters (lateral root number, lateral root length, taproot length and root volume) were made for each treatment. Subsequently, each plant part was oven-dried at 105 °C for 24 h and weighed using a sensitive balance.

Statistical analysis

Analysis of variance (ANOVA) was carried out with the SAS system for Windows-v8 (SAS Institute Inc. Cary NC, USA). Comparison between means was carried out according to Tukey test at $P = 0.05$ whenever the F-test declared significant differences. Data were also analyzed using the principal component and cluster analyses to describe the extent of variability among the wild *Coffea arabica* accessions. Graphs were prepared with SigmaPlot SPW9.0 (SYSTAT Software, Inc.).

RESULTS

Branch and leaf orientation

One-way ANOVA depicted that coffee seedlings of the 12 accessions uniformly managed under partial shading differed significantly ($P < 0.001$) in the orientation (angle) of the primary branch measured from the main stem axis. Consequently, the progenies from Harenna (I-1 = 48.83° and I-2 = 46.67°) and Yayu (IV-1 = 45.50° and IV-2 = 43.50°) exhibited maximum horizontal branch angles as opposed to the Berhane-Kontir progenies with reduced acute branch angles, ranging from 40 to 42° (Figure 1). On the other hand, coffee seedlings did not show significant variations in leaf angle measured at the base of the petiole. The results show that the acute leaf angle was greater than that of the primary branch. In the Harenna and Yaya accessions, leaf orientation was close to a right angle, i.e., almost double that of the branch angle. The most horizontal and vertical leaf angles were measured on the Yayu (IV-2 = 75°) and Berhane-Kontir (III-2 = 66°) accessions (Figure 1), respectively. In general, the degree of branch orientation followed the descending order: Yayu

> Harenna > Bonga > Berhane-Kontir, largely due to the difference in the growth stage of coffee seedlings. The Bonga accessions were intermediate in leaf and branching habits. Nevertheless, the pattern is similar to that of the branching habit and this was confirmed by the positive and significant correlation value (r = 0.27*) between branch and leaf angles (data not presented). Hence, based on leaf and branch orientation, the Harenna and Yayu seedlings had semi-horizontal, while those in Bonga and Berhane-Kontir showed semi-erect growth habit. This supports the crown habits of the respective mother trees in field conditions.

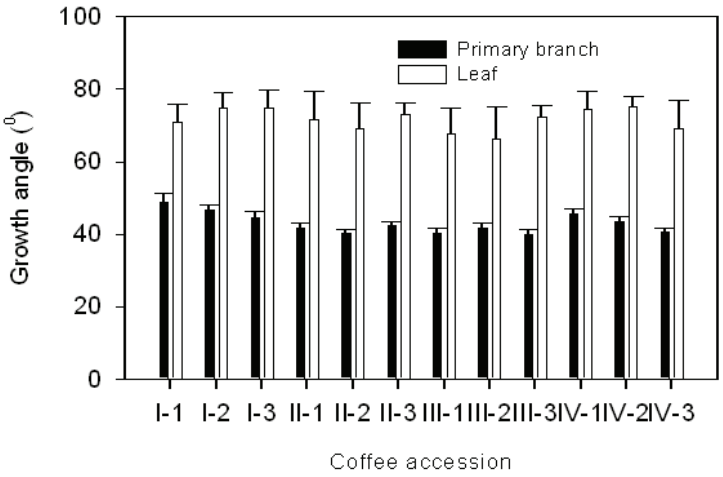


Figure 1. Primary branch and leaf orientation in seedlings of wild coffee accessions managed in modrate shade environments.

Shoot morphology

The extent of morphological variability among coffee seedlings was different during the different growth stages. The accessions showed significant differences in the early growth performance, but this disappeared with increased seedling age. The results of the extension growth parameters of one-year-old seedlings are presented in Table 1. The results depict no significant growth response among accessions in all the morphological parameters considered. There was even a change in the order of ranking among the accessions, like for example in height elongation pattern, between Yayu and Harenna seedlings. The Harenna seedlings exhibited the greatest average values for most variables such as seedling height, main stem diameter and length of internode on primary branch. The overall average seedling height was about 65 cm, while the undebarked main stem diameter was about 1.2 cm. Similarly, the Berhane-Kontir seedlings had reduced main stem nodes and long internodes on the primary branch, whereas the shortest seedlings with thin main stem diameter and short internodes on lateral branches were found in the Bonga coffee accession (II-1). This was followed by Yayu seedlings with higher values of leaf growths. These are some of the features of compact coffee types.

In a similar fashion, the average leaf areas of the seedlings were different among accessions, and maximum values were determined for Harenna and Berane-Kontir seedlings. This was in contrast to the reduced average leaf size in Bonga and Yayu. Accordingly, the total leaf area ranged from 1162.75 cm^2 to 1787.26 cm^2 for Berhane-Kontir (III-1) and Harenna (I-3) accessions, respectively. Despite the narrow and small leaf size, unlike Bonga coffees, Yayu accessions had maximum total leaf surface area and leaf area index (Table 1), indicating the indirect association between leaf number and leaf size. Hence, the leaf area index ranged from 3.36 to 5.10 for the same accessions, indicating the direct strong link between total leaf

surface area and leaf area index. Unlike the Bonga accessions, the higher leaf production in the Yayu accessions contributed to the increased leaf area index. Leaf length ranged from 9.82 to 10.68 cm for the corresponding accessions from the Harenna and Berhane-Kontir seedlings. However, the maximum leaf width was recorded in Harenna and the narrowest leaves in Bonga. The magnitude of variation was minimal for leaf dimensions (length and width), and mean leaf area was more dependent on leaf width. This resulted in the overall average results of 10.19 and 4.25 cm for leaf length and width, respectively. The accessions also showed maximum variations in the growth of primary branches, which was highest for Harenna as opposed to the lowest growth for Yayu accession. Further evaluation should, therefore, confirm whether or not such growth responses remain at the later stages in field conditions.

Shade treatments were applied in the later stage of the seedlings and lasted only for a 2-month period (between June and July 2005). As a result, no significant differences were observed for most extension and destructive parameters. However, slightly higher values of main stem diameter, number of lateral branches and number of nodes on main stem were recorded for sun-exposed seedlings than for those in shade plots. The influence of shade treatment was more noticeable on leaf growth as compared to stem and root parameters. Consequently, the seedlings produced a significantly ($P < 0.001$) higher leaf number in full sun than in shade environments (Figure 2). However, there were significant ($P < 0.05$) reductions in leaf dimensions (length and width) and thus average leaf area in unshaded plot, although higher total leaf surface area and leaf area index were obtained in partial shadow conditions.

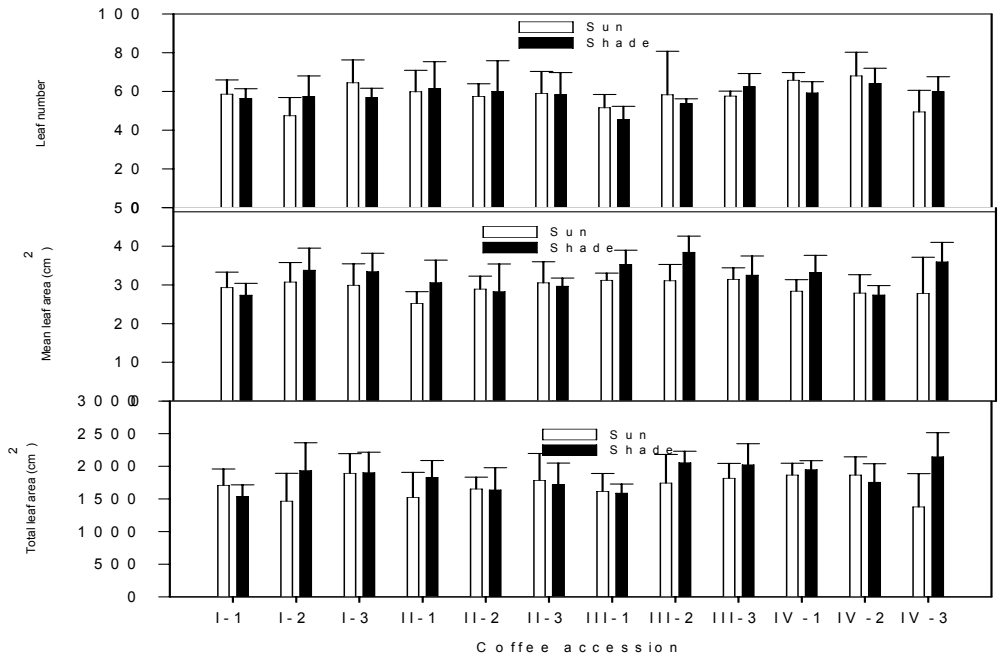


Figure 2. Leaf growth parameters of coffee seedlings as influenced by contrasting shade regimes.

Table 1. Morphological growth of seedlings of wild *Coffea arabica* accessions under controlled nursery conditions.

Accession	Height	Girth	NMSN	NPrBr	NBrN	BrIL	MSIL	LN	LL	LB	MLA	TLA	LAI
I-1	75.23	1.33	13.50	8.25	24.75	5.89	5.90	48.00	9.97	4.53	29.99	1385.27	4.00
I-2	74.45	1.27	12.75	7.50	21.00	5.54	7.28	47.00	9.82	4.36	28.46	1326.26	3.83
I-3	68.23	1.23	10.25	10.50	27.50	5.59	6.22	58.00	10.10	4.73	31.71	1787.26	5.17
II-1	58.38	1.18	12.50	8.75	28.50	4.82	6.27	58.00	10.29	4.32	29.57	1635.60	4.72
II-2	61.38	1.10	13.25	8.50	25.25	4.51	5.98	47.75	9.90	4.11	27.01	1269.05	3.67
II-3	60.75	1.09	12.25	7.50	23.25	4.90	6.39	49.50	10.21	3.81	25.50	1275.13	3.69
III-1	60.88	1.18	12.25	7.00	21.50	5.09	5.98	45.25	9.79	4.02	26.09	1162.75	3.36
III-2	61.55	1.13	12.00	8.50	21.25	5.44	6.91	48.50	10.97	4.59	33.29	1591.02	4.60
III-3	64.43	1.17	12.25	8.00	23.25	5.19	7.24	55.25	10.68	4.15	29.32	1575.88	4.56
IV-1	62.90	1.26	11.25	7.00	25.00	5.53	6.84	58.50	10.35	4.16	28.39	1657.16	4.79
IV-2	65.13	1.18	13.00	8.50	31.25	5.22	6.19	58.75	9.90	3.96	25.90	1506.59	4.36
IV-3	62.65	1.24	12.25	7.75	27.25	5.05	6.09	61.00	10.33	4.26	29.32	1664.95	4.81
Pr>F	**Ns**	**Ns**	**Ns**	**Ns**	**Ns**	**Ns**	**Ns**	**Ns**	**Ns**	**Ns**	**Ns**	**Ns**	**Ns**
Mean	**64.66**	**1.19**	**12.29**	**8.15**	**24.98**	**5.23**	**6.44**	**52.96**	**10.19**	**4.25**	**28.71**	**1486.41**	**4.29**
CV (%)	**7.85**	**7.04**	**9.00**	**17.41**	**21.89**	**7.21**	**10.09**	**14.64**	**4.11**	**6.54**	**10.09**	**11.97**	**11.99**

NS = Not significantly different from each other at P = 0.05. Abbreviations: NMSN = Number of main stem node, NPrBr = number of primary branch, NBrN = number of branch nodes, BrIL = branch internode length, MSIL = main stem internode length, LN = leaf number, LL = leaf length, LB = leaf breadth, MLA = mean leaf area, TLA = total leaf area and LAI = leaf area index.

