ASIC 2010

23rd International Conference on Coffee Science

Bali, Indonesia
October 3-8, 2010

ASIC
Association for Science and Information on Coffee
Association pour la science et l'information sur le café

VOLUME 2

23rd International Conference on Coffee Science
Bali (Indonesia), 3rd – 8th October 2010

23ème Colloque Scientifique International sur le Café
Bali (Indonesia), 3 – 8 octobre 2010

Table of Contents

VOLUME 2

Coffee Agronomy & Biotechnologies

Posters

- Characterization of *Coffea arabica* MADS-Box Gene Family
  de Oliveira, R.R., Mazzafera, P., Dornelas, M.C. (Brazil)

- Mapping Quantitative Trait Loci (QTLs) for Somatic Embryogenesis Ability and Yield Components in Robusta Coffee (*Coffea canephora*)
  Priyono, Rigoreau, M., Sumirat, U., Mawardi, S., Wahyudi, T., Broun, P., Petiard, V., Lambot, C., Crouzillat, D. (Indonesia)

- Establishment of Entomopathogens *Beauveria bassiana* as an Endophyte in Coffee Seedlings C*offea canephora* and *Coffea arabica*
  Sulistyowati, E., Aini, F.N., Mufrihati, E., Susiani (Indonesia)

- Protein and Lipid Oxidation Level in Just Prepared Coffee Beans
  Rendon, M., Salva, T., Braghini, M.T., Braganolo, N. (Brazil)

- The Importance of the Resting Period in the Coffee Grain Aspect and Beverage Quality
  Rendon, M., Salva, T., Braghini, M.T., Braganolo, N. (Brazil)

- Morphological Response to Drought of *Coffea arabica* (L.)
  Charmetant, P., RaKocevic, M. (France)

- Ecophysiological Differentiation for Drought Tolerance in *Coffea arabica* (L.) Genotypes
  Rakocevic, M., De Souza, F.S., Charmetant, P. (Brazil)

- Preliminary Results on Phenotypic Plasticity of Coffee (*Coffea arabica* Cv. Rubi and Iapar59) Plants in Response to Water Constraint Under Field Conditions
  Rodrigues, G.C., Rojas, J.S.D., Roupsard, O., Leroy, T., Pot, D., Moreira, M.Z., Verdeil, J.L. Dauzat, J., Jourdan, C., Andrade, A. C, Marraccini, P. (Brazil)

- Effects of Water Stress on Bean Biochemical Composition of *Coffea arabica* cv. Rubi
  Vinecky, F., Davrieux, F., Alves, G., Mera, A., Leroy, T., Bonnot, F., Pot, D., Rocha, O., Guerra, A., Rodrigues, G., Marraccini, P., Andrade, A. (Brazil)

- High-Throughput Sequencing of cDNA Extracted from Meristems of *Coffea arabica* cv. Rubi and Iapar59 Submitted to Different Water Field Conditions
  Vidal, R., Leroy, T., De Bellis, F., Pot, D., Rodrigues, G., Pereira, G., Andrade, A., Marraccini, P. (France)

- Current Status and Management of Coffee Leaf Rust Disease in Rwanda
  Gatarayiha, C., Mushimiymana, S., Bigirimana, J.. Phiri, N.A. (Rwanda)

- Evaluation of Coffee Varieties Derived from Diverse Genetic Sources of Resistance for Prospective Exploitation. An International Cooperative Effort
  Prakash, N.S., Kumar, M.M., Padmajyothi, D., Sudhakar, S.B., Hanumantha, B.Y., Daivasikamani, S., Suresh, N.R., Soumya, P.R, Asha, M.B., Madhura, M., Divya, M.H., Varzea, V.M.P., Silva, M.D.S., Jayarama, Phiri, N.A., Gichimu, B.M. (India)

- Fourteen Years of Coffee Breeding in Ghana: Achievements and Prospects
  Anim-Kwapong, E., Anim-Kwapong, J.G., Adomako, B. (Ghana)

- Quality Assessment of Coffee Genotypes from Brazil
  Cavaco Bicho, N.C., Lidon, F.C., Ramalho, J.C., Leitao, A.E. (Portugal)

- Analysis of Population Genetic Diversity and Differentiation in *Hemileia vastatrix* by Molecular Markers
  Batista, D., Guerra-Guimarães, L., Talhinhas, P., Loureiro, A., Silva, D., Gonzalez, L., Pereira, A. P., Vieira, A., Azinheira, H.G., Struck, C., Silva, Maria Do Céu, Paulo, O. S., Várzea, V. (Portugal)

- Chlorogenic Acid Content in Coffee Leaves: Possible Role in Coffee Leaf Rust Resistance
  Leitao, S., Guerra-Guimarães, L., Bronze, M.R., Vilas Boas, L., Sá, M., Almeida, M.H.G., Silva, M.C. (Portugal)

- Cellular and Molecular Responses in Host and Nonhost Coffee-Rust Interactions (*Hemileia vastatrix* and *Uromyces vignae*)
  Diniz, I., Talhinhas, P., Azinheira, H.G., Várzea, V., Oliveira, H., Fernandez, D., Silva, M.C. (Portugal)

- *Hemileia vastatrix* Gene Expression during the Infection Process of Coffee Leaves
  Vieira, A., Talhinhas, P., Loureiro, A., Duplessis, S., Fernandez, D., Silva, M.C., Paulo, O.S., Azinheira, H.G. (Portugal)

- A Preliminar Scan at Brazilian Coffea Genome Searching for MicroRNAs.
  Chalfun-Júnior, A., Silva, S.C., Silva, G.F.F., Valentim, F.L., Paiva, L.V., Andrade, A.C.

- Shine Gene Improving the Drought Tolerance of Chimeric *Arabidopsis thaliana* and *Coffea Arabica.*
  Stein, V.C., Chalfun-Junior, A., Paiva, R., Aarts, M.G.M.

- *In Silico* Characterization of Putative Members of the Coffee (*Coffea arabica*) Ethylene Signaling Pathway
  Lima, A.A., Ságio, S.A., Chalfun-Júnior, A.

- Modifications in the Carbohydrates Metabolism in Seedlings of Coffee Tree Progeny Siriema under Drought Conditions
  Chalfun-Junior, A., Melo, E.F., Fernandes, C.N., Barquero, L.O.B., Alves, J.D.

- Evolutionary Changes of the Gene Expression Pattern Mediated by Exonized Transposable Element Sequences in Coffee Genomes
  Lopes, F., Andrade, A.C., Marraccini, P., Teixeira, J.B., Carazzolle, M.F., Pereira, G.A.G., Carareto, C.M.A. (Brazil)

- Effect of Different Levels of Fertilization on Bean Biochemical Composition in *Coffea arabica* cv. Rubi
  Vinecky, F., Davrieux, F., Alves, G.S.C., Heimbeck, I.R., Mera, A.C., Leroy, T., Bonnot, F., Pot, D., Guerra, A.F., Rocha, O.C., Rodrigues, G.C., Marraccini, P., Andrade, A.C. (Brazil)

- Heterogeneous Characteristics during the Development of *Coffea arabica* Somatic Embryos
  Arimarsetiowati, R., Ismayadi, C., Priyono (Indonesia)

- In Vitro Inoculation of Arabica Coffee Derived from Somatic Embryogenesis with *Beauveria bassiana*
  Sulistyowati, E., Arimarsetiowati, R. (Indonesia)

- Predicting the Performance of Introduced *Coffea canephora* Germplasm under Recurrent Selection
  Anim-Kwapong, E., Anim-Kwapong, J.G., Adomako, B., Akpertey, A. (Ghana)

- Genotype X Environment Interaction on Yield of Selected *Coffea canephora* Clones in Ibadan, Oyo State, South Western Nigeria
  Dada, K.E., Omolaja, S.S., Oloyede, A.A., Adeyemi, E.A. (Nigeria)

- Identification and Partial Characterization of *Sepallata* Genes in *Coffea Arabica L.*
  Paula, M.F.B., Chalfun-Júnior, A., Barretto, H.G., Paiva, L.V.

- Coffee Seeds Isotopic Composition as a Potential Proxy to Evaluate Minas Gerais (Brazil) Seasonal Variations during Seed Maturation
  Rodrigues, C., Maia, R., Brunner, M., Carvalho, E., Ramalho, E., Prohaska, T., Maguas, C. (Portugal)

- Impact of Different Coffee Shade Trees on Soil Quality Parameters in Relation to Organic Coffee Farming Potential in Yayu District, Southwestern Ethiopia
  Tesfaye, A., Bobe, B. (Ethiopia)

- Impact of Different Shade Trees on Coffee Associated Parameters under Different Production Systems of Yayu District, Southwestern Ethiopia
  Tesfaye, A. (Ethiopia)

- Traditional Coffee Husbandry Practices in West Hararghe, Ethiopia
  Netsere, A., Endrise, S., Belachew, B. (Ethiopia)

- Pre-Sowing Arabica Coffee Seed Management in Ethiopia: a Review
  Netsere, A., Tefera,W., Belachew, B. (Ethiopia)

- Early Agronomic Performance of Some New and Existing Arabica Coffee Varieties in Kenya
  Gichimu, B.M., Omondi,C.O., Gichuru, E.K. (Kenya)

- Diversity of Shade Trees on Coffee Based Agroforestry System
  Priyadarshini, R., Hairiah, K., Suprayogo, D., Baon' J.B. (Indonesia)

- Effect of Higher Density Planting on Coffee Establishment and Growth in Nigeria
  Famaye, A.O., Iremiren, G.O., Oloyede, A.A. (Nigeria)

- Evaluation of Coffee Intercropped With Rice and Plantain at Early Stage of Field Establishment in Nigeria
  Famaye, A.O., Iremiren, G.O., Olubamiwa, O.O., Aigbekaen, A.E., Fademi, O.A., Oloyede, A.A. (Nigeria)

- Coffee Sector Efficiency and Equity: Lesson Learned from a Comparative Commodity Chain Analysis of Costa Rican and Kenyan Coffee Sector
  Pinard, F., Le Coq, J.F., Aithal, A. (Kenya)

- Status of Coffee Production and Marketing in Ghana
  Anchirinah, V.M., Baah, F., Oppong, F.K., Armon-Armah, F. (Ghana)

- The Dynamics of Predacious Mites, *Euseuis kenyae*, (Acari: Phytoseiidae) Under Coffee Sprayed with Chlorpyrifos
  Mugo, H.M., Mwangi, J.M., Ndoiru, S.K. (Kenya)

- The Benefits and Limitations of Shade Practices in Kenya Coffee
  Odeny D.A., (Kenya)

- Positive Effect of Bee Pollination on Coffee Production Is Highly Contingent of Irrigation in Coffee Agroforestry Landscape of Kodagu, Southern India
  Boreux, V., Vaast, P., Cheppudira, K., Madappa, L., Garcia, C., Ghazoul, J. (Switzerland)

- Beverage Quality Potential of Bourbon Selections for Specialty Coffee Production in Brazil
  Giomo, G.S., Borem, F.M., Fazuoli, L.C., Mistro, J.C. (Brazil)

- Validating the Agro-Ecological Systems of Coffee Land Evaluation in Tanzania Using the Parametric Approach
  Maro, G.P., Teri, J.M., Mosi, E.J. (Tanzania)

- Beverage Quality of Wild Ethiopian Arabica Coffee Accessions in Brazil
  Giomo, G.S., Borem, F.M., Silvarolla, M.B. (Brazil)

- Microbiological Soil Characteristics of Areas Cultivated with Conilon Coffee under Conventional and Organic Management Systems
  Partelli, F.L., Vieira, H.D., Ferreira, E.P.B., Viana, A.P., Martins, M.A., Urquiaga, S. (Brazil)

- Climate Change Adaptation and Mitigation in the Kenyan Coffee Sector (Sangana PPP)
  Linne, K., Schmitz-Hoffmann, C., Archer, J., Ng'Ang'a, J., Pensel, A., Kuhrt, C. (Germany)

- Standardization of Optimum Planting Densities and Training Methods for Dwarf Arabica Genotypes Grown under Shaded Canopy
  Biradar, I.B., Raghuramulu, Y., Hariyappa, N., Shivaprasad, P., Kamalabai, S., Jayarama (India)

- Study of Spacing and Fertilizer Requirement on Compact Coffee Varieties in Tanzania
  Mndolwa, E., Maro, G.P., Kilambo, D.L., Teri, J.M. (Tanzania)

- Integrating *Cedrela odorata* Trees into Robusta Coffee Production in Ghana as a Diversification Strategy – Establishment Phase
  Oppong, F.K., Anim-Kwapong, G.J. (Ghana)

- Population Dynamics of The Coffee Leaf Miner, *Leucoptera meyricki* (Ghesq.) (Lepidoptera: Lyonetiidae) in a Block of Unsprayed Coffee in Chipinge, Zimbabwe
  Kutywayo, D.A. (Zimbabwe)

- Effect of Organic Nursery Media on Coffee Seed Germination and Initial Growth
  Chemura, A., Mahoya, C., Kutywayo, D. (Zimbabwe)

- Selected Agroclimatic Factors Determining Water Stress of Coffee Plants
  Erwiyono, R., Bowo, C., Wibawa, A. (Indonesia)

- Influence of Arbuscular Mycorrhizal Inoculation and Phosphate Fertilizer Types on the Growth of Coffee Seedlings in Two Soil Types in Nigeria
  Iremiren, G.O., Ibiremo, O.S., Daniel, M.A., Oloyede, A.A., Adejumo, M.O. (Nigeria)

- Cup Taste Profile Evaluation on the Hybrid Progeny of Congolese and Guinea Groups of Robusta Coffee (*Coffea canephora*)
  Mawardi, S., Dwi Nugroho, Ucut Sumirat, Yusianto (Indonesia)

- Evaluation of Alternative Containers for Producing Cloned Seedlings of Coffee Conilon
  P*ires, A., Pinho, L., Fontes, P., Fontes, A., Pinheiro, A., Dos Santos, D., Sian, M., Carvalho, C. (Brazil)*

- Employment Generation in Brazilian Coffee Regions
  Bliska, F., Guilhoto, J., Imori, D., Sakon, F., Camargo, F., Vegro, C. (Brazil)

- Influence of Full Sunlight on Trapping of the Coffee Berry Borer *Hypothenemus hampei* Ferrari
  Dufour, B., Cilas, C., Ribeyre, F. (France)

- Yield Differences of "Conilon" Coffee Plants Propagated by Cuttings and Seeds along a Nine Year Period
  Partelli, F.L., Barbosa, D.H.G., Mauri, E.L., Vieira, H.D., Ramalho, J.C. (Brazil)

- Effects of Shade Tree Composition on the Coffee Quality in Agroforestry Systems of the Kodagu District, South-Western India
  Vaast, P., Raghuramulu, Y., Menon, S. (France)

- Coffee Agroforestry with Some Timber Shade Trees. Study on Carbon Stock, Mineral Cycle and Yield
  Prawoto, A.A., Yuliasmara, F. (Indonesia)

- Multiple Income Generation in Coffee Farms with *Paraserianthes falcataria* as Shade Trees
  Prawoto, A.A., Hartatri, D.F.S. (Indonesia)

- Characteristics of Anatomy, Morphology and Physiology as Indicators for Yield of Robusta Coffee
  Munandar, D.E., Abdoellah, S., Mardiono, E. (Indonesia)