There were no significant interactions between shade and coffee accessions for most of the morphological growth parameters studied. However, most accessions exposed to direct sunlight had higher results for leaf variables, except average leaf area. However, few accessions, particularly from Berhane-Kontir, had higher leaf growth in shade compared with the open sun conditions (Figure 2). In other words, the variations were very pronounced between the Harenna and Berhane-Kontir seedlings, which is in line with the results of seed germination and early stage growth performance of the seedlings as discussed above. In essence, although intra-population variations in morphological responses were evident, the wild coffee accessions were identified to have varying growth habits: open type (Harenna and Berhane-Kontir) and compact (Bonga and Yayu) canopy natures and hence, their adaptation strategies to specific environments could differ accordingly as reported by Burkhardt et al. (2006).

With regard to the coordination and classification of the accessions, the results of the principal component analysis for the extension data reveal that in principal axis-1 leaf growth parameters (total leaf area, average leaf size, leaf width, leaf number and leaf length) were the major variables for the variances among accessions. In principal axis-2, seedling height (50%), stem size (38%) and internode length of primary branch (44%) were responsible for the variability among accessions. Accordingly, coffee accessions were located along the axis on the basis of their geographical closeness, particularly between the southeast and southwest populations (Figure 3). Such differentiation was observed for the Bonga and Harenna accessions, which were located at a far distance. About 50% of the accessions, however, did not reveal this pattern. Likewise, the result of the cluster analysis for shoot morphological parameters also depicts the same patterns of grouping the coffee accessions. Consequently, at a rescaled distance between 10 and 15, the accessions were clustered into 5 classes: group 1 consisted of Yayu and Berhane-Kontir, group 2 of an accession from Berhane-Kontir, III-2, group 3 of a Harenna accession alone-I-3, group 4 of Bonga accessions, and group 5 of Harenna accessions (Figure 4).

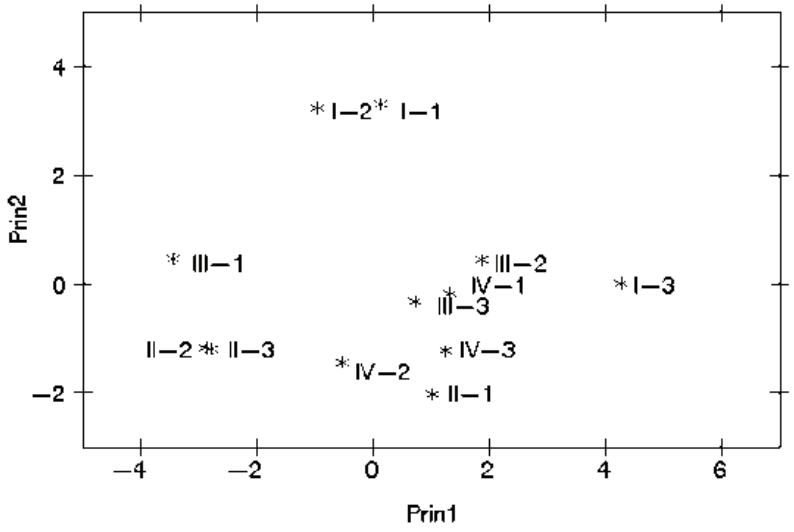


Figure 3. Principal component analysis for extension growth parameters of coffee seedlings under optimal shade and irrigation condition.

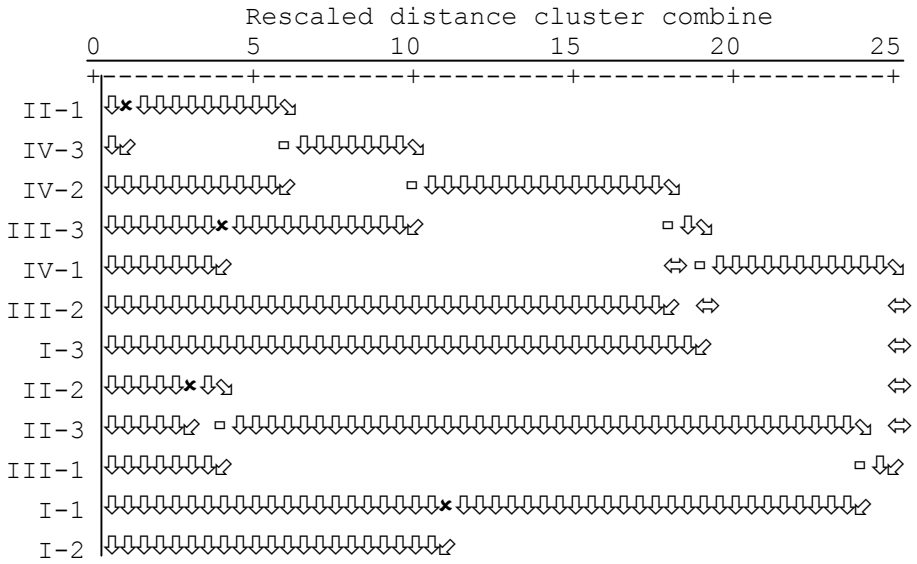


Figure 4. Dendrogram for clustering of wild coffee accessions according to results of extension morpho-agronomic parameters.

Root morphology

Although insignificant, the number and volume of lateral roots was higher for unshaded seedlings. Taproot and lateral roots were slightly longer in the shade, though not significantly different to those of unshaded seedlings. On the other hand, coffee accessions significantly differed in total root volume ($P < 0.001$), taproot length ($P < 0.05$) and length of lateral roots ($P < 0.01$). The accessions, however, did not differ in the number of lateral roots, though the respective maximum and minimum counts were obtained from the Harenna and Berhane-Kontir accessions. The longest and shortest lateral roots were obtained from Yayu (IV-1) and Harenna (I-3) seedlings, respectively. This reflects that coffee saplings can adapt to drought situations by extending the root system into deeper soil layers. Berhane-Kontir accessions had the significantly lowest root volume as opposed to the highest value for the Harenna seedlings (Table 2).

Table 2. Shoot and root growth parameters (means±SD) in coffee seedlings according to shade, irrigation and accession treatments.

Treatment	LDW (g)	SDW (g)	RDW (g)	RV (g cm^{-3})	TRL (cm)	LRN	LRL (cm)	R: S	TDM (g)
Shading	Ns	**	**	*	*	Ns	Ns	Ns	**
Full sun	13.58±1.59	17.48±2.49	8.93±1.64	38.52±7.32	29.57±5.91	38.73±4.17	17.97±1.05	0.29±0.03	39.99±5.31
Shade	13.06±0.91	15.60±1.55	7.89±1.52	36.03±7.09	32.03±4.20	37.84±4.64	18.42±1.46	0.28±0.04	36.54±3.13
Irrigation	*	*	**	Ns	***	*	Ns	Ns	**
Stressed	13.68±1.40	17.14±2.53	8.90±1.52	36.36±6.03	33.68±4.90	39.52±4.00	18.42±1.19	0.29±0.03	39.72±4.95
Watered	12.95±1.12	15.94±1.81	7.92±1.66	38.18±8.31	27.91±3.80	37.05±4.48	17.98±1.35	0.27±0.04	36.81±3.92
Accession	Ns	*	**	***	*	Ns	*	**	*
I-1	13.25±0.80	19.08±2.01a	10.02±1.11a	46.25±9.19 ab	34.45±5.00	41.10±2.68	19.62±1.35	0.31±0.04ab	42.34±3.43a
I-2	13.06±0.89	18.47±2.09ab	10.43±1.23a	48.50±2.78 a	35.42±6.13	43.20±5.60	19.45±0.63	0.33±0.01 a	41.95±4.03ab
I-3	13.73±1.85	16.63±1.78ab	9.22±0.95ab	42.70±2.55abc	34.06±6.35	38.43±0.97	19.19±0.45	0.31±0.02ab	39.57±4.52ab
II-1	13.63±1.78	16.65±2.47ab	8.30±1.44abc	34.95±4.99 cd	28.78±3.76	39.25±7.93	17.07±0.97	0.28±0.02abc	38.58±5.57ab
II-2	12.34±0.66	15.52±1.33ab	7.97±1.11abc	35.25±5.54bcd	31.74±5.06	39.70±3.50	17.77±0.77	0.28±0.03abc	35.82±2.90ab
II-3	12.81±0.68	15.29±1.66ab	8.15±1.34abc	37.55±4.35a-d	30.71±6.20	36.58±1.83	19.16±0.61	0.29±0.04abc	36.24±2.84ab
III-1	11.91±0.51	14.84±1.45ab	6.48±1.09c	32.70±5.13 cd	27.38±4.15	33.08±3.74	17.99±1.18	0.25±0.04 bc	33.23±1.93b
III-2	12.97±1.29	14.36±1.85b	7.22±1.66bc	31.20±4.98 d	28.73±4.53	36.50±4.89	18.11±1.41	0.27±0.04abc	34.54±4.21ab
III-3	14.37±1.45	16.11±2.41ab	6.86±1.20bc	28.80±4.67 d	27.39±6.56	36.60±2.15	17.31±0.91	0.23±0.03 c	37.33±4.65ab
IV-1	13.87±0.76	17.16±1.50ab	8.67±0.30abc	37.25±4.33a-d	27.14±2.74	38.15±4.83	17.03±0.99	0.28±0.02abc	39.69±2.09ab
IV-2	14.41±0.67	18.06±1.24ab	9.39±1.00ab	38.65±3.22a-d	30.58±1.25	39.75±2.71	18.38±1.34	0.29±0.01abc	41.87±2.81ab
IV-3	13.52±2.12	16.29±3.37ab	8.23±2.43abc	33.45±4.89cd	33.21±5.18	37.10±3.19	17.32±1.03	0.27±0.05abc	38.03±7.63ab
Mean	**13.32**	**16.54**	**8.41**	**37.27**	**30.80**	**38.29**	**18.20**	**0.28**	**38.26**
CV (%)	**7.95**	**9.35**	**10.72**	**10.61**	**10.38**	**9.77**	**5.05**	**9.31**	**8.20**

*Ns = Not significant; *, ** and *** = significant at P<0.05, P<0.01 and P<0.001, respectively. Means followed by same letter with in a column are not different from each other (Tukey test at P = 0.05). Abbreviations: LDW = leaf dry weight, SDW = stem dry weight, RDW = root dry weight, RV = root volume, TRL = taproot length, LRL = lateral root length, R:S = root to shoot ratio, TDM = total dry matter.*

CONCLUSION

The results reveal significant variability among seedlings of wild *Coffea arabica* accessions for most morpho-agronomic characteristics under contrasting irradiance and irrigation levels. This is in agreement with our report (Taye et al., 2004) on the *in-situ* growth variability among wild *Coffea arabica* populations, indicating the heritability of the attributes for breeding works. The morpho-agronomic descriptors and the interplay between root and shoot growth can be in part influenced by environmental factors and thus the magnitude of interaction between genotype by environment at each recommendation domain in each locality of origin remains to be investigated. Hence, it is important to further evaluate the *Coffea arabica* germplasm, which have been established in field conditions at the Jimma Research Center so as to identify and advance the most promising ones for each area of collection. There is also a need for complementary *in-situ* and *ex-situ* conservation strategy and utilization of *Coffea arabica* gene pools in Ethiopia.

REFERENCES

Burkhardt J., Kufa T., Beining A., Goldbach H. and Fetene M. 2007. Different drought adaptation strategies of *Coffea arabica* populations along rainfall gradient in Ethiopia. In: Proceedings of the 21st International Conference on Coffee Science Colloquium, 11-15 September 2006, Montpellier, France, pp. 1032-1036.