- Investigation on Main Factors Influencing the Arabica Green Coffee Quality
  Lambot, C., Goulois, E., Michaux, S., Pineau, N., De Smet, J., Husson, J., Broun, P. (France)

- Micro-Landscape Context Effects on the Dispersal of Coffee Berry Borer (*Hypothenemus hampei*) in Costa Rica
  Olivas, A., Rivera, C., Dufour, B., Hidalgo, E., DeClerk, F., Avelino, J. (Costa Rica)

- Landscape Context and Plot Incidence of Coffee Rust (*Hemileia vastatrix*), Coffee Berry Borer (*Hypothenemus hampei*) and the Root-Knot Nematodes *Meloidogyne* spp. in Costa Rica
  Romero, A., Cruz, H., De Melo, E., DeClerck, F., Avelino, J. (Costa Rica)

- Indonesian Green Arabica Coffee Growing Zones Characterization Using Near Infrared Spectroscopy
  Davrieux, F., Durand, N., Sianturi, J., Fischer, D.F. (France)

- Coffee Varieties Behavior (*Coffea canephora* Pierre) in Growing Area of High Altitude in the Northwest Region of Rio De Janeiro State - Brazil
  Barbosa, D.H.S.G., Partelli, F.L., Vieira H.D., Rodrigues, W.P., Pinto, J.F. (Brazil)

- Shade Level and Species Composition Affect Carbon Sequestration in Coffee Agroforestry Systems of the Kodagu District, South-Western India
  Vaast, P., Guillemot, J., Vignault, C., Charbonnier, F., Manjunatha, M., Devakumar, A. (France)

- The Influence of Primary Processing Methods on the Cup Taste of Arabica Coffee from the Indonesian Island of Flores
  Marsh, A., Yusianto, Mawardy, S. (Indonesia)

- Coffee Bourbon Pointu of Reunion Island: The Post-Harvest Process Is One of the Keys to Achieve the Best Sensorial Quality
  Aguilar, P., Berthiot, L., Descroix, F. (France)

- Support to Small Coffee Farmer in Thailand Through Large Scale Propagation of *Coffea canephora*
  Kunasol, T., Ducos, J.-P., Lambot, C., Kasinkasaempong, Y., Chantanumat, P., Broun, P. (Thailand)

- Coffee and the Environment. A Review
  Mayoli, R. (Kenya)

- Coffee Bourbon Pointu of Reunion Island: How to Define a Terroir to Obtain a «Gourmet» Coffee
  Descroix, F., Aguilar, P., Berthiot, L. (France)

- Scaling-Up LAI in Coffee Agroforestry Systems in Costa Rica
  Taugourdeau, S., Le Maire, G., Roupsard, O., Avelino, J., Gómez-Delgado, F., Jones, J., Marsden, C., Robelo, A., Barquero,A., Alpizar, A., Rapidel, B., Vaast, P., Harmand, J.-M. (France)

- Sustainable Coffee Production: an Analysis Model for Quality and Certification in the Coffee Agribusiness
  Leme, P.H.M.V., Machado, R.T.M. (Brazil)

- Economic Productivity of Coffee Intercropping Practices among Farmers in Kogi State
  Oduwole, O.O., Agbongiarhuoyi, A.E., Adejumo, M.O., Aigbekaen, E.O., Ipinmoroti, R.R. (Nigeria)

- Identification of Soil Organic Nitrogen Fraction as an Indicator of Coffee Plant Response to Nitrogen Fertilizer
  Bako Baon, J., Suwarti, Pandutama, M.H. (Indonesia)

- Volatilization and Efficiency of Urea Fertilization and Growth of Arabica Coffee Seedlings Applied with Filter Press Cake
  Bako Baon, J., Sari, Y., Pandutama, M.H. (Indonesia)

- Use of Sub-Surface Soil Water by Wick of Organic Matter to Mitigate Water Stress of Robusta Coffee
  Pujiyanto (Indonesia)

- Arthropod Diversity on Indonesian Coffee Ecosystems and Its Relationship on Main Insect Pests Infestation
  Wiryadiputra, S. (Indonesia)

- The Change of Climatology Components of Robusta Coffee Area and Their Impacts on Productivity: a Case Study in East Java, a Coffee Producing Area in Indonesia
  Soetanto Abdoellah (Indonesia)

- Livelihood Strategies of Smallholder Coffee Farmers in South Sulawesi and East Nusa Tenggara (Flores)
  Hartatri, D., Nielson, J., Arifin, B., Fujita, Y. (Indonesia)

- Efficiency Determinant Factors of Arabica Coffee Farming in Enrekang District, South Sulawesi
  Faila, H., Kadir, S. (Indonesia)

- Cost of the Use of Legumes as Source of Nitrate Fertilizers in Coffee Trees
  Konan, A., Legnate, H., Koffi N'goran (Côte d'Ivoire)

- Quality of Robusta Coffee in Côte d'Ivoire: Importance of the Shade
  Konan, A., Legnate, H., Yapo, A., Yoro, G., N'Goran, K. (Côte d'Ivoire)

- Biofertilizers – Effective Enricher for Sustainable Nutrition of Coffee Seedlings in Nursery
  Prasanna, S.M., Shivaprasad, P., D'Souza, M.V., Hareesh S.B, Hariyappa, N., Manjunath, A.N., Jayarama (India)

- Temporal Variations in the Abundance of Three Important Insect Pests of Coffee in Kilimanjaro Region, Tanzania
  Magina, F.L., Makundi, R.H., Maerere, A.P., Maro, G.P., Teri, J.M. (Tanzania)

Augmentation of Caffeine Alkaloids by Exogenous Indoleamines in *Coffea canephora* P. Ex. Fr. *in Vitro* Cultures: the Possible Involvement of Polyamines

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SUMMARY

The physiological roles of indoleamines serotonin and melatonin in plants have been of great interest since they were thought to exist exclusively in the animal kingdom. Though we can not rule out the antioxidant role of indoleamines in plants, the same could be having significant influence on secondary metabolites in plants. Polyamines putrescine (Put), spermidine (Spd) and spermine (Spm) are essential compounds for growth and development in plants. Polyamines are reported to influence on *in vitro* morphogenetic response and caffeine biosynthesis in *C. canephora*. Till today no reports are available on indoleamine mediated regulation of secondary metabolites in plants. Similarly information on correlation between indoleamines and polyamines in plants and its possible influence on caffeine alkaloids pathway are not available. Hence, a study was taken up on the profiles of serotonin and melatonin in *Coffea* sp. vis a vis endogenous polyamine pools and the content of caffeine alkaloids in *in viro* cultures of *Coffea canephora*. As caffeine levels too are affected by polyamines, their possible influence in alterations in caffeine levels under exogenous feeding of indoleamines SER and MEL was studied.

INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine; MEL) and serotonin (5-hydroxytryptamine; SER) are prominent indoleamines which participate in neural transmission in animals have also been reported in various genera of both cryptograms and phanerogams (Hernandez-Ruiz et al., 2005). SER has been found in more than 42 species (Roshchina, 2001). In angiospermic plants these indoleamines concentrations were usually in the range of picograms to nanograms per gram of tissue (van Tassel and ONeill, 2001). SER (5-hydroxytryptamine, 5HT) is reported in the coffee beans wax (Wurziger and Harms, 1968). Caffeine is the major alkaloid in *Coffea* sp. In caffeine biosynthesis pathway, methylation reactions are catalyzed by N-methyl transferases, and S-adenosyl methionine is the methyl donor for the methylation steps (Mazzafera et al., 1994). Though influence of both indoleamines (SER and MEL) and polyamines on plant morphogenesis is reported. The polyamines role on caffeine regulation in somatic embryos of coffee is well documented (Vinod Kumar et al., 2008). The endogenous polyamine profiles in both *in vitro* and *ex vitro* tissues of *C. arabica* and *C. canephora* along with their levels during the ontogeny of coffee fruit formation was documented recently (Sridevi et al., 2009). 5-hydroxy tryptamines being the precursors of IAA and also SER and MEL might have regulatory role secondary metabolites production in plants apart from their influence on growth and development in plants.

Till today no reports are available on indoleamine mediated regulation of secondary metabolites in plants. Similarly information on correlation between indoleamines and polyamines in plants and its possible influence on caffeine alkaloids pathway are not available. Hence, a study was taken up on the profiles on the influence of indoleamines on polyamine pools in *Coffea* sp. vis a vis endogenous content of caffeine alkaloids in *in viro* cultures of *Coffea canephora*.

MATERIALS AND METHODS

The indole compounds MEL, SER and polyamines Putrescine, Spermine, and Spermidine were obtained from Sigma-Aldrich, USA. All other chemicals and solvents obtained from Merck, India, Di-methyl sulphoxide (DMSO) from SRL, India. The solution of SER dissolved in sterile distilled water and MEL was dissolved in DMSO and filter-sterilized before addition to the autoclaved medium. Hypocotyl and leaf explants from *in vitro* germinated seedlings of *C.canephora* CxR variety were used for the experiment. Explants were placed on callus induction medium (van Boxtel and Berthouly, 1996) containing MS salts (Murashige and Skoog, 1962), 9.8 µM isopentenyladenine (2-iP), 2.2 µM 2,4-dichloroacetic acid (2,4-D) to initiate callus (55 days; 25±2 °C with a 16 h photoperiod) and then subcultured on to medium containing 4.5 µM 2,4-D and 17.6 µM benzyl amino purine (BA) to induce embryogenic callus (for two and a half months). The embryogenic callus was transferred to the EG medium containing MS salts, B5 vitamins, and 2% sucrose (w/v), 0, 1, 5, µM with IAA and without IAA, 0.25 mg/l BA, and 100 mg/L meso inositol (EG medium).

The embryogenic callus cultures were cultured on EG medium (without IAA) supplemented with B5 vitamins, 0.8 µM BA, 3% sucrose, 100 mg/l inositol and SER and MEL at 100 µM and indoleamine inhibitors viz., prozac (fluoxetine hydrochloride) (SER reuptake inhibitor) and p-CPA (SER to MEL conversion inhibitor) in the range of 20-60 µM were added individually or in combination to the MS medium.

Extraction and HPLC analysis of MEL and SER was performed as reported earlier (Murch et al., 2000) using C-18 Column (3.9x150mm, particle size 3 µm). Isocratic buffer consist of 0.1M sodium acetate, 0.1 M citric acid, 0.5 mM sodium octanylsulfonate, 0.15M EDTA, adjusted to pH 3.7 and 5% methanol was used as a mobile phase at a flow rate of 1 mL/min (Murch et al., 2000). MEL and SER were quantified from their peak areas in relation to the respective reference standards. Indolamines were monitored using Fluorescence detector (FD) at Em 348/Ex 280. MEL and SER were further confirmed by LC- MS-ESI according to Cao et al. (2006). The methanolic extracts obtained and analyzed by HPLC unit model- Alliance equipped with an electrospray ionization source (ESI) and coupled to Waters 2696 operation module and Waters 2696 photo diode array detector (DAD) with isocratic mode with methanol: water (3:2) with a flow rate of 1ml/min, column temperature maintained at 25 °C. Detector wave length of 286 nm. Extraction of caffeine alkaloids from embryogenic callus (50-100 mg) was performed and analysed by HPLC (Ashihara et al., 1996). Similarly the extraction and estimation of endogenous polyamines was carried out (Flores and Galston, 1982). An aliquot of methanol extract of experimental sample and standard sample were injected to C18 column of size 25x4.6 mm (High performance Liquid Chromatography model no: LC-6A, Shimadzu corporation, (Kyoto, Japan). The wave length set at 254 nm. Samples were run at isocratic condition using solvent mixture of methanol: water (64:36) with flow rate of 1.0 ml per min. Quantitative estimation of polyamines was done based on the peak area of specific concentrations of the sample.

RESULTS AND DISCUSSION

Incorporation of SER at 100 µM concentration to medium enhanced caffeine, theobromine and theophylline by 88%, 62.5% and 86.5% respectively compared to control wherein 210, 6 and 12 µg/ g dry wt. were detected (Table 1). But there was 30%, 22% and 41% increase in put, spd and spm respectively along with two fold increase in endogenous SER (2 folds) and MEL (1.5 folds). Similarly MEL addition to the medium at 100 µM augmented caffeine, theobromine and theophylline by 50%, 58.8% and 68% respectively. There was 27% (put), 28% (spd) and 34% (spm) increase in endogenous polyamines along with 2 folds increase in endogenous MEL profile (Table1). In order to substantiate the influence of SER and MEL on caffeine alkaloids profiles in callus cultures, indoleamine inhibitors p-CPA at 40 µM and Prozac at 20 µM were added to the callus culture medium, which reduced purine alkaloids production by 20-28% and 40-52% respectively (Table 1). The endogenous polyamine profiles too reduced by 45 & 39 % (put), 32% & 36% (spd) and 54 & 59 % (spm) respectively compared to the cultures treated with SER and MEL, under the influence of indole amine inhibitors Prozac and p-CPA respectively. Apart from this, the levels of tryptamine (2 µg/g FW) is less than that of SER (39 µg/g FW) and MEL (25 µg/g FW) in control cultures wherein the content of caffeine was 0.21% (not shown in table.1). Similarly all the three major polyamine profiles were low to moderate (Spm>put>spd).

Table 1. Influence of indoleamines and their inhibitors on caffeine alkaloids and endogenous polyamines in somatic embryos of *Coffea canephora.*

Treatment	Purine alkaloids (mg/100g dry wt.)			Polyamines (µg/g^{-1} dry wt.)		
	Caffeine	Theobromine	Theophylline	Putrescine	Spermidine	Spermine
EG medium (control)	210±2.56	12.03±0.8	06±0.02	82±2.2	58±4.6	11±0.95
EG+MEL (100 µM)	315±1.4	19.08±2.5	10.56±1.58	104.14±3.5	74.24±3.7	14.74±0.6
EG+SER (100 µM)	394.8±3.5	22.08±1.5	11.59±0.56	108.24±2.2	70.76±3.2	15.51±1.2
EG + Prozac (20 µM)	42.8±3.24	48.0±1.0	14.08±0.09	59.53±2.8	40.44±2.8	4.95±.025
EG + p-CPA (40 µM)	320.0±1.5	ND	28.00±1.26	50.04±1.5	37.12±2.2	3.51±.03

Values are mean of three determinants. DW-Dry weight; ND-Not Detected; ND-Not Detected ; EG medium (Embryogenic medium) ½ MS+IAA (0.5mg/L) BA (0.25mg/L); Data recorded on 60th day of culture.

Tryptophan is a precursor of indoleamines and methionine is a precursor of polyamines. In our study, we found that SER/MEL treatment, significantly elevated the levels of spermine, putrescine and spermidine. The interrelations between indoleamines and polyamines are not clear at the present time, which needs to be further explored. The relationship between polyamines (put, spd, spm) and caffeine alkaloids was well established (Vinod Kumar et al., 2008). There is strong evidence that there is competition for SAM between the biosynthetic pathways of polyamines, ethylene (Minocha et al., 1990) and caffeine biosynthesis (Ashihara and Suzuki, 2004). Although unconvincing attempts have been made by various researchers to hunt for the actual role for these indoleamines as a circadian, photoperiodic

(Reiter et al., 2001) and morphogenesis regulator in general and secondary metabolites that are specific to a plant species in particular (Kolar et al., 1997; van Tassel and O'Neill 2001) no clear evidence available about the precise function of MEL and SER in plant system. But in the present study, under the influence of MEL and SER changes in caffeine, theobromine and theophylline was evident. The indole amines influence was further substantiated by reversing their activity through their inhibitors Prozac and p-CPA. As a part of our study, we have noticed that, the indole amine inhibitors in culture medium had adverse effect on the endogenous pools of both SER and MEL (data not shown). Moreover, they reduced the levels of polyamines, which shows a possible interrelation ship between indoleamines and polyamines and also purine alkaloids.