Daniel G., Venkataramanan D., Awati M.G., D'souza G.F., Achar D., Saraswathy V.M., Udayakumar M. and Selvakumar M. 2004. Studies on genetic variability for root characteristics and water use efficiency in robusta coffee. In: Proceedings of 20th International Conference on Coffee Science (ASIC), 11-15 October 2004, Bangalore, India, pp. 1089-1093.

EARO (Ethiopian Agricultural Research Organization). 2002. Strategies and priorities document for national coffee research project. Crop research strategy document, Addis Ababa, Ethiopia.

IAR (Institute of Agricultural Research). 1996. Recommended Production Technologies for coffee and associated crops. Jimma Research Center Task Force, Addis Ababa, Ethiopia.

Kassahun T. 2006. Genetic diversity of wild *Coffea arabica* populations in Ethiopia as a contribution for conservation and use planning. Ecology and Development Series, 44, Cuvillier Verlag Göttingen, 137.

Montagnon C. and Bouharmont P. 1996. Multivariate analysis of phenotypic diversity of *Coffea arabica*. Genetic Resources and Crop Evolution 43(3): 221-227.

Pinheiro H.A., Da Matta F.M., Chaves A.R.M., Loureiro M.E. and Ducatti C. 2005. Drought tolerance is associated with rooting depth and stomatal control of water use in clones of *Coffea canephora*. Annals of Botany 96: 101-108.

Taye K., Burkhardt J. and Goldbach H. 2004. Ecophysiological variability of forest Arabica coffee populations in hydraulic characteristics along a climatic gradient in Ethiopia: Morphological and physiological variability. In: Proceedings of the 20th International Conference on Coffee Science (ASIC), 11-15 October 2004, Bangalore, India, 929-939.

Taye K., Tesfaye S. and Alemseged Y. 2002. Influence of media mixture and watering frequency on seed germination and seedling growth of Arabica coffee in Ethiopia. In: Proceedings of the 19th International Conference on Coffee Science (ASIC), May 14-18, 2001, Trieste, Italy.

Taye Kufa, Mesfin Abebe and Paulos Dubale. 2003. Contribution of Organic Amendment to Physico-chemical Conditions of Coffee Nursery Media. Pp 25-40. In: Tilahun Amede and Eylachew Zewdie (eds.). Proceedings of the Sixth Conference of the Ethiopian Society of Soil Science (ESSS) on Challenges of Land Degradation to Agriculture in Ethiopia, Feb 28-March 1, 2002, Addis Ababa, Ethiopia.

Walyero D.J. and Van der Vossen H.A.M. 1979. Early determination of yield potential in Arabica coffee by applying index selection. Euphytica 28: 465-472.

Wintgens J.N. (ed.). 2004. Coffee: Growing, Processing, Sustainable Production. A guide for growers, traders, and researchers. WILEY-VCH Verlag GmbH and Co.KGaA, Weinheim, Germany.

Study on the *Coffea arabica/Colletotrichum kahawae* Pathosystem. Impact of a Natural Elicitor (FEN 560) on this Pathosystem

N. DURAND[1], B. BERTRAND[2], B. GUYOT[1], J. GUIRAUD[1], A. FONTANA-TACHON[1]

[1]UMR Qualisud CIRAD-UMII, 34398 Montpellier, France
[2]UMR RPB CIRAD, 34398 Montpellier, France

SUMMARY

Using elicitors is a way of reducing the use of weedkillers, fungicides and insecticides by inducing the natural defence reactions of plants. FEN560 is a natural product extracted from Fenugreek (*Trigonella foenum-graecum*) and its elicitive action is currently known on many pathosystems. Coffee Berry Disease (CBD), due to *Colletotrichum kahawae*, causes fruit-rot (*Coffea arabica*) and can reduce yields by up to 40%. The objective of the study was to characterize the reactions induced by *Colletotrichum kahawae* on the *Coffea arabica* varieties *Caturra* (susceptible) and *Marseilleissa* (low susceptibility) and to verify the action of a natural elicitor (FEN560) on that pathosystem. The enzyme activity of phenylalanine ammonia-lyase (PAL) and the caffeine and chlorogenic acid (CGA) concentrations in hypocotyls of seedlings inoculated with the pathogen, then treated with FEN560, were quantified. The inhibitory effect of caffeine on *C. kahawae* was confirmed. Direct observation of the symptoms on seedlings demonstrated an effect of FEN560 on the studied pathosystem. However, in spite of the positive action of the elicitor, it did not seem to stimulate the biosynthesis of alkaloids (caffeine) or of polyphenols (CGA). Nevertheless, the pathogen triggered synthesis of caffeine and chlorogenic acid in *Coffea arabica,* which thus appeared to be worthwhile markers for studying Coffee Berry Disease resistance in *Coffea arabica*.

INTRODUCTION

Elicitors trigger natural resistance reactions in plants and can be used to reduce environmentally toxic pesticide treatments. FEN 560 is a natural extract of fenugreek (*Trigonella foenum greacum)* which has known elicitor activity (Siddi et al., 2005) in some pathosystems. Coffee berry disease (Bieysse et al., 2002), due to the mould *Colletotrichum kahawae*, causes arabica coffee berry rot that can cause crop losses of up to 40%. The aim of the present study was to analyse *Colletotrichum kahawae*-induced reactions on *Coffea arabica* and to assess the action of the natural elicitor FEN 560 on the same pathosystem. We monitored variations in the concentration of two biochemical compounds involved in coffee natural defense mechanisms, i.e. caffeine (Biratu et al., 1997) and chlorogenic acids (CGA) (Macheix et al., 1990), on *Colletotrichum kahawae*-contaminated coffee plantlets that had been treated with FEN 560.

MATERIALS AND METHODS

- Two *C. arabica* varieties: Caturra (Cat) (susceptible) and Marseilleissa (Mar) (relatively unsusceptible).
  - Elicitor treatment at D0: FEN 560:
  - 30 g/L
  - 0.5 L.m

- *C. kahawae* inoculations at D0 + 2:
  - 2.106 conidia/mL
  - 0.4 L/m^2

RESULTS

Pathosystem variations in the presence of FEN 560

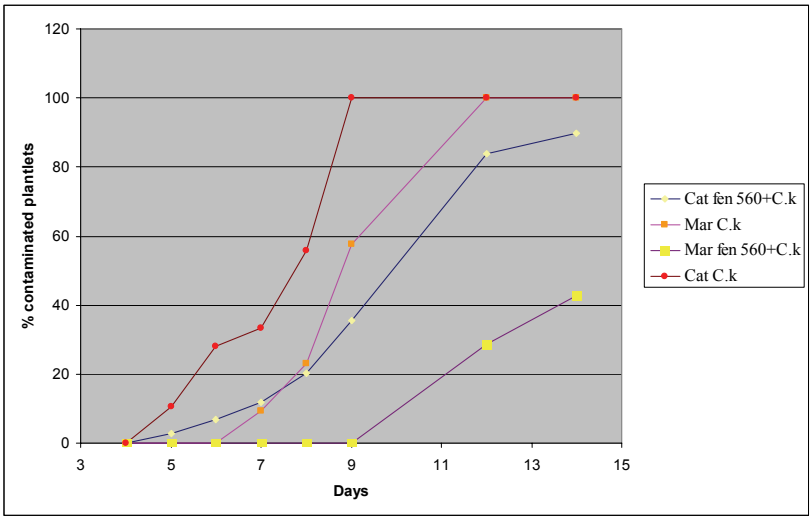


Figure 1. Variations in the number of contaminated plantlets according to varieties and treatments.

- Effects of FEN 560:
  - on symptom onset
  - on the contamination rate

- Responses of treated cv Caturra plants (susceptible) were similar to those of untreated cv Marseilleissa plants (relatively unsusceptible).

Figure 2. Caturra plantlets treated with FEN 560 and contaminated by *C. kahawae* 15 days postinoculation.

Figure 3. Caturra plantlets not treated with FEN 560 and contaminated by *C. kahawae* 15 days post-inoculation.

Variations in Caffeine contents

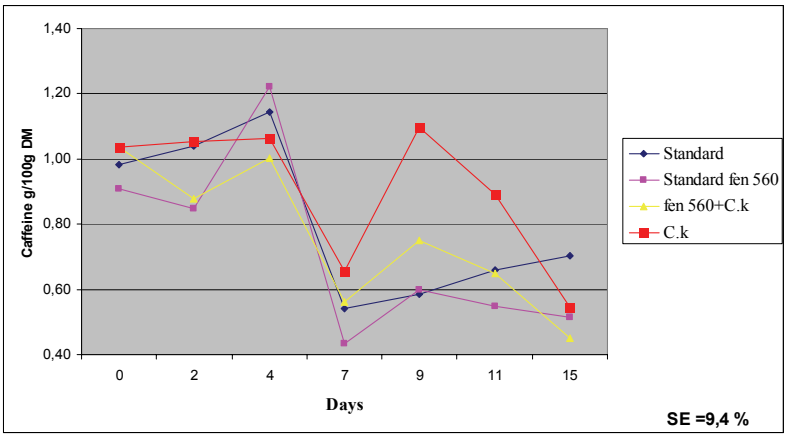


Figure 4. Caffeine concentration in hypocotyls of *Coffea arabica* cv Caturra (susceptible).

- Higher caffeine concentration in the presence of the pathogen.
- No elicitor effects.
- Slight impact of the elicitor in the presence of the pathogen.

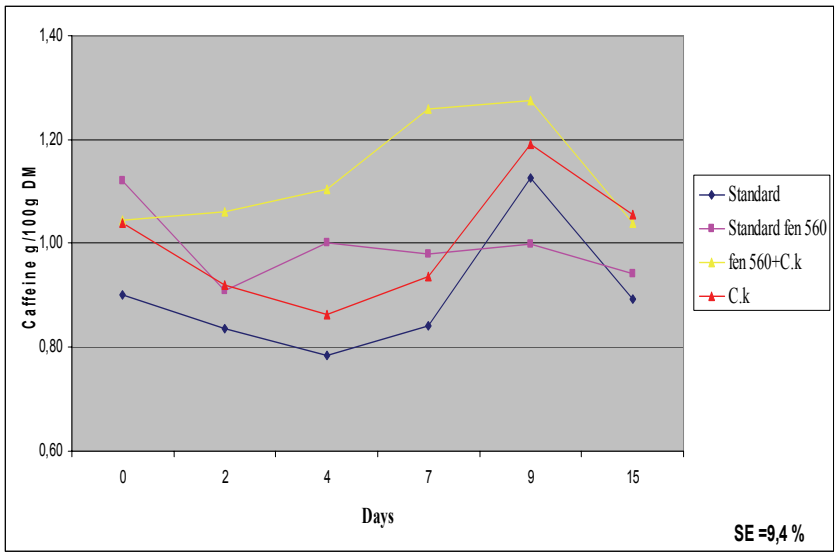


Figure 5. Caffeine concentration in hypocotyls of *Coffea arabica* cv Marseilleissa (relatively unsusceptible).

Variations in CGA contents

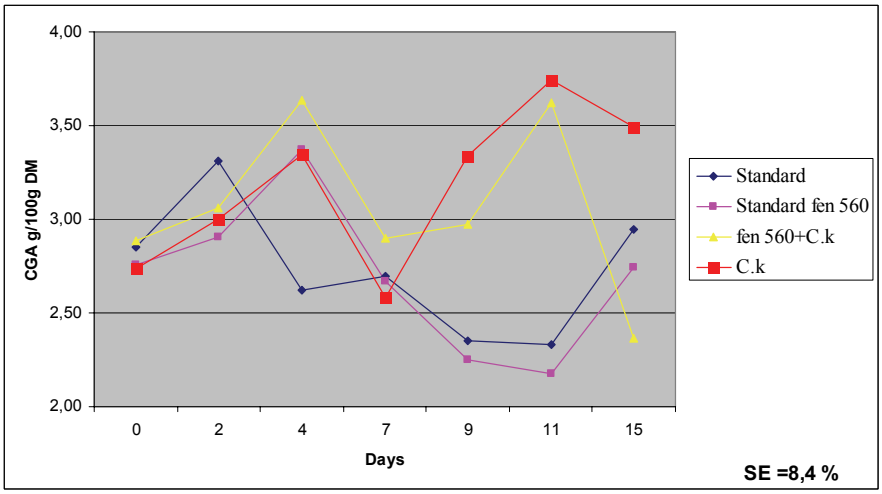


Figure 6. CGA concentration in hypocotyls of *Coffea arabica* cv Caturra (susceptible).

- Higher CGA concentration in the presence of the pathogen.
- No elicitor effects.
- Very little impact of the elicitor or the pathogen.
- The CGA concentration in cv Marseilleissa was in all cases twofold higher than in cv Caturra.

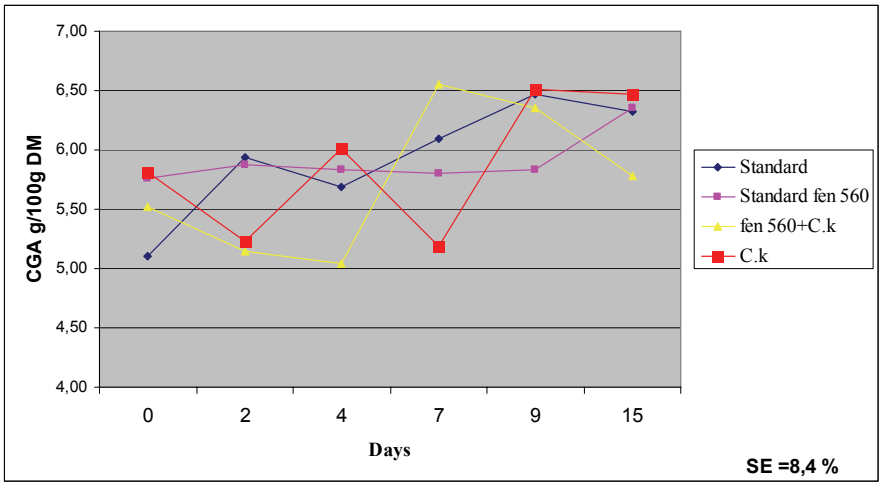


Figure 7. CGA concentration in hypocotyls of *Coffea arabica* cv Marseilleissa (relatively unsusceptible).

CONCLUSION

- We assessed symptoms directly in plantlets and noted a real effect of FEN 560 on the pathosystem regardless of the susceptibility of the coffee variety studied. However, despite a positive impact of the elicitor, it did not seem to stimulate alkaloid (caffeine) or polyphenol (CGA) biosynthesis pathways.
- In *Coffea arabica* cv Caturra (susceptible) plantlets, the pathogen did induce caffeine and chlorogenic acid synthesis, but this was not observed in the relatively unsusceptible variety which has a naturally high CGA content.
- Caffeine and CGA are thus markers of interest for investigating coffee berry disease resistance in *Coffea arabica,* which could be confirmed by studies on other varieties with different degrees of susceptibility.

REFERENCES

Bieysse D., Bella Manga M., Mouen Bedimo J., Ndeumeni J., Roussel V., Fabre J., Berry D. (2002). L'anthracnose des baies. Une menace potentielle pour la culture mondiale de l'Arabica. Recherche et caféiculture. CIRAD. Montpellier, France. 143-156.

Biratu T. (1997). Effects of caffeine on coffee berry disease/*Colletotrichum kahawae*. In 17th ASIC Colloquium. Nairobi. Kenya. 727-733.

Macheix J.-J., Fleuriet A., Billot J. (1990). Changes and metabolism of phenolic compounds in fruits. Fruit Phenolics. CRC Press. 149-237.

Siddi C., Lestienne I., Meytraud F., Marmey P., Baccou J.C., Nicole M., Martinez C. (2005). Un nouvel éliciteur FEN560 potentialise les mécanismes de défense chez plusieurs modèles plantes / agents pathogènes. VIème Congrès National Société Française de Phytopathologie. Toulouse, France.

Development of Selection Indices Based on Morphological Traits and Average of the First Two Years Yield at Early Bearing Stage of *Coffea arabica* L.

H. ERMIAS, B. BAYETTA AND H. SINGH

Ethiopian Institute of Agricultural Research, P.O. Box 2003, Addis Ababa

SUMMARY

Study was conducted to develop selection indices in order to select the accessions possessing high yield potential at early bearing stage in Western region of Ethiopia coffee germplasm. The experimental materials comprised of eighty one accessions collected in 1999 from western part of the country. The lines were planted in 2001 at Haru Research Sub-Center using a 9x9simple lattice design. The following characters were taken to construct selection indices due to their high genotypic correlation with average yield of the first two year and high direct effect. Girth at the base main stem, average length of primary and canopy diameter, number of main stem node and average yield of the first two year production. As a result higher genetic advance were obtained in selection indices based on four and three character combination, accordingly these indices also revealed higher relative efficiency than the direct selection for yield of the first two year production. However, selection indices based on two character combination with out average length of primary exhibited lower efficiencies compared to direct selection for yield. Therefore, selection of higher yielding land races may be conducting through the use of four, three and two character combination including the average length of primary at early stage in order to advance the genotypes for further breeding work at early stage.

INTRODUCTION

The premature identification of productive coffee genotypes with larger precision at the initial bearing stage is highly desirable in genetic improvement of a coffee plant. Index selection is an optimal procedure for selection, and it uses all information available about each parameter genotypic and phenotypic value combined to an index merit. It is the best linear prediction of an individuals breeding value and it takes of a multiple regression of breeding value of all source of information (Falconer, 1989).

Much work has not been done in establishing selection indices for early selection in Ethiopia for Coffee arabica L. However, results of some studies conducted elsewhere, indicated that a selection index for early determination of yield potential is a useful breeding tool. The study by Walyaro and Van der Vossen (1979) indicated that the genetic advance in the yield based on the first two years yield, stem girth, and percent bearing primaries was 97% efficient compared to selection based on ten years yield totals. Reviews made by Bayetta (1989) in crops other than coffee also revealed selection indexes based on combination of characters are in general more efficient than those based on single character. Sirinivasan (1982), reported that indices based on two and even a single character in coffee such as internodal length exhibited an efficiency which was as high as that based on yield initial bearing stages.

Sera (1987), using multiple regressions estimated the coefficient of determination (0.717) for selection index composed of production of fruits, height, canopy diameter and the first two years of production. The results showed the effectiveness of pre-selection for yield in coffee can be achieved using an index comprised of initial stages of production and certain growth characters that are highly associated with yield. Therefore, study was conducted on coffea arabica L.in Ethiopia to determine and evaluate the efficient selection indices at early bearing stage during 2001.

MATERIAL AND METHODS

The experimental materials were collected from different areas of Western Ethiopia in 1999. Four representative trees were selected per plot to measure the traits used for selection index. Analysis of variance for yield and yield related traits and CBD were analyzed using statistical software known as Statistical Analysis System SAS (1989).

Selections indices were constructed, by considering selection aimed at imparting single character (average yield of the first two year production) to conduct selection at early bearing stage of coffee. The following characters were taken to construct selection indices. Girth at the base of main stem, average length of primary and canopy diameter, based on their wide sense heritability, high genotypic correlation with the character of yield for the first two year of production, and the ease of measuring these character on coffee (Walyaro, 1983). The character number of main stem node was added, based on high direct effect on average yield in the present study.

The indices were constructed according to the formula described by (Falconer, 1989) by using the available estimates of phenotypic and genotypic variances and covariance. The relative weights of b_i, calculated using matrix analysis methods as described by (Singh and Chauhdary, 1995) and were used to compute the expected genetic advance from index selection formula (Falconer, 1989).Then after b values, have been rescaled in the respective indices. The relative efficiency of each indices computed by the ratio of genetic advance of the indices based on morphological characters to direct selection.

RESULT AND DISCUSSION

The analysis of variance revealed highly significant difference among the traits considered for making indices (Ermias et al., 2006). In addition, as pointed out by Ermias et al.(2006) the values of phenotypic variance and covariance of the characters and genotypic covariance of each of these traits with the average yield of the first two-year of production have been used for the development of selection indices (Table 1). Different b values for the characters, genetic advance and relative efficiency for the respective selection indices indicated in table 2.Higher genetic advance have been exhibited in selection indices based on four, and three character combination with the value 247.08 and 241.34 respectively. Accordingly these indices also revealed higher relative efficiencies than the direct selection for yield of the first two-year production.

With regard to selection indices based on single character, higher expected GA in yield were obtained for average length of primary (191.92) and followed by canopy diameter (140.97) and girth of the main stem (122.85) while for the character number of main stem node had the lowest value (61.86). As discussed in the in the correlation section, it has been exhibited that the character number of main stem node had significant genotypic and phenotypic correlation with the average yield, and had high direct effect. However, it had low heritability in broad sense, so that it may not be effective to use this character alone as an index to improve yield

of these land race genotypes. Selection index based on the average length of primary alone had relative efficiency more than one (1.01) and adding girth of the main stem the relative efficiency increased to (1.07). As well, adding also the third character, canopy diameter of the tree, and fourth character, number of main stem node, increases slightly relative efficiency from (1.28) to (1.31) respectively.

Table 1. Phenotypic variances (in brackets) and covariance's, and genotypic covariance's for these traits with the mean yield of two years (coulumn 5).

Characters	1	2	3	4	5
Girth of the main stem in cm	(0.13)	0.68	0.63	0.16	21.5
Average length of primariy in cm		(36.63)	0.78	0.06	563.61
Canopy diameter in cm			(144.04)	0.04	821.52
Number of main stem node				(5.87)	72.72
Average yield of the first two year production in gmper tree per annum					(132748.9)

Table 2. Expected genetic advance in coffee yield from use of various selection indices and their relative efficiency.

NO	Characters	Selection indices	Expected GA	Relative efficiency
1	X_1	$165.4\ X_1$	122.85	0.65
2	X_2	$15.4\ X_2$	191.92	1.01
3	X_3	$5.7X_3$	140.97	0.75
4	X_4	$12.4X_4$	61.86	0.33
5	X_1X_2	$1.05X_1+0.15X_2$	202.98	1.07
6	X_1X_3	$1.01X_1+0.04X_3$	174.88	0.92
7	X_1X_4	$1.00X_1+0.05\ X_4$	129.19	0.68
8	X_2X_3	$0.95X_2+0.35\ _{X3}$	263.88	1.25
9	X_2X_4	$0.96X_2+0.76\ _{X4}$	201.33	1.06
10	**X_3X_4**	**$0.35X_3+0.77\ X_4$**	153.89	0.81
11	$X_1X_2X_3$	$1.1X_1+0.23X_2+0.09\ X_3$	241.34	1.28
12	$X_1X_2X_3X_4$	$1.02X_1+0.29X_2\ 0.12X_3+0.22X_4$	247.08	1.31
13	X_5		189.29	1.00

Where X_1 Girth of the main stem in cm, X_2 Average length of the primary in cm,X_3canopy diameter in cm,X_4 number of main stem node andX_5 average yield of the first two year production in gm per tree per annum.