So from the study it is concluded that the administration of SER / MEL to the medium improved caffeine alkaloids profiles in somatic embryos and also the endogenous pools of polyamines compared to that of controls. Incorporation of SER re-uptake inhibitor Prozac or SER to MEL conversion inhibitor p-CPA to the medium leads to significant reduction in endogenous pools of both SER and MEL and also caffeine alkaloids levels. Respective polyamine levels also decreased that leads to poor response for somatic embryogenesis. Polyamines are known for improvement of somatic embryogenesis in *C. canephora*. Similarly our studies indicates a positive influence of indoleamines SER/MEL on somatic embryogenesis and caffeine alkaloids production. A re-examination of the classic systems of plant physiology may provide new clues to the role of MEL and SER that may be independent of other growth regulators, or act synergistically with other known growth regulators leading to morphogenesis and secondary metabolism.

ACKNOWLEDGEMENTS

The authors are thankful to DST, New Delhi, India for funding the project. The financial support from ASIC, Switzerland to Mr. P.Giridhar for attending the conference is highly appreciated.

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Genetic Study on Several Mechanical Properties of the Wood of *Coffea arabica* and *Coffea canephora* Coffee Trees

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SUMMARY

The physical characteristics of wood are not usually taken into account when breeding perennial species grown for their fruits or seeds. In the coffee tree, stem breakage during harvesting and lodging during the growth period are major defects in some cultivars. Such defects are linked to certain wood physical and mechanical properties, such as density or rigidity, which can be characterized by a parameter used in the resistance of materials: the modulus of elasticity. Wood density and the longitudinal modulus of elasticity of stem segments of *Coffea arabica* L and. *C. canephora* coffee trees were studied. *C. arabica* coffee trees derived from a diallel mating design, and *C. canephora* derived from a clone comparative trial. The modulus of elasticity was measured using an acoustic system based on an analysis of the vibrations produced by a blow to the end of a piece of wood of known geometry.

The modulus of elasticity and the wood density of the coffee tree stems were highly heritable in both species and the wood from *C. arabica* was stiffer on average than that from *C. canephora*. *C. canephora* clone classification according to wood stiffness revealed the clones most liable to lodging. In this species, wood stiffness displayed strong broad sense heritability (0.64), indicating that this trait can be efficiently improved. This parameter could also be used as a predictor of other traits of agronomic interest, such as resistance to borer insects. The classification of *C. arabica* parents according to the wood characteristics of their progenies depended on their rate of introgression by the species *C. canephora*. These traits could therefore be used as a measure of introgression in this species. Use of coffee tree wood could also be considered, notably to derive value from the wood arising from the different pruning and cutting back operations.

INTRODUCTION

In perennial species grown for their fruits or their seeds, such as coffee, the physical and mechanical properties of the wood are not traits that are taken into account in breeding programmes. Yet these properties can be involved in events that are prejudicial to crops, such as coffee tree lodging (De Reffye, 1976), which is primarily linked to the “elasticity” of the material. “Elasticity” is the tendency of a body to resume the shape and dimensions it had before stress, when that stress is removed. The coffee tree is one of the rare perennial plants that is susceptible to lodging, apart from a few fruit trees whose “weeping” or drooping growth habits are managed by cultural techniques. In the species *Coffea canephora* Pierre, which gives “Robusta” coffee, there is genetic variability for wood rigidity (or “stiffness”). “Stiffness” is the property of a solid characterizing the degree of deformation undergone for a given increase in stress; the slighter the deformation, the stiffer the body. The problems of lodging or of stem damage during harvesting are therefore returning to the forefront of producers' concerns. Susceptibility to lodging mainly depends on the elastic properties of the

wood and on stem geometry. Whilst lodging is often linked to insufficient rigidity in the wood, inversely high rigidity can lead to problems of main stem or branch breakage during harvesting. Indeed, on old coffee trees it is often necessary to bend the main stems to reach the fruits in the upper section of the coffee trees. When stems are too rigid, even slight deformation can lead to the elastic domain being overstepped, ruining the material. Optimum rigidity therefore needs to be sought in accordance with different cultural practices. A few studies have already been carried out on the rigidity of coffee tree wood (De Reffye, 1976; Cilas et al., 2000, 2002, 2006). We studied the variability and the inheritance of these traits (density and elasticity) on *Coffea canephora* and *Coffea arabica.*

MATERIALS AND METHODS

Wood density and the longitudinal modulus of elasticity were studied on stems of *C. canephora* L clones, and on stems of *C. arabica* L. coffee trees derived from a diallel mating design. The modulus of elasticity was measured using an acoustic system based on an analysis of the vibrations produced by a blow to the end of a piece of wood, with the "Bing" system.

Plant material

Ten clones of the species *C. canephora*, selected for their yield potential, were studied: clones 119, 126, 305, 461, 503, 526, 587, 588, 589, 609. The clones were in a clonal comparative trial at the CNRA research station (Centre National de Recherche Agronomique de Côte d'Ivoire) at Divo in central southern of Côte d'Ivoire.

On *C. arabica*, the stems studied came from the cutting back of a genetic trial set up at the Cicafé station in Heredia, Costa Rica, involving a diallel mating design of crosses between 10 parents: T17930, T17931, T17933, T18121, T18130, T18138, T18140, T18141, T8666, T8667. All these parents were of the compact type, due to the presence of the "Caturra" gene that conferred dwarfism. This incomplete diallel comprised 35 families, and 291 stems of the same age were analysed. The stems were obtained when the trees in the trial were cut back in 2001.

Methods

The study material was a segment of stem, with an average length of 60 cm and a slightly conical shape. On each of these wood samples (or test-pieces), the dynamic compression modulus of elasticity was measured by a vibrating analysis system developed at CIRAD: the "Bing" system (Baillères et al., 1998). The "Bing" system (Figure 1) is based on an analysis of the vibrations produced by a blow to a piece of wood of known geometry and density.

Once the information had been fed into the software, the longitudinal modulus of elasticity was calculated. The modulus E1 corresponding to the first frequency peak of the spectrogram f1 was estimated, along with the average modulus E corresponding to the mean of E1 and E2 (corresponding to the second frequency peak of the spectrogram f2). The specific modulus of elasticity (Er) is the ratio of the modulus (E) to the density (ρ).

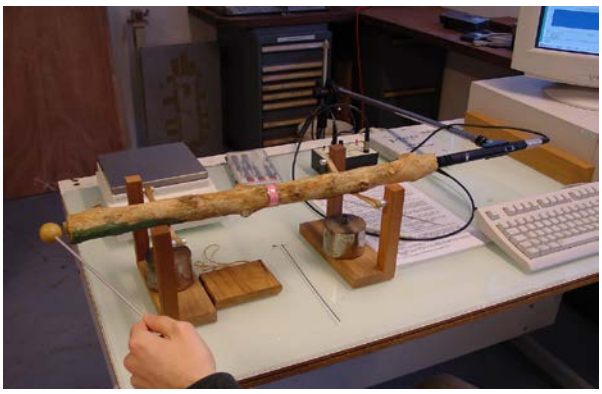

Figure 1. Laboratory version of the "Bing" system.

Statistical analyses

The clones were compared by a one-way analysis of variance and the broad sense heritability values were then estimated, along with the associated standard deviations, to obtain a confidence interval for the estimations. Estimations of heritabilities were given by the ratios of genetic variances ("clone" variances) and phenotypic variances.

Diallel analyses were carried out on the traits recorded: density (ρ), modulus of elasticity (E), specific modulus of elasticity (Er), and yields cumulated over 4 years (Y). Narrow sense and broad sense heritabilities were estimated for all the traits and the confidence intervals for those estimations were obtained by the Jackknife method. These statistical analyses were performed with "Diogene" quantitative genetics software (Baradat and Labbé, 1995).

RESULTS

The density of the wood is higher for *C. arabica* than for *C. canephora*; and the rigidity is higher for *C. canephora* (Table 1).

Table 1. Means for the two species.

Traits	*C. arabica*	*C. canephora*
Δ	820	760
E	7500	9500
Er	9	13

With: Δ : density of the wood (in kg/m^3); E : the specific modulus of elasticity (in Megapascals – MPa - i.e. in Newtons per mm²); Er : the specific modulus of elasticity - the ratio of the modulus (E) to the density (ρ).

On *C. canephora*, physics properties of wood have high broad-sense heritabilities, particularly the density of the wood, in comparison to agronomic traits like yield (Table 2).

Table 2. Heritabilities on *C. canephora.*

Traits	h_B^2 [95% confidence limits]
Δ	0.811 [0.664 ; 0.959]
E	0.671 [0.455 ; 0.888]
Er	0.576 [0.333 ; 0.819]
Y	0.474 [0.220 ; 0.728]

Y: Yield on several years.

We observed the things on *C. arabica*: heritabilities were higher for the wood properties than for yield (Table3).

Table 3. Heritabilities on *C. arabica*.

Traits	h_N^2 [95% confidence limits]	h_B^2 [95% confidence limits]
Δ	0.475 [0.336 ; 0.614]	0.538 [0.399 ; 0.677]
E	0.195 [0.066 ; 0.324]	0.228 [0.076 ; 0.380]
Er	0.385 [0.211 ; 0.559]	0.443 [0.287 ; 0.599]
Y	0.055 [0.000 ; 0.216]	0.180 [0.000 ; 0.368]

h_N^2 *: Narrow sense heritability;* h_B^2 *: Broad sense heritability.*

The properties of wood were linked for the 2 species, but not from the same manner (Tables 4 and 5). Yield was also linked to wood properties but the links need to be more investigated to better understand the relationships; in fact, yield increased when density decreased on *C. canephora*, but it was reverse for *C. arabica*.

Table 4. Genetic correlations between the traits on *C. canephora.*

Traits	***Y***	***Δ***
E	**-0. 448***	**0.605***
Er	-0.378	0.271
Δ	**-0.407 ***	

**Significant.*

Table 5. Genetic correlations between the traits on *C. arabica.*

Traits	***Y***	***Δ***
E	-0.146	**-0.423***
Er	-0.262	**-0.835***
Δ	**0.300***	

**Significant.*

CONCLUSION

The density of the wood is higher for *C. arabica* than for *C. canephora*; and the rigidity is higher for *C. canephora*.

Properties of wood (density and modulus of elasticity) are very heritable in the two species - more heritable than the yield. The heritabilities of these traits are higher in *C. canephora* than in *C. arabica.* Clones with a very low modulus of elasticity were effectively the ones with lodging problems in the field, notably clone 503 (*C. canephora*), for which the top of the stem can bend right down to the ground.

Wood density was significantly correlated to yield and wood elasticity. This trait should therefore be measured in breeding trials for use as a predictor. Studies on the ability of wood traits to predict other criteria of agronomic interest remain to be carried out. In particular, the physical properties of wood might explain the differences in coffee tree performance in relation to certain pests, notably stem borers such as *Bixadus sierricola* White, *Anthores leuconotus* Pasc. or *Apate monachus* Fabr. (Coste, 1989), whose penetration may depend on the mechanical properties of the wood.

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Coffee Cold Acclimation Ability and Its Relation to Oxidative Stress Control

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SUMMARY

Low positive temperatures (chilling) are often linked to the onset of oxidative stress conditions. This is of particular importance for coffee plants due to its cold sensitivity, reflected, *e.g*., on photosynthesis and crop yield. Experiments were carried out using 1.5-year-old coffee seedlings of Icatu and Apoatã, submitted to a gradual cold treatment and a recovery period. The less cold sensitive Icatu genotype showed higher enhancement of some enzyme (APX and catalase) activities and non-enzyme (ascorbate, zeaxanthin and lutein) contents, as well as a higher expression of a chitinase class III gene. Differences on the triggering of antioxidative mechanisms help to explain the different cold tolerances among *Coffea* spp. and could be an important tool to identify tolerant plants.

INTRODUCTION

Among environmental stresses, low positive temperatures constitute a major limiting factor, with impacts on photosynthesis, uptake of water and nutrients, as well as on crop production quality and post-harvest life (Ensminger et al., 2006; Chinnusamy et al., 2007). Chilling impairs all photosynthetic components. Chloroplasts are the major cellular source of reactive oxygen species (ROS), and under low temperature they are often the first and the most severely affected organelles (Kratsch and Wise, 2000). Therefore, the control of oxidative stress through the reinforcement of scavenging and detoxifying mechanisms is crucial to plant tolerance and survival under cold conditions (Mano, 2002; Logan, 2005). Despite its sensitivity to cold (Bauer et al., 1985; DaMatta et al., 1997; Ramalho et al., 2003) previous reports have highlighted different acclimation abilities within the *Coffea* genus, likely related to the tolerance of the photosynthetic apparatus and membrane stability in face of oxidative stress conditions (Ramalho et al., 2003; Campos et al., 2003). This work further investigates cold-induced adjustment of antioxidative mechanisms, supporting the hypothesis that they are decisive for cold tolerance in *Coffea* spp.

MATERIAL AND METHODS

Plant Material and Growth Conditions

The experiments were carried out as previously described (Ramalho et al., 2003), using 1.5 years old plants from the genotypes Icatu (IAC 2944 - *C. canephora* x *C. arabica*) and *C.*

canephora cv. Apoatã (IAC 2258). Potted plants were submitted successively to: 1) a gradual temperature decrease (0.5 °C day^{-1}) from 25/20 °C to 13/8 °C (day/night), to allow the expression of acclimation ability; 2) a 3 day chilling cycle (3x13/4 °C), where the plants were subjected to 4 °C during the night and in the first 4 h of the morning (with light) and 13 °C, throughout the rest of the diurnal period; 3) a 7 days rewarming period (25/20 °C), to plant recover. Photoperiod was set to 12 h, RH to 65-70% and irradiance to *ca*. 750-850 μmol m^{-2} s^{-1}.

Enzyme activities

Chloroplast Cu,Zn-superoxide dismutase (Cu,Zn-SOD- EC 1.15.1.1), ascorbate peroxidase (APX - EC 1.11.1.11) were determined as in Ramalho et al. (1998) and cellular catalase (EC 1.11.1.6) as in Lima et al. (2002).

Non-enzymatic antioxidants

Ascorbate evaluation followed (Romero-Rodrigues et al., 1992), while zeaxanthin and lutein contents were determined as in Campos et al. (2003).

Gene Expression Studies

Based on coffee cDNA sequence from ESTs NCBI data base, specific primers were designed (data not shown) in order to perform the mRNA expression studies by real time PCR as described in Batista-Santos et al. (2011 accepted).

Statistical analysis

A two-way ANOVA ($P < 0.05$) was applied to evaluate differences between temperatures and between genotypes, followed by a Tukey test for mean comparison (95% confidence level).