On the other hand, selection indices with out the average length of primary had relatively lower relative efficiencies. However, selection indices based on two character combinations, such as girth of the main stem and canopy diameter, canopy diameter and number of main stem node were closer to the value of direct selection for average yield.

Likewise Walyaro and Van der Vossen, 1979, indicated that using girth of the main stem and radius of the canopy in the indices increases the relative efficiencies of the selection indices. The relative efficiencies of the indices based on two characters combination, for stem girth and canopy diameter, canopy diameter and number of main stem node were close to unity with the value 0.92, and 0.81 respectively. Further more, it has been exhibited exclusively that selection indices based on two character combinations one being the character average length

of primary had higher genetic advance to yield, and their relative efficiencies were more than unity.

Generally the indices based on four, three as well as two character combination in most cases exhibited higher than or as high as relative efficiency as direct selection based on the average of the two-year yield. Similarly, the same result have been reported by Sirinivasan, 1982, that higher relative efficiency of selection indices based on five, four and three characters as well as indices based on single character (internodal length) have been exhibited. Similarly Sera 2000, indicated that using correlated characters (Canopy and Height) the coefficient of determination were larger than the production alone.

Unlike to the findings of the present study and the report of Sirinivasan, the study by Walyaro and Van der Vossen, 1979, presented the efficiency of the indices based on the morphological characters to be less than unity and none of the indices with out the first two-year yield exceeded the genetic advance based on straight selection. These might be due to the variation of the number of production years taken in which the selection bases, two year production were taken for the present study while Walyaro and Van der Vossen used a total of ten year production.

CONCLUSION AND RECOMMENDATION

Selection indices in the present study, it has given an interesting result, that the selection of the genotypes for yield at early bearing stage may be effective by considering morphological characters i.e. with out the inclusion of yield itself. It is also interesting, to note that in the present study, selection indices, which includes the character average length of primary, had higher genetic advance compared to the direct selection for yield of the first two-year production. Nevertheless, the indices based on two-character combination regardless of the character average length of primary had lower relative efficiency. Therefore in the present study, selection indices, with more than one character combinations that include average length of primary may be used to select the accession for higher yield performance.

Although, Selection index based on the average length of primary branch alone because of its higher relative efficiency, might not be effective to select productive accessions with higher yield performance, due to its very low direct and indirect effects via most of the considered traits. Therefore, in general the result of this study might need further genetic study for the applicability in other accessions and land races for a better advancement of genotypes to next breeding procedure and releasing potential varieties for coffee growers through selection indices.

REFERENCES

Bayeta B. (1977). A review:Arabica coffee breeding in Ethiopia.ASIC Nairobi Kenya 17:406-414.

Ermias H., Bayeta B. and Harjit S. (2006). Association of morphological. ASIC 21st Montepleire France September 6.

Falconer D.S., 1989. Introduction to Quantitative Genetics .Longman Scientific and Technical. Jhony Wiley and Sons, Inc. Newyork. 438p

SAS Institute Inc., 1999. SAS user's guide. Statistics, version 7 edition. SAS Institute Inc.,Cary,N.C.480p.

Sera T. (2000). Development of coffee cultivars in reduced time by using biotechnology in the iapar model for high density planting. Coffee biotechnology and quality. Proceeding of the third international seminar on biotechnology in the coffee agro industry, Dondria, Brasil.47-70.

Srinivasan C.S. (1980). Association of some vegetative characters with initial fruit yields in coffee. Jornal of Coffee Research 10 (2): 21-27.

Srinvasan C.S. (1982). Preselection for yield in coffee .Indian Journal of Genetics 42 (1):15-19.

Walyaro D.J. (1983). considerations in breeding for improved yield and quality in arabica Ph.D. thesis Agri. Uni.of Wageningen,Netherland.

Walyaro D.J. and Van der Vossen H.A.M. (1979). Early determination of yield potential in arabica coffee by applying index selection. Euphytica.28: 465-472.

Summary of a Decade of South Ethiopian Coffee Improvement Activity at Awada Coffee Research Center: Fruit of the Landrace Arabica Coffee Variety Development Strategy

K. MESFIN, T. ADDISU AND K. KETEMA

Ethiopian Institute of Agricultural Research, Awada Agricultural Research Center.
P O Box 205, Yirgalem, South Ethiopia. E-mail: mesfin04@yahoo.com

SUMMARY

Previously the Coffee improvement strategy of Ethiopia was aimed to develop widely adaptable and stable cultivars across all coffee growing regions of the country although there is a significant ecological variation that prevails between the major coffee growing regions. Assessing the feedback from users on the performance of released coffee cultivars, the national coffee research program realized the need to initiate coffee improvement programs for each coffee growing region that possesses specific coffee quality and fetch premium price in the world market. In effect, coffee improvement program was initiated for Awada Agricultural Research Center mandated to improve South Ethiopian coffee with the financial aid of the Government of Switzerland. To date about 580 accessions have been collected and maintained in the center in separate sets of collection, and are under evaluation. Forty two and sixteen selections are under variety trials, twelve selections are in variety verification trial and one high yielding cultivar that possess the typical quality of Yirgachefe coffee was released to coffee growers in the region. In this paper, coffee improvement activities, such as collection and evaluation of germplasm, variety development and genetic studies are reviewed.

INTRODUCTION

For more than two decades, Ethiopian coffee breeding program was aimed to search for improved coffee cultivars with wider adaptation by concentrating only in the southwestern part of the country. However, this research direction has failed especially in providing cultivars that are suitable for the coffee growing areas of the Southern and eastern part of Ethiopia due to problem of adaptation of the released coffee berry disease (CBD) resistant cultivars in these areas. In addition, these areas possess unique quality coffee types that are inherent only in the local varieties and land races of the respective locations. Hence, the national coffee research program initiated the Landrace Arabica Coffee Variety Development Strategy so that to establish coffee improvement programs for each coffee growing region that possesses specific coffee quality and fetch premium price in the world market (Bellachew and Labouisse, 2007). In order to improve the yield as well as quality of South Ethiopian coffee, collection of germplasm accessions from the representative areas were undertaken. Consequently, screening of the germplasm accessions for economically important characters commenced. In effect some promising cultivars were identified.

Awada Agricultural Research Sub-center is situated in the Tepid to cool semi arid mid highland agro-ecology. It is located at about 315 km south of Addis Ababa at $6^0$3' N of latitude and 38^0 E of longitude at an altitude of about 1740m a.s.l nearby Yirgalem town. The area has a semi-bimodal rainfall distribution characterized by double wet and dry seasons with an average precipitation of 1342 mm per annum (1988-1998). The *belg* starts in mid-February

and extends up to mid-May (i.e. the wet season is from March to May) and the *kiremt* extends from June to September/October (i.e. the wet season is from September to October).
The sub-center is mandated to run research activities on Southern Ethiopia coffee types in general and Sidama and Gedeo coffees in particular. Therefore, major emphasis has been given to the development and release of high yielding and disease resistant coffee cultivars that maintain the standard and/or known quality of these coffee types. Recently, one improved cultivar (Angefa) was released to growers and twelve promising selections are in verification trial. To date more than 580 coffee accessions have been collected and conserved at the center and most have been characterized using the IPGRI coffee descriptor.

ARABICA COFFEE IMPROVEMENT ACTIVITIES AT AWADA RESEARCH CENTER

Collection, characterization and evaluation of South Ethiopian coffee germplasm accessions

This trial was laid down at Awada on station comprising a total number of 538 accessions. The 1994, 1995 and previous Sidamo coffee collections (Batch I, 206 accessions and 5 checks), that are currently being evaluated for various desirable traits (cherry yield, resistance to CBD, CWD and CLR, and quality parameters) were planted in an augmented design of 4 blocks and 10 trees per plot in 1997. The 1996 collections (Batch II) field established in 1998 that comprises 56 accessions was laid down in the same pattern as indicated above. The 1997 collections (Batch III), which comprise 55 accessions, were planted in 1998 in RCB design of 3 replications and 6 trees per plot.

Recently new collections were added from 4 districts of Sidama Administrative Zone. 120 coffee accessions collected from Dale and Aleta Wondo districts in 2005 were transplanted in the field at Awada in July 2006 in augmented design. Similarly, 100 coffee accessions collected from Bensa and Dara districts in 2006 were transplanted in the field at Awada in July 2007. Currently the seedlings are at their required stage of growth. Batch I & II were managed in multiple stems as they were stumped due to the severe drought that occurred in 2000.

The first 318 accessions (1994, 1995, 1996 and 1997, and previous Sidamo coffee collections) were evaluated for cherry yield, resistance to major coffee diseases and quality parameters. The first batch 1994, 1995 and previous collection had 6 years yield data, the 1996 collection had 5 years yield data and the 1997 collection had 7 years yield data. In all the 3 batches of collections, the top ten high yielder of the respective collections were well above the standard checks, ranging from 17.29 to 26.30 q/ha of clean coffee. They were also moderately resistant to coffee berry disease at Awada condition. Most were free from the disease under visual assessment score; however, few accessions scored 3 to 8 % infection level under field condition.

Currently, 6 accessions were under variety verification trial and another 16 are under variety trial being evaluated in contrasting environments. Another 15 to 20 promising accessions will be promoted to variety trial in 2009 (JARC, 2007).

Variety and verification trials of South Ethiopian coffee selections

Three independent experiments were undergoing under this title. The first variety trial was established in 2 locations; Awada (mid altitude) and Wonago (high altitude). At Awada it was established in 1997 whereas at Wonago in 1999. The trial consists of 42 Arabica coffee

selections collected from South Ethiopia in 1970, 1977, 1981 and 1985 and 2 standard cultivars used as checks.
At Awada, combined analysis over four years showed that none of the top ten accessions did better than the best check included in the study. However, the entire top ten accessions performed better than the other check. Similarly, at Wonago, combined analysis over five years indicated that none of the accessions out yielded the best check. Nonetheless, all the top ten accessions did better than the other standard check included in the study. Except for 2 selections that showed wide adaptability both in terms of yield and resistance to CBD, the rest of the accessions performed differently confirming the fact that Ethiopian coffee landraces generally show specific location adaptation (Mesfin and Bayetta, 1997).

From the result we can recommend the above 2 selections for variety verification trial. However, both of the selections were promoted for verification trial at early evaluation confirming the precision of the previous selection criterion.

The second trial i.e. verification of Sidamo coffee selections comprising 14 accessions including two standard cultivars, was established only at two locations in a RCB design of three replications, spacing of 1.5 m x 2 m and plot size of 75 and 66 trees at Konga and Korkie demonstration sites, respectively in 2004. Currently, the coffee trees in both locations are in good conditions and the first cherry yield was harvested in this season.

Out of the 12 Sidamo coffee selections evaluated in both locations, all the selections except one excelled the 2 standard checks in this (2007) cropping season. However, relatively higher yield was recorded for all the selections in Konga as compared to Korkie. And coffee berry disease infestation was higher for Konga than Korkie.

The third trial was proposed to be undertaken in two sets; consequently, seedlings of 16 selections and 2 standard checks were transplanted at three sites (Awada, Wonago and Kumato) in August 2006. Currently, the seedlings are well established in the fields of the 3 locations. Set II will be established in 2009.

ANGEFA: - THE RELEASED SOUTH ETHIOPIAN COFFEE CULTIVAR

Few years back Awada Agricultural Research Center (AARC) has released an improved cultivar named “Angefa” which is high yielder and well adapted to Sidama and Gedeo coffee growing areas as it was evaluated in the same area. This cultivar was originated from this region and represents the local coffee types plus positive advantages i.e. resistant to coffee berry disease, high yield (24 qt/ha on research station of Awada) and also superior qualities (JARC, 2006).