RESULTS AND DISCUSSION

Previous evaluation of Icatu suggested a lower sensitivity to cold, related to a better ability of perform acclimation adjustments at the leaf level (Ramalho et al., 2003; Campos et al., 2003). Under low temperatures, a strong decrease in carbon uptake may lead to an excess of excitation energy and to an overproduction of highly reactive molecules (Adams et al., 2002), even at moderate irradiance. Therefore the difference in the triggering of antioxidative traits can be responsible for cold and photoinhibition tolerance. Icatu presented strong significant increases of Cu,Zn-SOD activity (that produces H_2O_2) together with an increased potential for H_2O_2 removal through chloroplastic APX (Figure 1A, B), the later being absent in Apoatã. This was confirmed by the decrease of H_2O_2 and the maintenance of $OH^{\bullet}$ (data not shown) contents close to control levels in Icatu but not in Apoatã, where it raised. Also, H_2O_2 levels could be further controlled in Icatu due to a higher catalase activity (Figure 1C), particularly under the harsh chilling conditions.

Ascorbate is a potent ROS scavenger that reacts with H_2O_2 in the reaction catalyzed by APX, and non-enzymatically with $^1O_2$, $O_2^{\bullet}$, $OH^{\bullet}$ and lipid hydroperoxides (Logan, 2005; Foyer, 2002). Thus, the highest ascorbate content shown by Icatu (Figure 2A), along with its higher APX activity, could also contribute to the observed decrease of H_2O_2 in this genotype under cold. Moreover, ascorbate is used by violaxanthin de-epoxidase to form zeaxanthin from violaxanthin (Logan, 2005). Therefore, its higher levels (Figure 2B) could allow efficient zeaxanthin production, which is consistent with the higher zeaxanthin pool shown by Icatu

compared to Apoatã. The high level of lutein could also complement thermal dissipation mechanisms particularly in Icatu (Figure 2C).

Complementary studies showed that only Icatu displayed strong transcriptional increases of the *cachi3-2* gene throughout the entire experiment (Figure 3), suggesting the involvement of this gene in cold tolerance, as found for chitinase class III genes in other plants under cold, drought, high irradiance and salt stresses (Sasaki et al., 2006).

In conclusion, Icatu showed the greatest ability to control oxidative stress and showed rapid recovery of most of the studied parameters after stress ending. These results support reports of lower impact and better recovery of the photosynthetic metabolism (Ramalho et al., 2003) and membranes (Campos et al., 2003). Despite the known sensitivity of the *Coffea* genus to cold, our results indicate important different acclimation capabilities that could be exploited in breeding programs for cold tolerance.

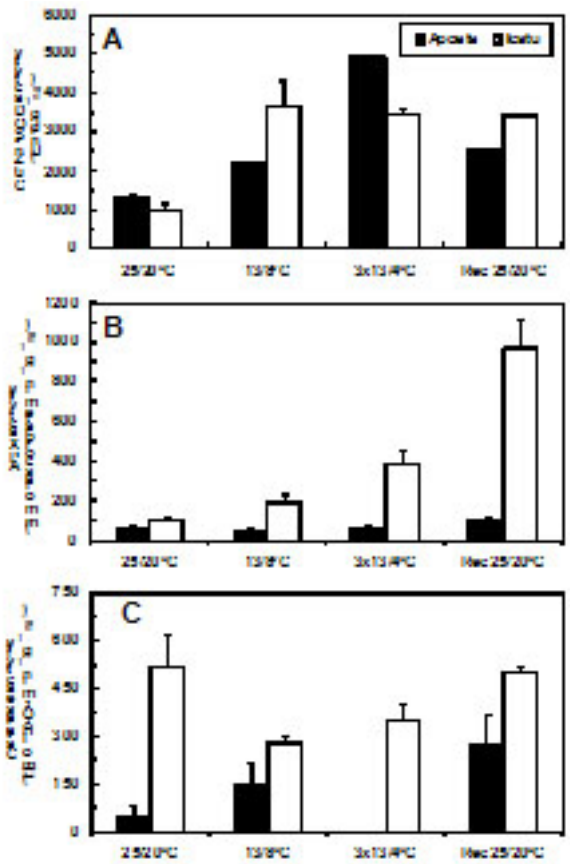

Figure 1. Changes in leaf maximal activities of chloroplastic A) Cu,Zn-superoxide dismutase (Cu,Zn-SOD) and B) ascorbate peroxidase (APX), and cellular C) catalase in the studied *Coffea* spp. genotypes under the imposed experimental conditions. Each value represents the mean ± SE (n=6-8).

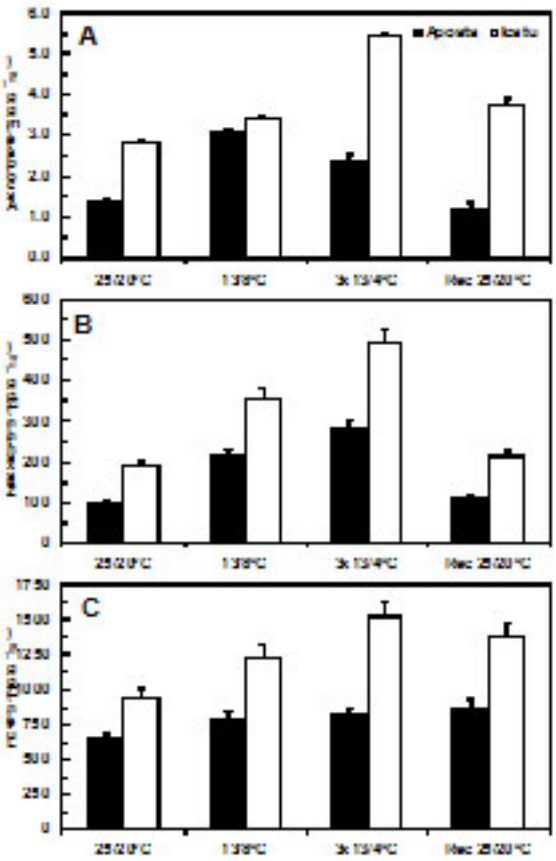

Figure 2. Changes in the cellular content of A) ascorbate, B) zeaxanthin and C) lutein in the studied *Coffea* spp. genotypes under the imposed experimental conditions. Each value represents the mean ± SE (n=6-8).

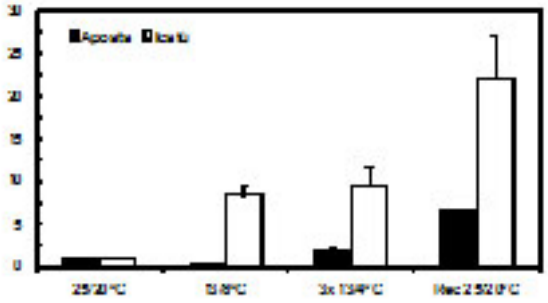

Figure 3. Real-Time PCR expression studies of chitinases class III, *Cachi3-2* in the studied *Coffea* spp. genotypes under the imposed experimental conditions. Each value represents the mean ± SE (n=4-8), from 3 independent biological assays.

ACKNOWLEDGEMENTS

This work was supported by FCT, through the projects REEQ/374/BIO/2005, POCTI/AGG/43101/2001 and PTDC/AGR-AAM/64078/2006 (partially financed by the European Fund FEDER) and by the grants SFRH/BPD/21884/2005 (A. Fortunato) and SFRH/18361/2004 (P. Batista-Santos), co-financed by the Portuguese PIDDAC program and European Social Fund, under the 3rd framework program. Conference attendance was co-financed by ASIC and Delta-Cafés (Portugal).

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Participatory Breeding for Improved Arabica Coffee Hybrids in Tanzania

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SUMMARY

A study carried out from 2004 to 2005 on participatory breeding for improved Arabica coffee hybrids in Tanzania revealed that for successful breeding programmes and ultimate adoption of research results it is important to involve farmers. From interactions with 192 coffee farmers; 144 males and 44 females in the Northern and Southern highlands coffee growing areas it was found that, farmers have specific criteria to select coffee clones. Criteria for evaluating the varieties included; good taste, drought tolerance, leafiness, big bean size, fast growth, high yield [big clusters with many berries at short internodes], disease resistance and insect-pest tolerance. Analysis of their selection criteria ranked high yielding variety as the most important selection criteria. Tools for evaluation of the clones were preference ranking, matrix and pair-wise ranking of the coffee varieties. Matrix as well as pair wise ranked coffee hybrids; KP423-1, N39-5, N39-2, N39-7, KP423-2, KP423-3, N39-6, N39-4, N39-3 and N39-1, higher than commercial varieties N39 and KP423. Farmers described N39 and KP423 to be susceptible to CBD and CLR, and of unstable yields. Therefore participatory breeding ensures that the best coffee clones are selected and adopted by growers.

INTRODUCTION

Programme to improve Arabica coffee in Tanzania started in 1930's. Trials were carried out to evaluate collection of varieties basing on yields, weight and size of beans, and cup quality. By 1940s varieties N. 39, KP 162 & 423 and H. 66 were selected and distributed to coffee growers. But all these varieties are susceptible to Coffee Berry Disease (CBD) incited by *Colletotrichum kahawae* Sp. Nov (Waller and Bridge) and Coffee Leaf Rust (CLR) caused by *Hemileia vastatrix* (Bert et Br). Efforts to identify improved Arabica clones resistant to CBD and CLR was initiated in 1950s and strengthened in early 1960s following the outbreak of CBD. By 2000 10 clones were established in on-farm trials in 25 sites throughout coffee growing areas. The aim was to involve farmers in the selection process to identify suitable coffee clones for growers and to local conditions. Therefore in 2004 and 2005 coffee research involved 192 farmers to get opinion on the performance of coffee hybrids (Lyimo and Sulumo, 2005; Lyimo and Owenya, 2004). The evaluation was of importance because new technologies like coffee hybrids which need to be part of the local society, its ecological and physical environment, its cultural experience and its socio-economic structure need to be evaluated thoroughly. The aim of this paper is to outline usefulness of participatory approaches in evaluation and selection of improved Arabica coffee hybrids in Tanzania.

MATERIALS AND METHODS

Tools used in the evaluation were preference and pair-wise comparison. Criteria for preference evaluation were disease tolerance, high yielding variety, big bean size, drought

tolerance, leafiness, fast growth and insect-pest tolerance. In matrix ranking, criteria raised by farmers were compared to coffee varieties and value assigned between 5 (Excellent), 4 (Good), 3 (Average) 2 (Satisfactory) and 1 (Poor). For pair wise ranking coffee varieties were listed in paired and farmers selected their preference and the reason for selection. Each coffee variety was counted for its frequency and ranked according to its total scores.

RESULTS AND DISCUSSION

Farmers' criteria for selecting coffee varieties

The evaluation was carried out on mature bearing trees when the crop was to a greater extent at a ripening stage. Farmer's criteria in the order of importance were high yielding variety, good cup taste, big bean size, drought tolerance, fast growth, leafiness, disease and insect-pest tolerance.

Use of matrix ranking in ranking the varieties and selected criteria

Matrix ranking revealed that good cup taste, drought tolerance, leafiness, big bean size, fast growth and high yield are the most preferred (Table 1 and 2). Coffee N39-1, N39-2, N39-3, KP423-1, and N39-4, ranked higher than N39 and KP423, because of bigger berry clusters, and able to endure drought. KP 423 was ranked higher than N39 because of higher yield and robustness.

Table 1. Matrix ranking of coffee varieties and criteria selected by farmers.

Criteria	Coffee varieties							Total	Rank
	N39-1	N39-2	N39-3	KP423-1	N39-4	N39	KP423		
Disease tolerance	5	5	5	5	5	1	3	29	4
High yield	5	5	5	5	5	2	4	31	3
Good cup taste	5	5	5	5	5	5	5	35	1
Big bean size	5	5	5	5	5	3	5	33	2
Drought tolerance	5	5	5	5	5	5	5	35	1
Leafiness	5	5	5	5	5	5	5	35	1
Fast growth	5	5	5	5	5	3	5	33	2
Insect-pest tolerance	3	3	3	3	3	1	2	18	5
Total	38	38	38	38	38	25	34		
Rank varieties	1	1	1	1	1	3	2		

Key for scores: 1-Poor; 2-Satisfactiry; 3-Average; 4-Good; 5-Excellent.

Coffee varieties N39-5, N39-6, N39-7, KP423-2, and KP423-3 ranked higher than N39 and KP423 (Table 2). Similarly KP 423 was ranked higher than N39 because of higher yield and robustness. Drought tolerance, leafiness and good cup taste ranked top position.

Table 2. Matrix ranking of coffee varieties and criteria selected by farmers.

Criteria	Coffee varieties							Total	Rank
	N39-5	N39-6	N39-7	KP423-2	KP423-3	N39	KP423		
Disease tolerance	5	5	5	5	5	1	2	28	5
High yield	5	5	5	5	5	2	3	30	4
Good cup taste	5	5	5	5	5	5	5	35	1
Big bean size	5	5	5	5	5	3	5	33	2
Drought tolerance	5	5	5	5	5	5	5	35	1
Leafiness	5	5	5	5	5	5	5	35	1
Fast growth	5	5	5	5	5	3	4	32	3
Insect-pest tolerance	3	3	3	3	3	2	2	19	6
Total	38	38	38	38	38	26	31		
Rank	1	1	1	1	1	3	2		

Key for scores: 1-Poor; 2-Satisfactiry; 3-Average; 4-Good; 5-Excellent.

Use of pair wise comparison/ranking of the varieties

Coffee varieties were paired for comparison, and selection of each variety in pair based on farmers preference. The results are presented in Tables 3 and 4.

Table 3. Pair wise ranking of coffee varieties.

	N39-1	N39-2	N39-3	KP423-1	KP423	N39	N39-4	Total	Rank
N39-1		N39-2	N39-3	KP423-1	N39-1	N39-1	N39-4	2	5
N39-2			N39-2	KP423-1	N39-2	N39-2	N39-2	5	2
N39-3				KP423-1	N39-3	N39-3	N39-4	3	4
KP423-1					KP423-1	KP423-1	KP423-1	6	1
KP423						KP423	N39-4	1	6
N39							N39-4	0	7
N39-4								4	3

Table 4. Pair wise ranking of coffee varieties.

	N39-5	N39-6	N39-7	KP423-2	KP423	KP423-3	Total	Rank
N39-5		N39-5	N39-5	N39-5	N39-5	N39-5	5	1
N39-6			N39-7	KP423-2	N39-6	KP423-3	1	3
N39-7				N39-7	N39-7	KP423-3	3	2
KP423-2					KP423-2	KP423-2	3	2
KP423						KP423-3	0	4
KP423-3							3	2

Pair wise ranking of coffee varieties in Tables 3 and 4 shows that varieties KP423-1, N39-5, N39-2, N39-7, KP423-2, KP423-3, N39-6, N39-4, N39-3 and N39-1 ranked higher than commercial varieties KP423 and N39. Selections of the varieties were linked to high yielding, absence of CBD and CLR symptoms and health green berries. Participatory approach has been used very effectively in crops such as finger millet (Gowda et al., 2000) and barley (Ceccarelli et al., 1997) to facilitate not only in selection but also in variety adoption. Similarly in this study participatory approach has been shown to be effective in selecting and ranking the best coffee varieties, as well as in adopting them.

It should be noted also that disease resistance *per se*, was not a major selection criterion to the farmers, but criteria such as clones with many and big clusters and high yield was preferred. This shows that farmers' main concern is yield and avoidance of crop failure. Aikpokpodion et al. (2003) observed a similar experience when conducting farmer-researcher participatory on farm selection of improved cocoa varieties in Nigeria. However when farmers ranked drought-tolerance on top of high yielding clones (Table 1 and 2), it shows the importance of searching for drought-tolerance clones by coffee breeders. Farmers' participation in this study assisted to rank varieties KP432-1, N39-5, N39-2, N39-7, KP423-2, KP423-3, N39-6, N39-4, N39-3 and N39-1 higher than commercial varieties N39 and KP423 (Table 1 to 4). Socio-economic studies done recently on the adoption of new varieties indicated that new varieties are cost-effective (Chimilila et al., 2008). It is to the expectation of the coffee breeding programme that the new varieties will contribute to increasing productivity and improve incomes of smallholders.

CONCLUSION

Participatory breeding entailing farmer-researchers ensures sustainable coffee production adapted to farmer-managed situations. Farmers should be considered as true partners and involved in all stages of selection process. Farmers' participation in this study assisted to rank varieties KP432-1, N39-5, N39-2, N39-7, KP423-2, KP423-3, N39-6, N39-4, N39-3 and N39-1 higher than commercial varieties N39 and KP423.

ACKNOWLEDGEMENT

We thank European Union (EU) and Coffee Stakeholders' for the financial support of this study. The commitments and contributions of the farmers we visited went far beyond our expectations and made this work possible. We also thank Selian Agricultural Research

Institute to allow Mr. S. D. Lyimo, Mr. P. F. Sulumo and Mrs. M. Z. Owenya for their expertise in probing farmers' preferences and documenting the reports.

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Progress in Accelerated Multiplication and Distribution of Seedlings of Improved Hybrid Coffee Varieties in Tanzania

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SUMMARY

One of the major achievements of the Tanzania Coffee Research Institute (TaCRI) is the release of hybrid coffee varieties that combine resistant to Coffee Berry Diseases (CBD) and Coffee Leaf Rust (CLR), high yielding and good cup quality. However, the accelerated multiplication and distribution of the new hybrids to coffee growers pose a considerable challenge in order to maintain their genetic stability. TaCRI has already perfected two methods, namely clonal propagation and grafting which are currently used by coffee growers to accelerate the multiplication of hybrid seedlings of coffee varieties in all coffee growing zones. The other two methods of tissue culture or somatic embryogenesis and hybrid seed production are still under research and their research progress is encouraging.

This paper focuses on sustainable and cost effective clonal propagation method that farmers have been using to achieve massive multiplication of clonal seedlings of improved hybrid coffee varieties in Tanzania.

INTRODUCTION

Coffee can be propagated conventionally by seeds or vegetatively using cuttings. Vegetative propagation of coffee by cuttings guarantees uniformity as it maintains the genetic make-up of the mother plants which are usually heterozygous (Rehm and Espig, 1991; Juma et al., 1994; Nzallawahe et al., 2004; Beyl and Trigiano, 2008). In such plants, vegetative propagation ensures that the characteristics a breeder has achieved are maintained without further selection, while the variety is being multiplied vegetatively; and the progenies arising from plants propagated by clonal materials are exactly "true – to – type". For grafting method, the scions of hybrid varieties are grafted on root stocks of traditional coffee varieties (TaCRI, 2008 and 2009).

In recent years, technological advancement has also discovered the application of tissue culture techniques in clonal multiplication. These methods are being studied all over the world to improve the performance of the crop species (Benyl and Trigiano, 1981). The application of tissue culture techniques such as somatic embryogenesis permits massive production of uniform and identical plants to the mother plants (Chaleff, 1981; Beyl and Trigiano, 2008). This method, however, has several limitations including high investment in terms of cost and time in the development and application of this technology (Chaleff, 1981).

One of the major achievements of the Tanzania Coffee Research Institute (TaCRI) is the release of hybrid coffee varieties that combine resistant to Coffee Berry Diseases (CBD) and Coffee Leaf Rust (CLR), high yielding and good cup quality. However, the accelerated

multiplication and distribution of the new hybrids to coffee growers pose a considerable challenge in order to maintain their genetic stability.

TaCRI has already perfected two methods, namely clonal propagation and grafting which are currently used by coffee growers to accelerate the multiplication of hybrid seedlings of coffee varieties in Tanzania; and research is going on to explore the use of other two methods, namely somatic embryogenesis and hybrid seed production.

This paper focuses on sustainable and cost effective clonal propagation method that farmers have been using to achieve massive multiplication of clonal seedlings of improved hybrid coffee varieties in Tanzania.

METHODOLOGY

Structure of stakeholders involved in hybrid seedlings multiplication

TaCRI has supported the establishment of primary, secondary, and tertiary nurseries (Figure 1) in all coffee growing zones. The nurseries are served by TaCRI through its sub-stations. In each of the sub-stations, TaCRI is working with farmer groups, district councils, coffee co-operatives, estates and individual coffee farmers.

Primary nurseries

These are TaCRI managed nurseries established in each coffee growing zone. They are currently serving as primary nurseries for multiplication of hybrid seedlings for secondary, tertiary, co-operatives and individual nurseries. Their mother gardens are under intensive with sprinklers and overhead irrigation facilities installed. Under good management, each mother plants will produce 50-100 seedlings annually.

Secondary nurseries

District nurseries are managed by District Agricultural and Livestock Development Officers (DALDOs) and a few non-governmental organizations (NGOs). TaCRI is assisting them through capacity building, technical backstopping and provision of free seedlings. Seedlings produced from these nurseries are distributed to tertiary nurseries and some are sold to farmers are at a subsidized price.

Secondary nurseries (Coffee estates and co-operatives)

These are nurseries owned by large scale producers or coffee association and seedlings activities are carried out by skilled staff. Seedlings produced from these sources are for planting in their farms, distributed to their members (primary societies) and some are passed to neighbour coffee growers

Tertiary nurseries

Tertiary nurseries are managed by farmer groups and individual farmers. The sizes of the plots for planting clonal mother gardens vary from 200 to 1,000 mother plants due to limited land. The groups contain 25-30 farmers. TaCRI in collaboration with district extension officers, provide free inputs for use in the clonal mother gardens, regular training and technical backstopping to strengthen these groups.

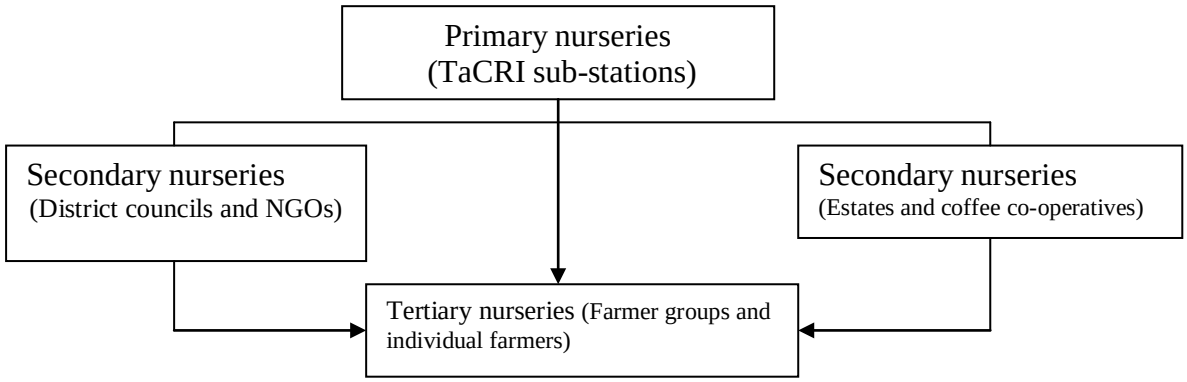

Figure 1. Structure of stakeholders involved in hybrid seedlings multiplication.

Support to secondary, tertiary, estates and co-operatives nurseries

Secondary, tertiary and other stakeholders are being improved through provision of free seedlings and inputs for use in their mother gardens as well as construction of simple propagation structures for nursing seedlings of improved coffee varieties. This provision is being supported by other donors and the government of Tanzania through the annual budget for subsidy that is given to TaCRI to accelerate multiplication of the new hybrid coffee varieties in an effort to speed up the rejuvenation of the sub-sector, improving the livelihoods of the growers and hence playing a positive role in the national poverty reduction strategies.

The process of hybrid seedlings multiplication by clonal propagation method

Figure 2. Clonal mother garden with single stem (a); clonal mother garden with multiple stems (b); harvesting shoots (2); preparation of cuttings for planting (3); half node cutting (4a);single node cutting (4b); three nodes cuttings (4c); planting of cuttings into propagation boxes(5); propagation boxes covered with polythene sheets under shade (6); rooted cuttings from propagation boxes (7); potted cuttings (8); Hardened seedlings for distribution (9) and seedling ready for planting (10).

RESULTS AND DISCUSSION

Number of farmer groups, district councils and coffee co-operatives

A total of 208, 411 coffee growers (80% males and 20% females) have been trained and currently TaCRI is working with over 846 framer groups of 25-30 members, 85 coffee co-operatives and primary societies and 100 individual coffee growers scattered in 33 coffee growing districts. These groups have been trained on seedlings multiplication along with the provision of free planting materials for planting in their mother gardens.

There are over 800 farmer groups with clonal mother gardens with 300,000 mother plants which are used as sources of cuttings for hybrid seedlings multiplication. Seedlings produced by farmer groups are divided among the members of the groups and the excess are sold at the agreed price set by the members.

Progress on multiplication and distribution of hybrid seedlings

Since TaCRI inception in 2001, the production of hybrid seedlings has been on an exponential increase (Figure 3). A total of 32,268,991 seedlings have been multiplied and distributed to various coffee growers and a large part of seedlings is produced by farmer groups. This increase is the result of increased demand for hybrid seedlings by coffee growers and cost effective technologies that are currently used for multiplication of seedlings which do not require heavy investment.

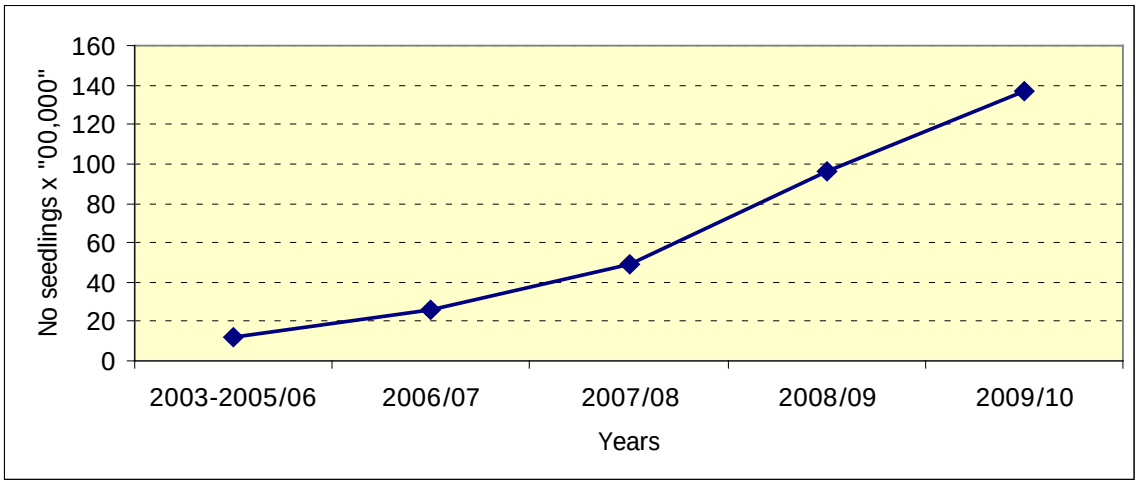

Figure 3. Progress on multiplication and distribution of planting materials of improved coffee varieties, 2002/03-2009/10 across coffee growing regions in Tanzania.

CONCLUSION

Clonal propagation is a viable, successful and easily adopted method by farmers for rapid multiplication of clonal seedlings. Tanzania is one of the few countries that has used this method through farmer groups and achieve a massive hybrid seedlings multiplication on a commercial scale. Therefore, sustainable technology development is a key to successful technology adoption.

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Diversity of *Coffea canephora* in the Tanzania Germplasm Collection

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SUMMARY

Diversification analysis is of importance in order to have identity of the accessions for protection, and the extent to which a crop can be improved through selection process. Diversity analysis of 10 out of 656 *Coffea canephora* accessions was conducted in Robusta germplasm at Maruku Kagera in 2009. The germplasm contains accessions selected from farmers' fields in Bukoba, Karagwe, Misenyi, Muleba and Ngara Districts of Kagera region and established at Maruku in 1998. Descriptors for coffee developed by the International Plant Genetic Resources Institute (IPGRI) were used to diversify the accessions. The IPGRI descriptor uses phenotypic features like vegetative plant parts, fruits and inflorescence to assign distinctive characteristic(s) to accession. Results showed distinctive characteristics of the accessions in the leaf petiole colour, young shoot colour, mature leaf colour, bud wax colour, bud wax thickness, calyx limb persistence, leaf width and leaf length, leaf apex shape, fruit colour, fruit disc shape, ripe fruit colour, and fruit shape. These results suggest that there are accessions within farmer's fields and in the germplasm established at Maruku having genetic variability which can be used to improve the *C. canephora* breeding programme, and in case one of the accessions qualifies for commercial release distinctive features can assist in protecting the variety.

INTRODUCTION

Robusta coffee, *Coffea canophera* is one of important cash crop grown by more than 250,000 families in Kagera region in the western part of Tanzania ((TaCRI 2006). It accounts 30-50% of coffee production in the country and contributes to 20-25% of the national foreign exchange from coffee sales (TaCRI, 2008). Despites the importance of robusta coffee in the country, the available commercial varieties are of inferior type especially on disease resistance to coffee wilt disease (CWD) which causes serious yields and monetary losses to farmers in Kagera (CABI, 2003; TaCRI, 2003). Therefore characterization of *C. canephora* accessions within the germplasm forms an important step to determine genetic diversity for improvement of the existing commercial varieties through crossing programme or selection of the best accessions for breeding advancement.

There are several approaches to characterize crop varieties including molecular markers which provide information on phylogenic relationships, and diversity of crop population (Musoli et al., 2008; Anthony et al., 2002). However use of phenotypic features is also useful in characterizing genetic diversity within a crop plant. For example in genetics of *Coffea arabica* it is commonly known that *typical* usually posses dark bronze young leaves and that the green colour of young leaves is very common among *bourbon* plants (Filho and Carvalho,

1988). Therefore it is very easily to discriminate typical from bourbon when the plants are in the field. This can serve in breeding programme when selecting and making advancement for improved varieties. It is for this reason that *Coffea canephora* accessions established at Maruku were characterized using phenotypic features to facilitate selection and advancement in Robusta improvement programme particularly in the effort to encounter CWD problem.

MATERIALS AND METHODS

Accessions characterized were 1/62, 13/61, KR 23, BK 27, NG20, NG 10, MR 10, ML 2, MS2 and MS3. Characters studied were plant habit, plant height, overall appearance, vegetative development, branching habit, angle of insertion of primary branches, stipule shape, stipule aristal length, young leaf colour, leaf shape, leaf apex shape, leaf length, leaf width, leaf petiole length, leaf petiole colour, young shoot colour, mature leaf colour, venation pattern bud wax colour, bud wax thickness, number of days from rainfall to flowering, inflorescence position, inflorescence on old wood, number of flowers per axil, number of flowers per fascicles, number of fascicles per node, inflorescence stalk length, corolla tube length, number of petals per flower, anthers insertion, number of stamens per flowers, fruit colour, fruit shape, absence/presence of fruit ribs, fruit disc shape, calyx limb persistence, fruit length, fruit width, fruit thickness, pulp thickness, harvest duration, seed length, seed width, seed thickness, seed colour, seed shape and ripe fruit colour. The procedure for the measurement of these characters was based on adapted IPGRI standard coffee descriptors (IPGR, 1996). Accessions and characters under study where presented in a matrix form, and distinctive characters identified per each accession.