In relation to the previously released coffee berry disease resistant cultivars originated southwest of Ethiopia, Angefa is highly preferred by coffee farmers of Sidama and Gedeo Zones for its high vigor, yield advantage and quality characters and it fits in well with the government’s strategy of strengthening the development of local landrace coffee varieties. Currently Awada Agricultural Research Center is the only source of seed for this cultivar and the demand for this cultivar in the country is very high.

Angefa was initially collected from Quoti Kebele of Wonago district in Gedeo Zone of South Ethiopian region. It can be described as follows; it has an open type of growth habit, bronze leaf tip color, can grow at an altitude range of 1700 to 2000 m. The rainfall requirement of this cultivar is well above 1200 mm per annum. It grows best in Nitosol type of soil with the application of 125kg DAP and 81kg of Urea fertilizers per hectare. It can give 11 to 17

quintals of clean coffee per hectare on farmers' field. It requires 50% shade using common shade trees like Milletia, Cordia, Albizia, Sesbania and Acasia species. A spacing of 2 m x 2 m is the best recommended practice as the cultivar has open type of growth habit. In reaction to major coffee diseases, it is resistant to CBD and moderately resistant to CLR under field conditions both at Yirgalem and Wonago areas. It is also characterized by Yirga Chefe type of cup test with best raw and roast quality.

GENETIC STUDIES

Genetic diversity analysis was conducted on 41 South Ethiopian coffee selections collected from 6 districts of Gedeo, Sidama, and Wolayta Administrative Zones along with 2 released coffee berry disease (CBD) resistant cultivars originated from Southwest Ethiopia (Mesfin et al., 2007). Data on 7 morphological agronomic characters vis-à-vis stem girth, plant height, number of primary branches, number of stem nodes, length of longest primary branches, canopy diameter and internodes length of the main stem; percent disease infestation levels on CBD and coffee leaf rust (CLF) and average of 3 years clean coffee yield was obtained on the 43 genotypes. The ANOVA showed a highly significant difference among the genotypes for the 7 morphological agronomic characters and yield.

The cluster analysis grouped the 41 south Ethiopian coffee selections and the 2 southwest Ethiopian origin CBD resistant cultivars in to 9 clusters; suggesting the prevalence of wide phenotypic variations in the coffee populations.

The intra and inter-cluster distance (D^2) analysis showed a highly significant ($P < 0.01$) difference among clusters. The smallest inter-cluster distance (18.6) was observed between clusters VI and VII while the highest (134.7) was between clusters V and VIII. In most of the cases, the genotypes among the clusters were significantly ($P < 0.001$) divergent from each other.

The first four principal components with Eigen values greater than unity explained 82.63 percent of the total variation among the 43 genotypes for the 10 quantitative characters measured. Principal component one accounted nearly one third (32.52%) of the total variation. In light of the results obtained from the PCA, it may be possible to deduce that more than half (53%) of the variation obtained was primarily due to number of nodes, primary branches, and plant height (data not shown).

REFERENCES

Bayetta B. and Labouisse J-P. 2007. Arabica Coffee (*Coffea arabica* L.) Local Landrace Development Strategy in its Center of Origin and Diversity. 21st Int. Sci. Colloq. on Coffee. ASIC, Montpellier, France.

Jimma Agricultural Research Center (JARC). 2006. Progress report of Awada Research Sub-Center. EIAR, Addis Ababa.

Jimma Agricultural Research Center (JARC). 2007. Progress report of Awada Research Sub-Center. EIAR, Addis Ababa.

Mesfin A. and Bayetta B. 1997.Genotype-environment interaction and its implication on selection for improved quality in arabica coffee (*Coffea arabica* L.).ASIC, 17e colloque, Nairobi, Kenya.

Mesfin K., Bayetta B. and Seyoum S.. 2007. Diversity in the South Ethiopian Coffee. 21st Int. Sci. Colloq. on Coffee. ASIC, Montpellier, France.

Agronomic Characterization of *Coffea canephora* Germplasm

M.A.G. FERRÃO[1], R.G. FERRÃO[2], A.F.A. da FONSECA[3], E.M. RIVA-SOUZA[2], P.S. VOLPH[2], A.C. VERDIN FILHO[2]

[1]Embrapa Café/Incaper, Vitória, ES, Brasil
[2]Incaper, Vitória, ES, Brasil
[3]Embrapa Café, Brasília, DF, Brasil

SUMMARY

The Capixaba Institute of Research, Technical Assistance and Rural Extension (Incaper) initiated the genetic improvement program in 1985 with the specie *Coffea canephora*, selecting, initially, mother plants in various municipalities and evaluating them in advanced trials. At present, Incaper maintains an Active Germplasm Bank (BAG), containing 375 accessions. The initial 181 accessions planted were evaluated for 43 different morpho-agronomic characteristics and reaction to the diseases, including 19 quantitative and 24 multicategorical. Based on the means of these 43 characteristics, a matrix of genetic dissimilarity was calculated, through mean Euclidian distance, and a clustering of the genotypes utilizing the optimization method of Tocher was conducted. A great genetic variability between the germplasm in the different characteristics and formation of 47 groups was verified. The 15 genetic accessions most dissimilar were those of numbers 138, 01, 81, 139, 95, 51, 07, 78, 122, 166, 34, 160, 28, 116 and 72; while those most similar were those of numbers 82, 170, 126, 38, 98, 162, 20, 108, 64, 152, 55, 143, 11, 99 and 35. Maintenance of the genetic reserve in this collection has objectives to help prevent possible genetic losses and planning of new germplasm collections, as well as contribute positively to development of new genotypes with specified characteristics.

INTRODUCTION

The species *Coffea canephora* has great economic and social importance in Espírito Santo. The Capixaba Institute of Research, Technical Assistance and Rural Extension (Incaper) initiated the genetic improvement program in 1985, selecting, initially, mother plants in various municipalities and evaluating them in advanced trials. Based on the experimental results five clonal varieties and one multiplied by seed were developed and recommended. In this research program, there has been a great concern with the maintenance and amplification of the genetic base, in the sense that use of clonal varieties in the state does not cause a notable reduction in the diversity of material cultivated and consequent vulnerability.

MATERIALS AND METHODS

At present, Incaper maintains an Active Germplasm Bank (BAG) of the species *Coffea canephora*, at the Marilândia Experimental Farm in the municipality of Marilândia, ES, in a uniform area of soil group Latossolo Vermelho Amarelo Distrófico, hilly topography, altitude 140 m, latitude 19º 24', longitude 40º 31', mean annual temperature of 26 ºC, and mean annual precipitation of 1140 mm.

Initially, 181 genetic materials of interest, chosen after experimental evaluation, were cloned and planted in rows of 10 plants with spacing of 3.0 x 1.5 m in May of 1998. In the years

2004 to 2006, 194 accessions of additional genetic stuff of the species were gradually included, with the BAG currently containing 375 accessions.

The initial 181 accessions planted were evaluated for 43 different morpho-agronomic characteristics and reaction to the diseases, including 19 quantitative and 24 multicategorical. Based on the means of these 43 characteristics, a matrix of genetic dissimilarity was calculated, through mean Euclidian distance, and a clustering of the genotypes utilizing the optimization method of Tocher was conducted.

RESULTS AND DISCUSSION

A great genetic variability within the germplasm was verified, as indicated by results obtained for some characteristics: leaf length (10.5-19.0 cm); leaf width (4.0-8.0 cm); relationship between raw bean and processed bean weight (3.01-5.08); fruit size (very small – big); fruit shape (round – elliptic – oblong); plant form (cylindrical – cone – inverted cone); plant height (short – very high) pulp adhesion rank of the seed (weak – strong); fruit ripening (early – late); reaction to the rust (1-9); dought tolerance (1-9); and so on.

Based on the means of these 43 characteristics, a matrix of genetic dissimilarity was calculated, through mean Euclidian distance. The genotypes 1 and 97 presented the greatest distance (3.016) and the numbers 151 e 161 the smaller distance (0.6017).

By the Tocher optimization method, the genotypes were classified into 47 groups (Table 1). The 15 genetic accessions most dissimilar were those of numbers 138, 01, 81, 139, 95, 51, 07, 78, 122, 166, 34, 160, 28, 116 and 72; while those most similar were those of numbers 82, 170, 126, 38, 98, 162, 20, 108, 64, 152, 55, 143, 11, 99 and 35.

Maintenance of the genetic reserve in this collection has objectives to help prevent possible genetic losses and planning of new germplasm collections, as well as contribute positively to development of new genotypes with specified characteristics, obtained by intra and interspecific breeding utilizing important alleles that can be transferred via controlled hybridization. It is worth roting that the genetic variability increase of this BAG is currently the main goal of the improvement program of from our Institute, which has devoted a great effort for the introduction of new genetic materials.

Table 1. Groups established by the Tocher oprtimization method, based on the genetic dissimilarity matrix in 181, *Coffea canephora* acccessions.

Groups	Genotypes					
1	82	170	126	38	98	162
2	20	108	64	152		
3	55	143	11	99		
4	35	79	167	123		
5	65	109	21	153		
6	104	148	60	16		
7	22	66	154	110		
8	25	69	157	113		
9	26	70	158	114		
10	29	117	161	73		
11	128	172	40	84		
12	33	77	121	165		
13	32	76	164	120		
14	59	147	103	15		
15	43	131	87	175		
16	46	90	178	134	2	
17	86	130	174	42		
18	136	180	48	92	4	
19	85	129	173	41		
20	68	112	24	156		
21	13	101	145	57		
22	30	118	74			
23	47	179	135	91	3	
24	39	83	171	127		
25	71	115	27	159		
26	56	100	144	12		
27	17	149	61	105		
28	37	169	125			
29	89	177	45	133		
30	36	168	124	80		
31	119	163	31	75		
32	14	102	58	146		
33	88	132	176	44		
34	5	181	49	137	93	
35	8	140	52	96		
36	10	54	142			
37	19	63	107	151		
38	6	94	50			
39	18	62	106	150		
40	53	141	97	9		
41	23	155	111	67		
42	72	116	28	160		
43	34	166	122	78		
44	7	51	95	139		
45	81					
46	1					
47	138					

ACKNOWLEDGEMENTS

The authors thank the Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café - CBP & D-Café (Brazilian Consortium of Coffee Research and Development) for financial support and scholarships and Mark P. Culik, Incaper, for review and translation into English of this work..