RESULTS AND DISCUSSION

Table 1 shows characterized accessions of *C. canephora* and their distinctive characteristics. The distinctive characters included leaf petiole colour, young shoot colour, mature leaf colour, bud wax colour, bud wax thickness, calyx limb persistence, leaf width and leaf length, leaf apex shape, fruit colour, fruit disc shape, ripe fruit colour, and fruit shape.

Diversity in accessions was noticed in the vegetative parts, inflorescence and flowering, fruits colour and shape, and colour of the ripe cherry (Table 1). It is also interesting to note differences in the bud wax colour and their thickness, and colour of the young shoot. Filho and Calvalho (1988) found differences within *C. arabica* varieties in the colour of the young leaves. Accession MR10 was found consistently with very peculiar characteristic of having very prominent fruit disc shape (Table 1). This description agreed with distinct characteristics of some of *C. liberica* as outlined by Wellman (1961). ML2 was found with a peculiar character of having purple colour of its cherries. Cherries appearing yellow, pink, and orange were noticed within *C. arabica* varieties in El Salvador (Anon, 2010). The colour of the cherries was reported to be associated with *xanthocarpa* gene which usually rules the colour of the fruit. The diversity noticed by characterizing only a portion of *C. canephora* accessions at Maruku basing on phenotypic characterization suggest that there are genetic variability. This allows for a more detailed study of the accessions on genetic diversity using molecular markers such as inter simple sequence repeat (ISSR), simple sequence repeats (SSRs) and amplified fragment length polymorphism (AFLP). For example Ruas et al. (2003) successfully used inter simple sequence repeat (ISSR) markers for genetic differentiation of *Coffea* species. Application of these methods can be used to shorten selection of the best breeding lines in the programme to develop CWD resistant varieties.

Table 1. Distinctive characteristics of six *Coffea canephora* accessions.

Accession	Plant part	Description	Remarks
KR23	Plant height	Very short	Distinct
	Leaf petiole colour	Dark green	Distinct
	Young shoot colour	Dark green	Distinct
	Mature leaf colour	Dark green	Distinct
	Bud wax colour	Orange	Distinct
	Bud wax thickness	Thick	Distinct
	Calyx limb persistence	Persistence	Distinct
1/62	Leaf width (mm)	Very wide	Distinct
BK27	Leaf length (mm)	Very long	Distinct
13/61	Leaf width (mm)	Very narrow	Distinct
	Fruit colour	Green	Distinct
NG10	Bud wax colour	Cream	Distinct
	Bud wax thickness	Thin	Distinct
NG20	Leaf petiole length (mm)	Very short	Distinct
MR10	Fruit disc shape	Very prominent	Distinct
ML2	Ripe fruit colour	Purple	Distinct
MS3	Bud wax colour	Yellow	Distinct
MS5	Fruit shape	Obovate	Distinct

CONCLUSION

- This study shows that within accession established at Maruku there is genetic diversity. The diversity was noticed in examining vegetative plant parts, inflorescence and flowering, fruits colour and shapes, and colour of the cherries.
- Some of the distinctive characteristics found in the accessions under study were also quantified in *C. arabica* and *C. liberica*, therefore urge for a more detailed study of the accessions using molecular makers.
- The study should be extended to characterize remaining 646 accessions using phenotypic description and thereafter use of molecular markers.

ACKNOWLEDGEMENT

The authors acknowledge the permission of the Chief Executive Director, TaCRI to publish this paper. Also the participation of support staff Mrs. Elinasoe Mosha in this study.

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Multiplication of Tanzanian Coffee Arabica Hybrids and Robusta Clones by Somatic Embryogenesis

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SUMMARY

A series of experiments involving Tanzanian *Coffea arabica* hybrids and Robusta clones were carried out at Nestlé R&D Centre-Tours (France) from December 2008 to November 2009.

Nine Tanzanian genotypes (four *C. arabica* hybrids and five *C. canephora* clones) were tested for their potential for multiplication by somaticembryogenesis. The callogenesis procedure used, followed the one commonly used at the Centre. The disinfection were more efficient when the primary explants came from rooted cuttings acclimatized in the NR&DC-T greenhouse rather than from leaves coming directly from the field. High frequency somatic embryogenesis was observed for 8 of the genotypes and the embryogenic calluses were successfully multiplied. Five to six months after disinfection, embryogenic calluses were particularly abundant for two Robusta (G1/62 and NG20) and one Arabica (N39-6). All the tested genotypes showed high potential for multiplication by use of somatic embryogenesis. The somatic embryos developed were transferred to Tanzania at different stages of development, successfully harvested, hardened and potted ready for field planting.

MATERIALS

Leaf samples from four *C. arabica* hybrids N39-3, N39-5, N36-6 and KP423-1 and five *C. canephora* clones ML2, MR10, NG20, BK27 and 1/62 were used in this experiment. Two types of samples were used.

The first type was leaf samples from these genotypes straight from the field which were received for *C. arabica* hybrids and *C. canephora* from Tanzania while the second type was leaf samples derived from the rooted cuttings which were received from Tanzania and raised in the Centre's quarantine facility.

For the field samples, callogenesis was initiated in January and March 2009 for *C. arabica* and *C. canephora* respectively. For the rooted cuttings callogenesis was initiated in April and June 2009 for *C. arabica* and *C. canephora* respectively.

METHODS

Field received leaf samples, was first checked for their phytosanitary condition before they were washed with tap water then dipped for 30 seconds in a 70% solution of ethanol. The leaves were surface sterilized for 30 minutes in a solution of Calcium hypochlorite ($CaCl_2O_2$) at 40 g/l and rinsed three times in sterile water. The leaves were cut in small explants (3x5 mm) avoiding mid veins, margins, apical and basal portions. A glass beads sterilizer at 250°C

and hot flame were used frequently to sterilize the tools during the manipulation. Fifteen explants were plated per Petri dish (diameter 10 cm), containing 40 ml of Pierson (Pierson et al., 1983) media and M15A8 (Murashige and Skoog, 1962) media for *C. arabica.* For *C. canephora* clones, they were plated on 23A8 (Yasuda et al., 1985) and M15A8 media respectively. The upper epidermis was in contact with the medium. The explants were incubated in darkness at 25±1 °C for 1-2 months after which they were sub cultured on fresh media of the same protocol. Then after two sub cultures, the media was changed to 23A15 and cultures were placed in the same conditions. During sub culture, the aqueous callus and contaminated Petri dishes were discarded. Evaluation was done at 10 and 7 months for *C. arabica* and *C. canephora* respectively. In the case of the quarantine raised rooted cuttings, young fully expanded leaves were collected, washed with tap water then dipped for 30 seconds in a 70% solution of ethanol. The leaves were surface sterilized for 25-30 minutes in a solution of Calcium hypochlorite ($CaCl_2O_2$) at 40 g/l depending on the tenderness of the leaves. Then they were rinsed three times in sterile water and cut in small explants (3x5 mm) avoiding mid veins, margins, apical and basal portions. A glass beads sterilizer at 250 °C and hot flame were used frequently to sterilize the tools during the manipulation. Fifteen explants were plated per Petri dish (diameter 10 cm), containing 40 ml of T1B (Van Boxtel and Berthouly, 1996), Pierson and M15A8 media for *C. arabica* hybrids and 23A8 medium for *C. canephora*. The upper epidermis was in contact with the medium. The explants were incubated in darkness at 25 ±1 °C then; all the cultures were sub cultured on the fresh media of the same protocol 1 month after; except for the cultures on T1B which were sub cultured on T2B medium on the same conditions. During the sub culture the aqueous callus were removed from the explants and the contaminated Petri dishes were discarded accordingly. The performance evaluation was carried out after 7 and 4 months for *C. arabica* and *C. canephora* respectively.

RESULTS AND DISCUSSION

From Table 1, it is clearly shown that the Robusta samples from the field are highly characterized by the high level of contamination 64 % for protocol 23A8 and 83% for protocol M15A8. Genotypes performed differently on the different protocols both on HFSE and LFSE. Genotype 1/62 performed exceptionally better than other genotypes when considering toghether both protocols on the HFSE aspect while genotype MR10 had the best results on protocol M15A8. Although genotype NG20 did not perform so well on the aspect of HFSE on M15A8 protocol, it gave the best results on LFSE on protocol 23A8. The potential for production of embryogenic callus on protocol 23A8 ranged from 0-31% while on protocol M15A8 ranged from 0-15%. On production of direct embryo, protocol 23A8 gave the range of 10-65% while protocol M15A8 ranged from 0-26%.

Table 2 shows that 100% of material from the quarantine green house was uncontaminated. Both NG20 and G1/62 produced HFSE; 88.50% and 48.50% respectively. While BK27 never produced HFSE, had the highest percentage of LFSE, 78.50%. NG20 and G1/62 produced LFSE but at a lower percentage; 9.50% and 21.60% respectively.

Arabica genotypes tested clearly gave the same picture of high level of contamination for leaf samples direct from the field (Table 3). The clean explants on Pierson protocol indicate low potential for HFSE with only one genotype KP423-1 able to display HFSE of 4%. On the other hand, all the tested genotypes except N39-5 were able to give direct embryos (LFSE), but all were less than 14%.

Table 1. Callogenesis Robusta clones samples from the field 7 months after culture.

Genotype	Protocol 23A8-23A15							Protocol M15A8-23A15						
	A	B	C	D	%	E	%	A	B	C	D	%	E	%
MR10	100	88	12	2	2	10	10	100	77	33	7	7	26	26
NG20	100	35	65	0	0	65	65	100	100	0	0	0	0	0
BK27	100	79	21	0	0	21	21	100	82	8	1	1	7	7
ML2	100	77	23	2	2	21	21	100	96	4	2	2	2	2
G1/62	100	43	57	31	31	26	26	100	60	40	15	15	25	25
Total	500	316	178	35	35	143	143	500	415	85	25	25	60	60
Mean		64.4	35.6	7	7	28.6	28.6		83	17	5	5	12	12
%		64.4	35.6	7		28.6			83	17	5		12	

A: Initially cultured explants; B: Contaminated explants; C: Clean explants at the time of evaluation; D: Explants with embryogenic callus(HFSE); E: Explants with direct somatic embryos(LFSE).

Table 2. Performance of Tanzania Robusta from Quarantine after 5 months.

Genotype	Protocol 23A8- 23A15						
	A	B	C	D	%	E	%
NG20	324	0	324	287	88.5	31	9.5
BK27	340	0	340	0	0	267	78.5
G1/62	268	0	268	130	48.5	58	21.6
Total	932	0	932	417	137	356	109.6
Mean	310.7	0	310.7	139	45.7	118.6	36.5
%		0	100	44.8		38.2	

A: Initially cultured explants; B: Contaminated explants; C: Clean explants at the time of evaluation; D: Explants with embryogenic callus(HFSE) High frequency somatic embryogenesis; E: Explants with direct somatic embryos (LFSE) Low frequency somatic embryogenesis.

From Table 4 conspicuously we can observe the high percentage of clean Arabica explants (100%). Two protocols were compared; all the genotypes were able to produce both HFSE and LFSE. Protocol Pierson gave the response of 0-4.87% on HFSE while protocol Van Boxtel and Berthouly gave from 24.20-66.80% on the same aspect. For LFSE the two protocols gave the ranges of 7.76-22.40% and 2.62- 17.50% respectively. Genotype N39-6 gave the highest percentage of HFSE in both protocols 4.87% and 66.80% for Pierson and Van Boxtel and Berthouly respectively. On the other hand; genotype KP423-1 gave the highest percentage of LFSE on both protocols 22.40% and 17.5% respectively.

Generally all the tested genotypes displayed very good embryogenic potential especially on Van Boxtel and Berthouly protocol (24.2-66.8%).

Table 3. Arabica genotypes samples from the field 10 months after callogenesis.

Genotype	Protocol PIERSON A8-23A15						
	A	B	C	D	%	E	%
N39-3	100	88	12	0	0	12	12
N39-5	100	100	0	0	0	0	0
N39-6	100	86	14	0	0	14	14
KP423-1	100	94	6	4	4	2	2
Total	400	368	32	4	4	28	28
Mean		92	8	1	1	7	7
%		92	8	1		7	

A: Initially cultured explants; B: Contaminated explants; C: Clean explants at the time of evaluation; D: Explants with embryogenic callus(HFSE) High frequency somatic embryogenesis; E: Explants with direct somatic embryos (LFSE) Low frequency somatic embryogenesis.

Table 4. Callogenesis Arabica genotypes samples from quarantine after 7 months of culture.

Genotype	Protocol PIERSON							Protocol Van BOXTEL						
	A	B	C	D	%	E	%	A	B	C	D	%	E	%
N39-3	194	0	194	2	1.0	17	8.7	196	0	196	107	54.6	16	8.1
N39-5	219	0	219	0	0.0	17	7.7	196	0	196	50	25.5	29	14.8
N39-6	246	0	246	12	4.8	48	19.5	229	0	229	153	66.8	6	2.6
KP423-1	263	0	263	7	2.6	59	22.4	211	0	211	51	24.2	37	17.5
Total	922		922	19	8.4		58.3	832		832	361	171	88	51.1
Mean	231		231	4.7	2.1		14.6	208		208	90.3	42.8	22	12.8
%			100	2						100	43.4		10.6	

The embryos obtained from genotypes Arabica N39-6 and Robusta NG20 were filtered from the liquid multiplication media, dried on sterile Whatman filter papers and parked in sterile Petri dishes before they were transferred to Tanzania for further development. Currently the plantlets derived from this work are potted ready for field planting.

GENERAL CONCLUSION

- All the tested genotypes displayed very good response to produce embryogenic callus.
- There is a clear genotype difference in performance.
- It is very important to start with clean material from the greenhouse or if possible from in vitro collections.
- For the Arabica genotypes, Van Boxtel and Bethouly protocol seem to be the best.

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Characterization of *Coffea arabica* MADS-Box Gene Family

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SUMMARY

We have analyzed the Brazilian Coffee Expressed Sequence Tag database (CafEST) (Vieira et al., 2006) and searched for all possible orthologs of MADS-box genes in *Coffea arabica*. We have performed sequence comparisons and built filogenetic trees to determine the putative *Coffea* orthologs of most MIKC-class members of MADS-box proteins, including APETALA1 (AP1), APETALA3 (AP3), PISTILATA (PI) and AGAMOUS (AG).We have used RT-PCR and *in situ* hybridization techniques to study the expression patterns of the *C. arabica* MADS-box genes during development and found that many of them are expressed preferentially during reproductive development.

INTRODUCTION

The MADS-box gene family encodes transcription factors that acts as key regulators in many developmental stages in diverse organisms (Coen and Meyerowitz, 1991). In plants, these genes are involved with the determination of the identity of floral meristems and floral organs as well as with controlling flowering time and fruit development (Theissen, 2001). The differential expression patterns of MADS-box genes such as APETALA1 (AP1), APETALA3 (AP3), PISTILATA (PI) and AGAMOUS (AG) are the key regulators of the identity of floral organs in a diverse range of flowering plants (Theissen, 2001). Due to the economical importance of coffe fruits, the understanding of the molecular processes involved with flower and fruit development in *Coffea* are of major interest. The Brazilian Coffee Expressed Sequence Tag database (CafEST) (Vieira et al., 2006) has provided a large collection of EST sequences derived from a diverse range of tissues and cell types, thus allowing the search for putative *C. arabica* homologs of the MADS-box genes. The aim of our work was to exhaustively search for coffee MADS-box homologs in the CafEST database and investigate their expression patterns during development using RT-PCR and *in situ* hybridization techniques.

MATERIALS AND METHODS

Sequence analysis

The chromatograms obtained from Brazilian Coffee Expressed Sequence Tag database (CafEST) (Vieira et al., 2006) were processed using PHRED (Ewing et al., 1998) and consensus contigs representing the most complete sequences were obtained using CAP3 (Huang and Madan, 1998) algorithm available in the BioEdit software (Carlsbad, CA). Contig sequences were compared to public databases at NCBI using the BLAST algorithm (Altschul et al., 1998) to access putative gene identities. The presence of conserved protein motifs in the deduced protein sequences derived from the obtained sequences, was investigated using Pfam (Finn et al., 2008). Multiple sequence alignments of the obtained contig sequences and the

complete set of MADS-box protein sequences from *Arabidopsis* were performed using CLUSTALX (Thompson et al., 1994). Distance trees were obtained from neighbor-joining matrices (Saitou and Nei,, 1987), with Bootstrap calculated from 1000 replicates and visualized with TreeView (Page, 1996). Parsimony trees were obtained using hand-corrected sequence alignments with MEGA (www.megasoftware. net).

RT-PCR

After DNAse (Ambion) treatment, first-strand cDNA samples were obtained using the Superscript First Strand Synthesis kit (Invitrogen) with a 17mer oligo-dT primer, from total RNA from different *C. arabica* tissues. Samples were normalized using a pair of primers designed for a *C. arabica* actin homolog. RT-PCR was performed using normalized cDNA samples and the gene-specific primers under the following PCR conditions: initial denaturation at 94 °C for 3 min; 30 cycles of 94 °C for 40s; 60 °C for 40 s and 72 °C for 1 min. The obtained fragments were migrated together with a molecular marker in a 1 % agarose gel, visualized under UV light and documented.

In situ hybridization

The *in situ* hybridization technique was basically as described by Dornelas et al. (2000) with the following adaptations: paraffin was removed from sections by two consecutive 5min washes in xylene followed by 5 min washes in 3:1, 1:1, 1:3 (v/v) ethanol:xylene. The slides were then briefly rinsed in ethanol and air-dried. After they were dried, the slides were individually treated with Proteinase K solution (10 µg.mL^{-1} in Tris-HCl pH 7.5) at 37 °C for 10 min. After the proteinase treatment, the slides were washed twice with 0.01 M Tris-HCl pH 7.5. A hybridization mix containing 50% deionized formamide; 0.1 M Tris-HCl pH 7.5; 0.05 M NaCl; 0.01 M EDTA; 100 ng.mL^{-1} yeast tRNA and 10% sodium dextransulphate was used to dilute 500 ng.mL^{-1} of DIG-labeled antisense or sense (control) RNA probes (the entire cDNA clone was used as probe template). Probe labeling was according to the labeling kit manufacturer (Roche) instructions. Hybridization was carried out at 42 °C for 16 h. The excess non-hybridized probe was washed out during four rinses (20 min each at 42 °C), the first two rinses were with 4 ×SSC followed by two rinses with 2 ×SSC. The slides were then rinsed twice with DB1 buffer (0.01 M Tris-HCl pH 7.5) at room temperature, and kept in a blocking buffer (2% w/v Blocking Agent, Roche, in 0.01 M Tris-HCl pH 7.5) for 8 min at 37 °C. An alkaline phosphatase-conjugated anti-DIG antibody (Fab-fragments, Roche) was added (1:2000 dilution) and the samples were kept at 37 °C for an additional hour. The slides were then rinsed twice with DB1 buffer and maintained for 10 min in DB3buffer (0.01 M $MgCl2.6H_2O$; 0.01 M Tris-HCl pH 9.0) at room temperature. The DB3 buffer was then replaced by a phosphatase substrate (NBT/BCIP plus suppressor, Pierce, USA) and the slides were kept overnight at room temperature in the dark. The staining reaction was stopped by two rinses with DB4 buffer (0.05 M EDTA; 0.01 M Tris-HCl pH 8.0). The hybridization signal was observed under a Zeiss Axioskope 50 microscope model equipped with a Zeiss AxioCam HRc digital camera.

RESULTS AND DISCUSSION

The hundreds of reads obtained from the BLAST search could be organized in 25 clusters. From these, 19 were non-redundant and could be compared to their *Arabidopsis* counterparts after additional sequencing of their respective clones to complete their open reading frames.

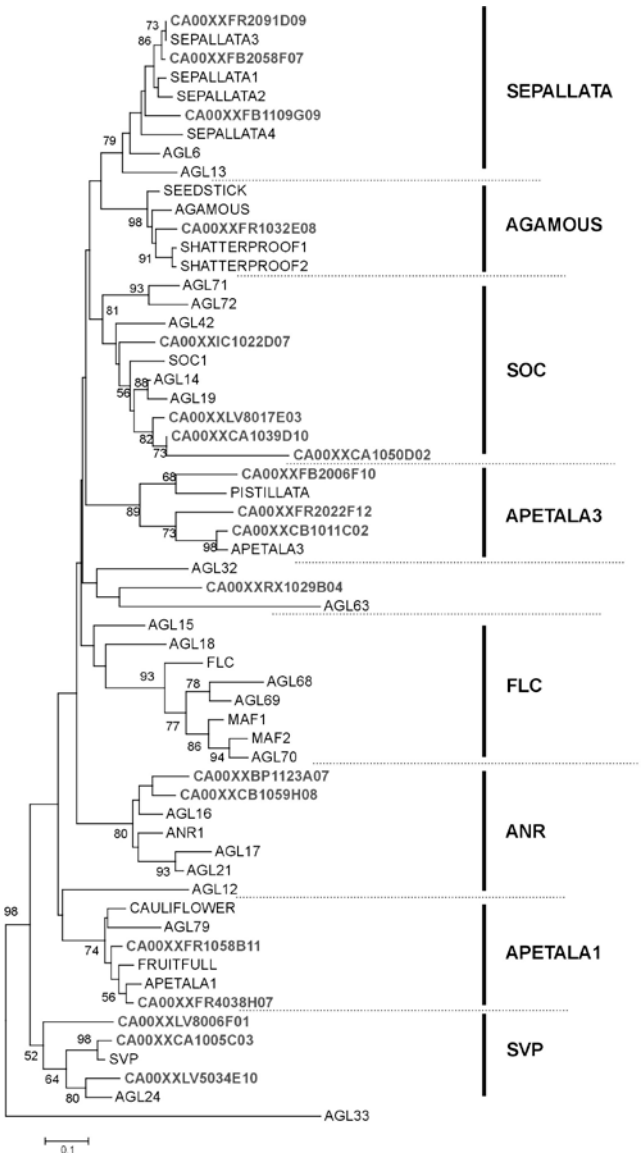

Figure 1. Comparative analysis of *C. arabica* MADS homologs belonging to the MIKC subfamily and their *Arabidopsis* counterparts. Only Bootsrap values above 75% are shown. The known functional groups are shown on the right-hand side of the figure.

The alignment of the MADS motif, translated from of all the obtained *Coffea* sequences, with the *Arabidopsis* MADS homologs showed a high degree of sequence similarity among the *Coffea* and *Arabidopsis* proteins. A cladogram built from distance matrices obtained from the sequence alignment showed that most of the coffee sequences belonged to the type II MADS proteins and were placed among the MIKC-class (Figure 1). We have obtained *Coffea* homologs for all the known functional groups within the MIKC-subfamily. The genes belonging to this MADS subfamily are known to be key controllers of development (Coen and Meyerowitz,, 1991). As the differential expression patterns of MADS-box genes are associated to their function, we performed RT-PCR to investigate the MADS genes transcript distribution in different *Coffea* tissues. Among the results obtained, we have observed a preferential accumulation of transcripts in reproductive tissues for the well-known ¨ABC model¨ genes (Theissen, 2001), with special emphasis on AP1, PI and AG (Figure 2). We have named the *C. arabica* homologs *CaAP1*, *CaPI* and *CaAG*, respectively. The *in situ* hybridization results corroborated the potential roles for these coffee MADS homologs in controlling early floral development.

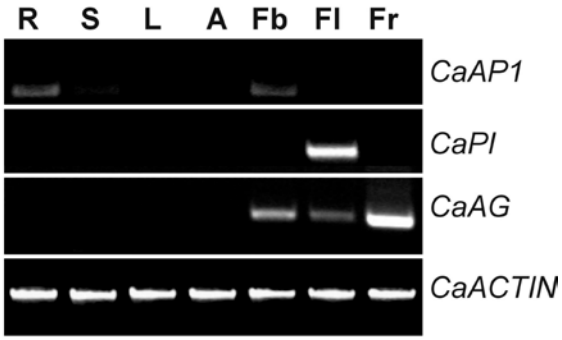

Figure 2. Expression analysis of *C. arabica* MADS homologs based on RT-PCR. CaAP1: *C. arabica* homolog of APETALA1; CaPI: *C. arabica* homolog of PISTILLATA; CaAG: *C. arabica* homolog of AGAMOUS; CaACTIN: *C. arabica* homolog of ACTIN (control). R: root; S: vegetative shoot; L: leaves; A: vegetative apex; Fb: Floral bud; Fl: flower; Fr: Immature fruits.

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Mapping Quantitative Trait Loci (QTLs) for Somatic Embryogenesis Ability and Yield Components in Robusta Coffee (*Coffea canephora*)

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SUMMARY

Traditional plant breeding methods have made a significant contribution to Robusta coffee improvement, but they have been slow in targeting complex traits like yield, green bean quality and plant propagation. Moreover, superior clones of Robusta coffee are commonly long to be released to smallholders due to several technical constraints in vegetative propagation.

Biotechnology brings new tools to plant breeders which can assist them to increase the coffee production imposed by land competition with other crops. In this regards, QTL analysis have been successfully introduced to analyse complex quantitative traits like coffee somatic embryogenesis ability or yield components determinism.

A set of three Robusta progenies, growing in two locations in Indonesia, was used for the QTL study related to agronomic traits of interest. A shared set of molecular markers based on SSRs allowed the definition of a genetic framework for the quantitative analysis of direct somatic embryogenesis (DSE) ability, the yield and the weight of green bean in Robusta coffee.

One QTL associated with the frequency of somatic embryogenesis (SE) was located on the linkage group G.Two QTLs linked to the green coffee yield were identified on the linkage groups C and H and one QTL for the weight of green bean was also identified on the linkage groups H.

However, the two QTLs for these two green bean characteristics are located on two different genome areas of the linkage group H. BP409 parental clone carried favourable alleles for DSE, green bean yield and the weight of green bean, although Q121 carried only favourable alleles for the green bean yield. Identification of QTLs provides valuable tool to improve the selection of genotypes with higher yield, better green bean characteristic and higher SE ability.

INTRODUCTION

Depending on the genotype, coffee yield show a wide variation however favourable growing condition reduces the differences between genotypes (Eskes and Leroy, 2004**)**. Somatic embryogenesis (SE) ability has been considered as an important aspect for the distribution of

improved clones to farmers. Due to that, the selection for yield in coffee breeding could be compromised by a low SE ability.

The use of molecular markers for quantitative trait loci (QTLs) analysis has provided an effective approach to dissect complex quantitative traits into component loci to study their relative effects on the trait (Doerge, 2002). Furthermore, knowledge regarding the number, genomic location, and effect of QTL should facilitate marker-assisted selection and the development of clone with desirable characteristic.

Although QTL analysis is one of an important technique to guide and speed up coffee breeding, few data about QTLs in *Coffea* sp have been published such as: QTL linked to trigonelline bean content (Ky et al., 2001), pollen viability (Coulibaly et al., 2003), period of cherry maturation (Akaffou et al., 2003), and agronomic traits (N'Diaye et al., 2007). More recently, the QTLs for the SE and cutting abilities were identified by Priyono et al. (2010). The goal of this study was to localize the QTLs linked to yield and SE ability in three populations of *C. canephora*.

MATERIAL AND METHOD

Plant Material

The coffee trees observed were derived from three cross pollinated populations: BP961 x Q121 (CPA), BP409 x Q121 (CPB) and BP409 x BP961 (CPC). A total of 77, 87 and 92 progenies for CPA, CPB and CPC, were respectively used.

Phenotypic evaluation

The SE evaluation was based on direct somatic embryogenesis protocol (Priyono et al., 2010). The yield evaluation was based on the weight total of green bean per tree and weight of 100 green beans.

Genetic mapping and QTL detection

The genetic maps were performed on the basis of the reference genetic map established by Crouzillat et al. (in preparation). The three genetic maps were constructed by using JoinMap® software (Ooijen, 2006) with Kosambi's mapping function (Kosambi, 1944).

The software MapQTL5® (Van Ooijen, 2004**)** was used for QTLs detection on the genetic maps. In a first step, the non-parametric Kruskal-Wallis (KW) test was applied to detect individually significant association (α= 0.05) between markers and traits. In a second step, interval mapping (IM) analysis was used to detect QTL. A threshold of LOD values of 4 and 3 for CP and BC segregations respectively, have been defined and used to declare the presence of a QTL. A QTL is validated when both LOD and K tests are significant. The genetic linkage groups carrying QTLs were represented using MapChart software.

RESULT AND DISCUSSION

Three characters for SE ability were recorded: frequencies of SE (FSE), number of somatic embryos per reactive explants (ANERE), and number of somatic embryos per Petri dish (ANEP). This study shows that the FSE frequency is ranging from 0 to 88% and 0 to 100% for CPA and CPB, respectively. ANERE is ranging from 1.3 to 22 and 1 to 55 for CPA and

CPB, respectively. The ANEP score is ranging from 0 to 322 and 0 to 667 for CPA and CPB, respectively. The yield data is ranged 17 to 1222, 119 to 1445, and 55 to 1239 grams per tree for CPA, CPB, and CPC, respectively. The weight of 100 green beans is ranging from 13 to 23, 9 to 29, and 13 to 26 grams for CPA, CPB, and CPC, respectively.

Three QTLs linked to the three characters for SE ability were overlapping on the linkage group G. The favourable allele is coming from the same parental clone (Q121) and a strong correlation (R= 0.65, 0.82, and 0.84) is observed between these traits for SE ability. Thus, these three QTLs were suggested to be a unique QTL. Although pleiotropic gene action might be another explanation for the co-location of these three QTLs detected for SE ability.