Agronomic Performance of Short Plant Arabica Coffee Progenies*

J.C. MISTRO[1], L.C. FAZUOLI[1,], M.R. PETEK[1], A.C.B. OLIVEIRA[2], M.T. BRAGHINI[1], P.B. GALLO[3]**

[1]Instituto Agronômico (IAC/APTA), Campinas, SP, Br
[2]Empresa Brasileira de Pesquisa de Minas Gerais (EPAMIG), Viçosa, MG, Br
[3]Pólo Regional do Nordeste Paulista (APTA Regional), Mococa, SP, Br
*Study partly supported by the Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café
**Awarded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) with a scholarship for scientific productivity

SUMMARY

The development of highly productive arabica coffee cultivars (*Coffea arabica* L.) with agronomically desirable characteristics, such as short plant and rust resistance, is very important to modern coffee culture as such cultivars allow for reduced spacing, mechanical harvesting and, above all, increase the longevity of the plants and the profitability of coffee farming. Throughout the years, the Arabica Coffee Genetic Improvement Program of the Instituto Agronômico de Campinas (IAC/APTA) has been introducing plant materials carrying genes capable of enhancing the above-mentioned characteristics and which were, and still are, used in hybridizations with Brazilian cultivars, with the aim of improving the latter. Within this context, special focus should be placed on hybridizations between introduced gene-carrying materials, such as the Timor X BA 10 Hybrid, and Brazilian cultivars of the Catuaí and Icatu germplasms. The objective of the present study is to evaluate the agronomic performance of Arabica coffee progenies derived from such hybridizations, in order to select and further develop the most promising combinations, which in the future may result in a new cultivar. In 2003, an experiment was carried out by the APTA Regional, in Mococa (BR), with 26 progenies and four control cultivars in a random bloc design, with four replicates and 5 plants per plot, planted at a spacing of 3,50 x 1,25 m. The treatments investigated were: 1 to 8 (derived from Catimor), 9 to 14 (short plant Icatu), 15 to 21 (Catuaí x BA 10), 22 and 23 (Catuaí x Catimor), 24 (Catuaí x Icatu), 25 (Obatã Amarelo), 26 (Obatã IAC 1669-20), 27 (Ouro Verde IAC 5010-5), 28 (Bourbon Amarelo), 29 (Catuaí IAC 81) and 30 (Ouro Amarelo IAC 4397). The following characteristics were evaluated: cherry coffee yield, vegetative vigor, plant height, stem diameter, fruits ripening and fruits size, and incidence of coffee leaf rust. The results of an analysis of variance showed the existence of genetic variability for cherry coffee yield, plant height, stem diameter and incidence of coffee leaf rust. Progenies 1, 2, 3, 4, 13, 14, 17, 19, 20, 21, 22, 23 and 24 detached in relation to yield; progenies 1, 16, 18 and 20 presented short stature, while progenies 2, 3, 4, 8, 11, 14, 16, 18, 22, 24 and 25 were either immune or resistant to leaf rust.

INTRODUCTION

A modern coffee cropping requires the development of high yield Arabica coffee cultivars (*Coffea arabica* L.) with desirable agronomic characters, such as low plant height and high rust resistance, because these characters favor the coffee dense cropping system and mechanical harvest, and on the overall, increase plant longevity and crop profits.

The leaf-rust disease (*Hemileia vastatrix* Berk. et Br.), the main coffee fungus disease, may severely damage plants under dense cropping system and during high yielding years, causing production losses between 35 and 50%. High air relative humidity and temperatures of 20-25 °C are favorable environmental conditionsfor the pathogen development (Zambolin et al., 2002).

The Arabica Coffee Breeding Program at Instituto Agronômico (IAC)/Agency of Agricultural Research and Technology (APTA), State of São Paulo, Brazil, is aiming at breeding and selecting, along the years, an unique coffee cultivar presenting short plants and high rust resistance. Important hybrid crossings have been obtained between Catuaí or Icatu cultivars (from the IAC coffee germplasm collection), and foreign material such as the Timor Hybrid and BA 10.

The use of rust resistant cultivars has been the most efficient technology against this constraint. Breeding plants for a disease resistance requires the development of multigenic resistant cultivars, conditioned by several genes that make plants more effectively resistant to different pathogen races (Metha, 1978). According to Várzea et al. (2002), the continuous appearance of new physiological races has broken down the rust resistance of some coffee cultivars, as the 'Cauvery' (Caturra x Timor Hybrid) that, at India, has shown rust susceptibility. The 'Cauvery' is a commonly used germplasm in the current coffee cultivars, and its rust resistance breakdown turns unpredictable the durability of the Timor hybrid rust resistance and of their derivates.

The objective of this research work was to evaluate the agronomic performance of Arabica coffee progenies, originated from those hybrid crossings, in order to select and improve the most promising combinations, which in the future may result into a new cultivar.

MATERIAL AND METHODS

An experiment was carried out during 2003-2006 in the Experiment Station (IAC/APTA-Agency of Agricultural Research and Technology), at Mococa, State of São Paulo, Brazil. Twenty six coffee progenies and four control-cultivars were used, in a randomized complete block design, with four replications and five plants per plot (experimental unit), spaced 3.50 x 1.25 m.

The treatments were: 1 to 8 (originated from Catimor), 9 to 14 (Icatu, short plants), 15 to 21 (Catuaí x BA 10), 22 and 23 (Catuaí x Catimor), 24 (Catuaí x Icatu), 25 (Obatã Amarelo), 26 (Obatã IAC 1669-20), 27 (Ouro Verde IAC 5010-5), 28 (Bourbon Amarelo), 29 (Catuaí IAC 81) and 30 (Ouro Amarelo IAC 4397).

The following plant characteristics were evaluated: coffee yield, vigor (rated 1 to 10, where 1 = very low vigor and 10 = very high vigor); shoot tree height and diameter, fruit maturation (rated 1 to 5, where 1 = early; 2 = medium-early; 3 = medium; 4 = medium-late; and 5 = late); fruit size (rated 1 to 5, where 1 = small; 2 = medium-small; 3 = medium; 4 = medium-large; 5 = large); and leaf rust resistance. Leaf rust disease was evaluated by the visual symptoms using the scale described by Fazuoli (1991): 0 = immune, no visual infection (Immune); 1 = yellowing lesions, small spots, no sporulation (Resistant); 2 = yellowing lesions, sporulation in the lesion borders, small pale-yellow spots, sporulation beginning, with small lesions and little sporulation (Moderately Resistant); 3 = yellowing lesions, spots increase in size, lesions associated with spots and characteristic pustules of the 2 and 4 reaction types, with low, medium or high level of sporulation (Moderately Susceptible); 4 = lesions with intense sporulation, presence of many large pustules (Susceptible).

The obtained data was submitted to analysis of variance (F test) and means were compared by the Scott Knott test ($P < 0.05$). The statistical analysis was run by means of the GENES software for genetics and statistics (Cruz, 2001).

Table 1. Agronomic characteristics of coffee progenies and control-cultivars evaluated in an experiment carried out in the experiment station of the Agency of Agricultural Research and Technology (APTA), at Mococa, State of São Paulo, Brazil, during 2005 and 2006.

T	Yield	Plant vigor	Plant height	Shoot diameter	Fruit maturation*	Fruit size*	Leaf-rust score*
	kg/plant		——— cm ———				
1	3,084 a	7.3 b	93.5 d	138.1 b	3 b	2 b	1 c
2	3,667 a	6.8 b	104.5 b	148.6 a	4 a	3 a	0 d**
3	3,230 a	7.1 b	104.9 b	143.6 b	3 b	3 a	0 d
4	3,052 a	7.0 b	105.6 b	138.6 b	3 b	3 a	0 d
5	2,190 b	6.4 c	106.5 b	145.3 a	4 a	2 b	1 c
6	2,627 b	6.8 b	110.6 a	159.0 a	4 a	3 a	1 c
7	2,627 b	6.8 b	111.9 a	152.5 a	3 b	2 b	1 c
8	2,489 b	6.4 c	112.2 a	153.5 a	3 b	2 b	0 d
9	1,795 b	5.7 c	110.3 a	129.6 b	4 a	2 b	3 b
10	1,772 b	6.2 c	110.1 a	137.2 b	3 b	2 b	2 b
11	2,272 b	6.6 c	115.7 a	135.8 b	3 b	2 b	0 d
12	2,239 b	6.6 c	110.2 a	139.6 b	3 b	2 b	1 c
13	4,093 a	6.9 b	115.4 a	139.0 b	4 a	2 b	2 b
14	3,597 a	6.6 c	115.2 a	153.7 a	4 a	2 b	1 c
15	2,344 b	6.8 b	101.5 c	143.2 b	3 b	3 a	3 b
16	2,462 b	7.0 b	91.6 d	136.6 b	4 a	2 b	0 d
17	2,965 a	6.9 b	104.2 b	142.6 b	4 a	2 b	1 c
18	1,932 b	6.2 c	93.0 d	138.6 b	4 a	2 b	0 d**
19	3,157 a	8.2 a	97.5 c	151.1 a	4 a	2 b	1 c
20	3,261 a	7.2 b	92.3 d	130.8 b	4 a	2 b	1 c
21	3,665 a	7.6 a	99.7 c	141.3 b	4 a	2 b	1 c
22	2,906 a	6.9 b	108.4 b	145.7 a	4 a	3 a	0 d
23	3,261 a	7.1 b	102.2 c	145.5 a	4 a	2 b	2 b
24	3,595 a	6.7 b	105.9 b	151.0 a	4 a	2 b	0 d
25	2,579 b	7.1 b	98.4 c	134.0 b	4 a	2 b	0 d**
26	3,412 a	6.5 c	103.3 b	136.0 b	4 a	3 a	1 c
27	2,350 b	6.2 c	99.1 c	131.5 b	3 b	2 b	4 a
28	2,427 b	6.0 c	110.4 a	152.3 a	4 a	2 b	4 a
29	2,351 b	6.3 c	109.2 a	145.8 a	4 a	2 b	4 a
30	3,192 a	6.5 c	110.4 a	142.5 b	4 a	2 b	4 a

**Scores given to - Plant vigor score 1 to 10 (1 = low vigor; 10 = high vigor). Fruit maturation: 1 = early; 2 = medium-early; 3 = medium; 4 = medium-late; and 5 = late. Fruit size: 1 = small; 2 = medium-small; 3 = medium; 4 = medium-large; 5 = large. Leaf-rust resistance: 0 = immune; 1 = resistant; 2 = moderately resistant; 3 = moderately susceptible; and 4 = susceptible.*

***All plants of progenies 2, 18 and 25 were immune to the leaf-rust disease.*

RESULTS AND DISCUSSION

The agronomic characteristics of twenty six progenies and four control-cultivars used in the experiment are presented in Table 1 (average data of 2005-2006).

Significant differences among treatments were obtained for all characters evaluated by the F test ($P < 0.05$), indicating the existence of genetic variability within the studied population for such characters, which is primordial condition to carry on the selection in a breeding program. Progenie 13 (short tree 'Icatu') showed the highest yield 4,093 kg/plant of coffee berry, but it was not statistically different from the yields of progenies 1, 2, 3, 4, 14, 17, 19, 20, 21, 22, 23, 24, and the control-cultivars 26 ('Obatã IAC 1669-20') and 30 ('Ouro Amarelo IAC 4397').

Progenies 19 and 21 were the most vigorous coffee progenies from the Catuaí x BA 10 crossing. These progenies were also among the most productive ones, what is a relevant fact, once there is a known negative relationship between plant vigor and yield.

Shorter trees were observed among the progenies 1, 16, 18 and 20, with 91.6; 93.0 and 92.3 cm of height, respectively, meanwhile the progenies 5, 6, 7, 8, 14, 19, 22, 23, 24 and the control-cultivars 28 and 29 presented larger shoot diameters. Progenie 20 presented short tree and small shoot diameter, which are both desirable characteristics for the coffee dense cropping system.

As concerned to the fruit maturation period and fruit size, it was observed medium and medium-late periods, and medium to small-medium fruits. The (larger) plant spacing used in this experiment might have influenced the fruit maturation period.

As concerned to the leaf rust agent attack, progenies 2, 3, 4, 8, 11, 14, 16, 18, 22, 23, 24 and 25 were given score = 0, that is, they were immune to the disease. All plants of progenies 2, 18 and 25 showed no symptoms of leaf rust infection. Progenies 1, 5, 6, 7, 12, 14, 17, 19, 20, 21 and 26 were classified as resistant (score = 1). The most productive progenie (13) presented small lesions with little sporulation and was classified as moderately resistant. The control-cultivars 27 ('Ouro Verde IAC 5010-5'), 28 ('Bourbon Amarelo'), 29 ('Catuaí IAC 81') and 30 ('Ouro Amarelo IAC 4397') were susceptible to the disease.

Progenies 1, 2, 3, 4, 21 and 24 are highlighted for their high yields, low plant heights and high resistance (immune or resistant) to the leaf rust disease.

CONCLUSIONS

1 The studied coffee population presented genetic variability among progenies, indicating genetic potential to be effectively explored.
2 The most productive coffee progenie 13 (short plant 'Icatu') was moderately resistant to the leaf-rust disease.
3 Progenies 1, 2, 3, 4, 21 and 24 showed high yields, shorter plants and immunity or resistance to the leaf-rust disease that are highly desirable characteristics for the coffee dense cropping system.

REFERENCES

Cruz C.D. Programa GENES: versão Windows. Aplicativo computacional em genética e estatística. Viçosa: Editora UFV, 2001. 648p.

Fazuoli L.C. Metodologia, critérios e resultados da seleção em progênies do café Icatu com resistência a *Hemileia vastatrix*. Campinas (SP), 1991. 322p. Tese (Doutorado em Ciências) − UNICAMP.

Mehta Y.R. Doenças de trigo e seu controle. Ceres, São Paulo, n.20, 190p., 1978.

Várzea V.M.P., Rodrigues Junior C.J., Silva M.C., Gouveia M., Marques D.V., Guerra-Guimarães L., Ribeiro A. Resistência do cafeeiro a Hemileia vastatrix. In: Zambolim L. (Ed.) O estado da arte de tecnologias na produção de café. Viçosa: Editora UFV, 2002. p.297-320.

Zambolim L., Vale F.X.R., Costa H., Pereira A.A. And Chaves Gm Epidemiologia e controle integrado da LRRugem-o-cafeeiro. In: Zambolim L. (Ed.). O estado da arte de tecnologias na produção de café. Viçosa, Departamento de Fitopatologia, UFV, 2002. p.369-450.

Selection of Robusta Coffee Stock Plants at Mococa, Brazil*

L.C. FAZUOLI[1], J.C. MISTRO[1], M.T. BRAGHINI[1], P.B. GALLO[2]**

[1]Instituto Agronômico (IAC/APTA), Campinas, SP, Br
[2]Pólo Regional do Nordeste Paulista (APTA Regional), Mococa, SP, Br
*Study partly supported by the Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café
**Awarded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) with a scholarship for scientific productivity

SUMMARY

Since 1970, IAC develops and maintains a robusta coffee (*Coffea canephora* Pierre ex A. Frohener) improvement program. As part of this program, numerous stock plants and progenies have been selected over the years. The objective of this paper is to report on the selection of highly productive robusta stock plants which additionally exhibit a number of other agronomically favorable characteristics and check the feasibility of developing them into future clones. The experiment was conducted with 67 progenies from previous selections in an experimental area established in Mococa, SP, Brazil, in 1994, totaling 1012 plants. Cherry coffee yield were harvested twice over a total period of three years, time during which the following agronomic characteristics were visually evaluated: vegetative vigor and yield, ripening of the fruits, outturn coffee beans, rust resistance, bean size by passing through a medium size sieve, percentage of flat berries, peaberries and elephant beans, the weight of 100 flat beans, total soluble solids and caffeine contents of the seeds. The results showed enormous variability among the coffee plants regarding the characteristics investigated and allowed for the selection of 60 highly productive plants, with an average annual cherry bean production ranging from 23,7 to 35,3 kg per plant over the two-year period studied and high average sieve size varying between 15,7 and 18,9. All selected stock plants were immune to rust. The soluble solids content of the plants evaluated varied from 29,3 to 34,7%, whereas the caffeine content ranged from 1,6 to 2,8%. The selected plants are currently being used in a series of clone and progeny experiments.

INTRODUCTION

Brazil is the second major producer country of robusta coffee (*Coffea canephora* Pierre ex A. Frohener). Areas considered marginal lands to Arabica coffee cropping are feasible to cultivate robusta coffee, characterized by low altitudes and high air temperatures (Silva e Costa, 1995). Robusta coffee is an allogamous species, diploid (2n = 2x = 22 chromosomes) and auto-incompatible, what means its reproduction occurs only by crossed fecundation. This species is divided in two genetic groups: Congolese and Guinean. The Robusta, Guarini and Apoatã cultivars of Congolese group present fruits and seeds of greater size, larger leaves, more vigorous and productive plants. The Conilon and Robusta Tropical cultivars of Guinean group present smaller seed size and narrower and smaller leaves. Cultivars of both groups are generically nominated as “robusta coffee”, since ‘Robusta’ has a worldwide economical expression.

The robusta coffee characteristics such as plant rusticity, tolerance to pests, diseases and nematodes, plant adaptation to lower altitudes and to warm and humid regions, turn viable this species cultivation in marginal lands to Arabica coffee. Besides, the robusta coffee beans

show adequate chemical properties to the soluble coffee industry and they have been frequently used in coffee blends with Arabica coffee (Fonseca, 1999).

Since 1970, a Robusta Coffee Breeding Programme has been developed in the Instituto Agronômico (IAC) / Agência Paulista de Tecnologia Agropecuária (State Government Agency of Research and Technology in Agriculture - APTA), at Campinas, State of São Paulo, Brazil. The fundamental step of any breeding program begins with the knowledge of the available germplasm, which is essential for the development of new cultivars. A great number of robusta coffee matrix plants and progenies have been evaluated and selected from the germplasm bank of IAC/APTA.

The objective of this work was to select robusta coffee matrix plants for high productivity added to other desirable agronomic characteristics, in order to develop, in the near future, clonal cultivars for the State of São Paulo.

MATERIALS AND METHODS

Since 1994, an experiment has been carried out with robusta coffee in the APTA experiment fields, at Mococa, State of São Paulo, Brazil. This experiment consisted of 67 robusta coffee progenies, a total of 1,012 plants, originated from previous selections obtained in other locations of the State of São Paulo. The coffee crop was established using 4.00 x 3.00 m plant spacing without stem conduction.

Two cherry coffee harvests were obtained and the following characteristics were visually evaluated during three years: plant vigor; productivity; fruit ripening; green coffee yielding; rust resistance; seed size through medium sieve; percent of flat beans, peaberries and elephant beans, the weight of 100 flat beans, total soluble solids and caffeine concentrations of the seeds.

RESULTS AND DISCUSSION

The data presented in Table 1 is referred to the following variables: average yearly yield of two harvests (kg of coffee cherry per plant), fruit ripening, percent of flat beans, peaberries and elephant beans, the weight of 100 flat beans, seed size through medium sieve, and seed soluble solid and caffeine concentrations of 30 robusta type plants of robusta coffee, selected from 1,012 analyzed plants.

The average yearly yield of 30 plants ranged from 15.1 to 35.3 kg coffee cherry per plant, evidencing the yielding potential of the selected coffee plants. Fruit ripening varied from medium-early until medium-late.

The percent of flat beans varied from 70.1 to 92.3%, evidencing the feasibility of selecting plants with high percentage of flat seeds which is important for better yielding of matrix plants.

The weight of 100 flat beans ranged from 13.4 to 23.2 g, evidencing an excellent flat seed size, which was corroborated by the medium sieve data (16 to 19). In the average of 30 selected matrix plants, the seed soluble solid concentrations varied from 29.33 to 34.67 % and caffeine varied from 1.9 a 2.8 %. All selected plants were immune to the leaf rust disease.

The results evidenced the feasibility of selecting robusta coffee plants presenting high productivity, high leaf rust resistance and low seed caffeine concentrations (close to 2%).

Table 1. Average yearly coffee cherry yield (kg of coffee cherry per plant) and agronomic characteristics of thirty robusta coffee elite matrix plants selected in experiment carried out at the "Pólo Regional do Nordeste Paulista" (APTA Regional), at Mococa, State of São Paulo, Brazil.

Plant	Yield	Rip *	Beans						
			Flat	Peaberry	Elephant	Weight	Size	SS **	Caffeine
nº	kg/pl		%			g		%	
7	21,1	ML	81,5	18,5	0,0	18,7	18,79	31,77	2,4
10	15,1	M	73,7	26,3	0,0	17,1	17,14	34,67	2,2
18	17,4	M	80,0	20,0	0,0	13,4	17,42	31,77	2,0
23	17,5	M	73,8	26,2	0,0	13,6	16,00	32,89	2,5
73	23,5	ME	75,8	24,0	0,0	19,9	18,67	32,66	-
75	19,7	L	92,2	7,8	0,0	22,3	18,43	31,70	2,0
99	24,4	M	77,9	22,1	0,0	16,5	18,19	30,29	2,3
105	27,0	ML	83,9	16,0	0,0	17,6	17,63	27,00	2,2
107	27,7	M	73,7	26,3	0,0	20,0	18,95	29,60	2,3
108	20,8	M	86,0	14,0	0,0	18,0	17,37	30,36	2,5
112	31,0	M	81,8	18,2	0,0	15,1	16,63	31,18	2,2
115	26,3	M	78,9	21,1	0,0	19,4	18,49	29,53	2,1
116	23,1	M	72,3	27,7	0,0	17,6	17,94	32,21	2,2
141	20,6	ME	70,1	29,9	0,0	17,4	17,52	32,52	2,6
160	17,5	M	79,2	20,8	0,0	17,6	17,58	31,39	2,5
178	21,7	M	85,2	14,8	0,0	16,0	17,71	31,70	2,4
189	20,4	E	89,7	10,3	0,0	23,2	18,85	31,70	2,2
280	30,1	M	92,3	7,7	0,0	16,3	18,90	30,43	2,5
285	30,9	ML	78,6	21,4	0,0	15,0	17,70	31,25	2,2
614	23,4	M	75,3	24,7	0,0	17,5	18,60	31,56	2,8
628	24,7	M	80,1	19,9	0,0	18,9	19,00	30,29	2,5
633	22,1	M	87,4	12,6	0,0	22,0	18,60	31,63	2,2
636	23,0	M	78,3	21,3	0,4	21,0	18,77	29,33	2,2
644	35,3	M	88,9	11,1	0,0	15,0	17,01	30,29	1,9
708	22,1	M	84,8	15,2	0,0	20,1	18,69	31,32	2,2
738	25,1	ME	81,3	18,3	0,4	18,1	18,03	31,18	2,3
743	22,7	ME	90,4	9,6	0,0	16,4	17,18	32,96	2,2
752	25,9	M	80,2	19,8	0,0	13,2	16,54	31,11	2,4
761	28,1	M	76,2	23,8	0,0	16,7	17,63	31,56	2,3
846	19,4	M	78,7	21,0	0,3	16,2	17,93	33,85	2,5

** Fruit ripening: E = early, ME = medium early, M = medium, ML = medium late e L = late.*
*** SS = total soluble solids.*

CONCLUSIONS

About 100 robusta coffee matrix plants were selected for the characteristics of high yield and vigor, leaf rust resistance, differentiated fruit ripening, high values of medium sieve, high percentage of flat seed type, varied soluble solid contents and low caffeine contents. Such selected coffee plants may, in a near future, be developed as clonal cultivars for the State of São Paulo.

REFERENCES

Fonseca A.F.A. 1999. Análises biométricas em café conilon (*Coffea canephora* Pierre). Tese de doutarado, Curso de Pós-Graduação em Genética, UFV, Viçosa, 121p.

Silva A.E.S., Costa E.B. Importância econômica e social. Manual técnico para a cultura do café no Espírito Santo. Vitória: SEAG. p. 9-10, 1995.