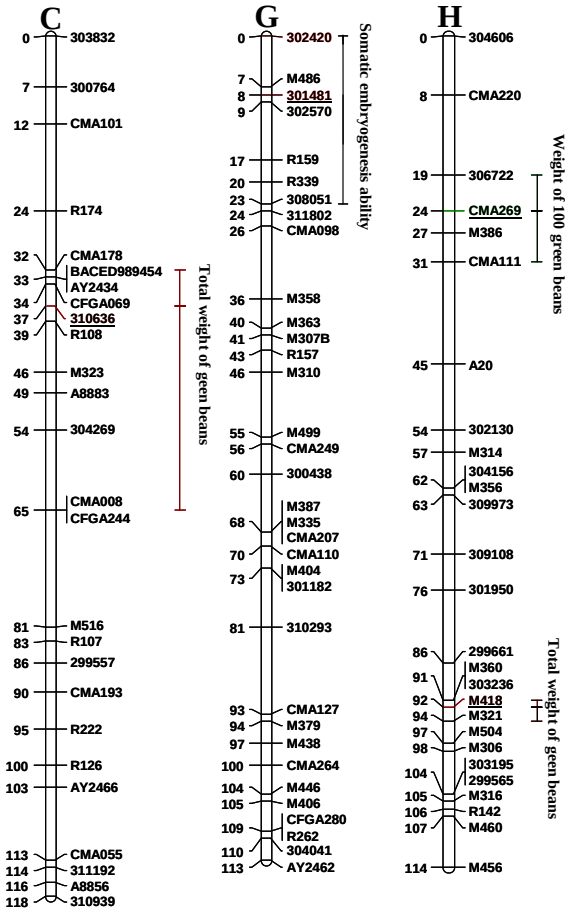

Figure 1. QTLs detected for SE ability, yield, and weight of 100 green beans of *Coffea canephora*. The vertical line is estimating the location of the different QTLs with α = 5%. The underlined locus for each QTL shows the nearest locus associated with the trait analysed.

Although the yield scores of both parental clones, in each population, are not significantly different and that the heritability of these traits is recorded as low, the study shows that three QTLs for yield traits are detected from the three different populations. This multiple population approach has the advantage that the QTLs from the three parental clones can be evaluated in different genomic backgrounds. Two QTLs linked to the total weight of green bean are detected on the two linkage groups (C and H) with a LOD score of 4.2 and 5.1, respectively. Another single QTL for the weight of 100 green beans is also detected with a LOD score of 3.3 on the group H. However, the two QTLs found on the linkage group H for these two yield components are located on two different genome areas. Moreover, our study

shows that there is no significant correlation between SE ability and yield traits. These results suggest that QTLs linked to SE ability and to yield characters are independent. Finally, a total of four genomic areas are involved in the QTL study **(**Figure 1**).**

The three QTLs associated with yield traits are detected in three chromosomal regions on linkage groups C and H. Favourable alleles from BP409 parent are associated with the characters related to the weight of 100 green beans and to the total weight of green beans both on the linkage group H. Another favourable allele from Q121 clone is detected for this last trait on the linkage group C. These results illustrated that these yield QTLs from BP409 and Q121 may be successfully associated to improved field performance in Robusta.

At the end a new generation of Robusta varieties cumulating yield performance and somatic embryogenesis or cutting abilities could be delivered through a marker assisted selection based on key agronomic traits for Robusta.

ACKNOWLEDGMENT

The authors acknowledge Nestlé Indonesia for their financial and logistic supports.

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Establishment of Entomopathogens *Beauveria bassiana* as an Endophyte in Coffee Seedlings C*offea canephora* and *Coffea arabica*

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SUMMARY

Establishing of *B. bassiana* as an endophyte is important first step in the possible use of this fungus as biocontrol agent. The research with aim to study the possibility of *B. bassiana* established as an endophyte in coffee by inoculating of coffee seeds with *B. bassiana* suspension has been conducted in laboratory. The results showed that the fungal entomopathogen *B. bassiana* became established as an endophyte in in vivo coffee seedling. *B. basiana* was recovered as an endophyte in culture from root, stem and leaves of coffee seedling up to 5 months postinoculation. Application of *B. bassiana* on coffee seed without parchment is better compare to coffee seed with parchment seed, and yielding the highest percentage of recovered *B. bassiana* as an endophyte. Three months and a half after inoculation of 4 g/10 l spore concentration on unparchment seed, *B. basssiana* as an endophyte was recovered from root (20.00%), stem (6.67%) and leaves (10.0%); whereas on parchment seeds *B. basssiana* was recovered from root (6.67%) stem (6.67%) and leaves (3,33%). Inoculation of *B. bassiana* of 8 g/10 l spore's concentration yielding more higher percentage of recovered *B. bassiana* as an endophyte, were 26.67, 16.67 and 20,0% respectively on root, stem and leaves of unparchment seed, whereas 10.0 and 16.67, and 3.33% on root, stem and leaves of parchment seeds. The highest of percentage of recovery *B. bassiana* as an endophyte on Arabica coffee was indicated that inoculation of *B. bassiana* on Arabica coffee more easier than Robusta coffee.

BACKGROUND

Beauveria bassiana was known as one of the effective biological agents of coffee berry borer in Indonesia. One possible pest management mechanism against the coffee berry borer, involves the inoculation of coffee plants with entomopathogen fungi, *B. bassiana* in attempt to establish the fungus as an endophyte. This would ideally result in the establishment of a systemic biocontrol agent inside the plant. If effective, such a technique could become a very valuable pest management strategy. Recently *B. bassiana* have isolated as endophyte in coffee plants in Columbia (Posada and Vega, 2005).

B. bassiana can be introduce as an endophyte of Zea mays, where it causes mortality of thr European corn borer, Ostrinia nubilalis (Bing and Lewis, 1991 cit. Posada and Vega, 2005). *B. bassiana* has also been known as an endophyte in potatoes, jimsonweed, cotton, and cocklebur. Establishing of *B. bassiana* as an endophyte is important first step in the possible use of this fungus as biocontrol agent. The objective of this study was to determine whether it could be possible to inoculate coffee seedling with *B. bassiana*.

MATERIAL AND METHODS

Research with aim to determine the possibility of *B. bassiana* established as a endophyte in coffee was carried out at the laboratory of pest and diseases Indonesian Coffee and Cocoa Research Institute (ICCRI), since December 2009. The experiment was arranged by completely randomized design in three factorial and 4 replications. The factors were :

- coffee plant materials Arabica coffee (*Coffea arabica* S795, *Coffea arabica* AS1) and Robusta coffee,
- two types of coffee seeds (with parchment and without parchment),
- concentration of *B. bassiana* spores are 0 (Control), 0.4, and 0.8 g spore/l

Application of *B. bassiana* has been done by dipping of coffee seeds (with parchment and without parchment) on various spore concentration of *B. bassiana*. After dipping, the seeds planted in sterilized soil medium. Assesment of *B. bassiana* colony recovered from coffee plant were conducted on 2, 3, 4 and 5 months post-inoculation when the seeds has growth, by cutting the roots, stems, and leaves of coffee and disinfected by bleach solution 0,5%, alcohol 70% (350 ml ethanol 95% + 150 ml H_2O) for 2 minutes, rinsed with sterile water, and dried on sterile paper towels (Arnold et al., 2001 cit. Posada and Vega, 2006). The tissues were cut into 2 to 2 mm^2, five of which were placed in Petri dishes containing PDA.

RESULT AND DISCUSSION

The results showed that the fungal entomopathogen *B. bassiana* became established as an endophyte in in vivo coffee seedling. *B. bassiana* was recovered as an endophyte in culture from root, stem and leaves of coffee seedling up to 5 months postinoculation.

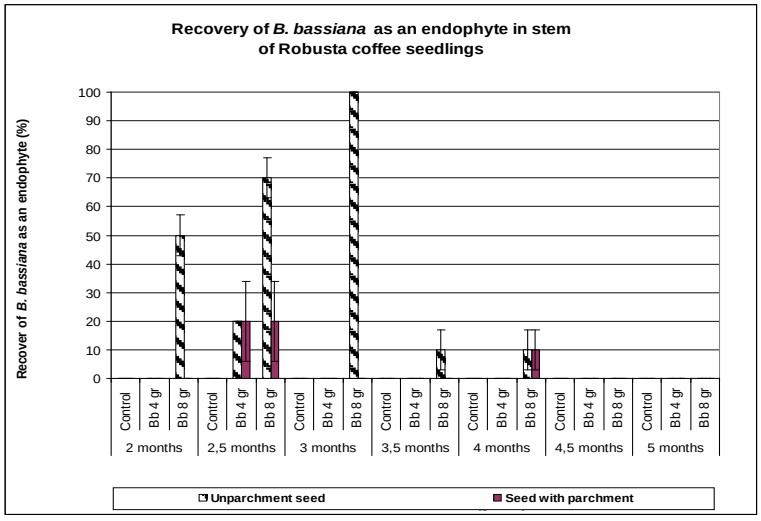

Figure 1. Percentage of recovery of *B. bassiana* as an endophyte in stem of Robusta coffee seedlings post-inoculated with *B. bassiana.*

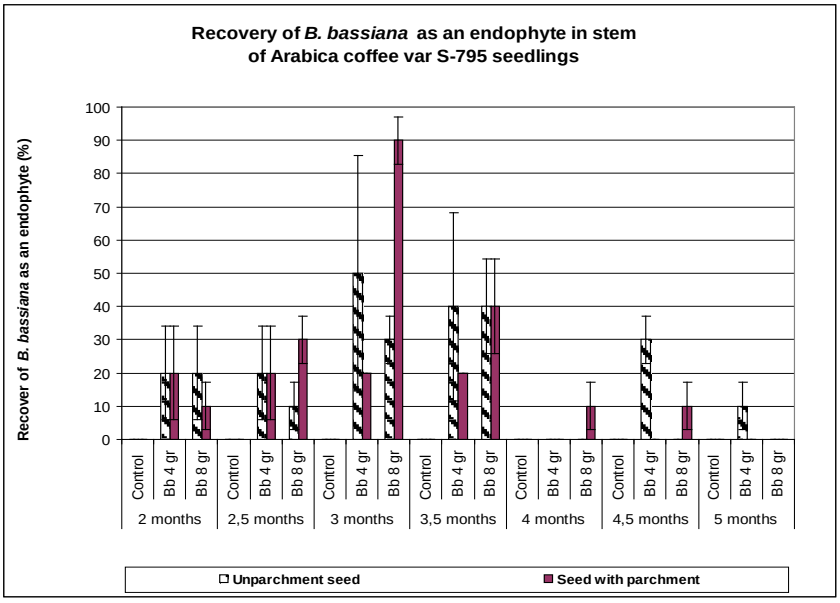

Figure 2. Percentage of recovery of *B. bassiana* as an endophyte in stem of Arabica S 795 coffee seedlings post-inoculated with *B. bassiana*.

Table 1. Percentage of colony of *B. bassiana* recovered in roots, stems and leaves of coffee seedlings 3, 5 months post-inoculated with *B. bassiana.*

Treatments	Control			*B. bassiana* 4 g/10 l			*B. bassiana* 8 g/10 l		
	root	stem	leaves	root	stem	leaves	root	stem	leaves
Seed without parchment									
Robusta coffee	0	0	0	0	0	30	0	10	0
Arabica coffee S 795	0	0	0	60	40	10	80	40	60
Arabica coffee AS-1	0	0	0	0	20	20	0	0	0
Averages	**0.00**	**0.00**	**0.00**	**20.00**	**20.00**	**20.00**	**26.67**	**16.67**	**20.00**
Seed with parchment									
Robusta coffee	0	0	0	0	0	0	10	0	0
Arabica coffee S 795	0	0	0	20	20	10	30	40	10
Arabica coffee AS-1	0	0	0	0	0	0	10	0	0
Averages	**0.00**	**0.00**	**0.00**	**6.67**	**6.67**	**3.33**	**16.67**	**13.33**	**3.33**

Posada and Vega (2006) has been inoculation coffee seedling grown in vitro with the fungal entomopathogen *B. bassiana* suspension in the redicle, and the results was known that *B. bassiana* was recovered as an endophyte 30 and 60 days postinoculation. Application of *B. bassiana* on coffee seed without parchment is better compare to coffee seed with parchment seed, and yielding the highest percentage of recovered *B. bassiana* as an endophyte. Three months and a half after inoculation of 4 g/10 l spore concentration on unparchment seed, *B. basssiana* Bb 715 isolate as an endophyte was recovered from root (20.00%), stem (6.67%) and leaves (10.0%); whereas on parchment seeds *B. basssiana* was recovered from root (6.67%) stem (6.67%) and leaves (3.33%). Inoculation of *B. bassiana* of 8 g/10 l spore's

concentration yielding more higher percentage of recovered *B. bassiana* as an endophyte, were 26.67, 16.67 and 20,0% respectively on root, stem and leaves of unparchment seed, whereas 10.0 and 16.67, and 3.33% on root, stem and leaves of parchment seeds. The highest of percentage of recovery *B. bassiana* as an endophyte on Arabica coffee was indicated that inoculation of *B. bassiana* on Arabica coffee more easier than Robusta coffee. Others fungus were also detected as an endophyte in the coffee seeds, *Curvularia* sp.,*Rhizoctonia* sp, and some unidentified of fungus and bacterial endophyte. These indicating that they were as endophyte in the seed.

CONCLUSION

- The fungal entomopathogen *Beauveria bassiana* became established as an endophyte in coffee seedling, inoculated by dipping coffee seed in *B. bassiana* suspension.
- *B. basiana* was recovered as an endophyte 2, 3, 4 and 5 month post-inoculation from roots, stems and leaves
- Application of *B. bassiana* on coffee seeds without parchment is better than parchment seeds, and yielding the highest percentage of recovery of *B. bassiana* as an endophyte
- The highest percentage of recovery *B. bassiana* as an endophyte on Arabica coffee indicated that inoculation of *B. bassiana* on Arabica coffee more easier than Robusta coffee

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Protein and Lipid Oxidation Level in Just Prepared Coffee Beans

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SUMMARY

The lowering of stored meat and soy products quality during the storage time has been attributed to the presence of oxidized lipid and proteins. Since coffee beans contain considerable quantities of these components, lipid and protein oxidation could be responsible for changing in the beverage quality during the green bean storage as well. Taking into account these possible changes in the coffee beans composition and beverages, it was proposed a study aiming to follow the oxidation level of proteins and lipids during the stored green beans as well as the effect of the cultivar and processing method on such event

Fully ripe coffee fruits were processed by dry and semi-dry methods and sun dried until around 10% moisture. The carbonyl groups concentration in the green bean just after drying was employed to express the protein oxidation level. The content of substances which reacted with tiobarbituric acid (TBARS) was used to express the lipid oxidation level. TBARS values ranged from 7.9 to 11.9 nmol MDA (malondialdehyde) gr^{-1} dry matter and the carbonyl groups content was between 1.7 and 5.0 nmol mg^{-1} protein.

INTRODUCTION

The drying process is possibly the most important step in the coffee processing. It may take from 7 to 20 days depending on the weather, processing method and drying conditions. The process of oxidative stress may start when the bean moisture level favors the production of reactive oxygen species (ROS) and the seed metabolism becomes inefficient to neutralize the amount of ROS formed (Leprince et al., 1994). If the oxidative stress does occur during the drying of coffee, it can be responsible for the decreasing of the level of glutathione and ascorbate as well as for the increasing of the free fatty acids (FFA) content. Coffee unsaturated fat acids, which prevail in free fat acids and in triacylglycerols molecules, could on its turn favor the oxidation of the coffee lipid fraction (Dussert et al., 2006; Nikolova-Damyanova et al., 1998).

Beyond ROS, primary and secondary products generated during the lipid oxidation could react with seed proteins, causing their oxidation. Protein cleavage and cross-links protein-protein and lipid-protein, beyond protein radicals, damaged amino acids and carbonyl groups in proteins molecules may be formed as result of the protein oxidation (Gardner, 1979; Stadman and Levine, 2003).

Results reported herein refer to a part of a study concerning to a) the lipid and protein oxidation levels of stored green coffees, b) the effect of the processing method on the protein

and lipid oxidation and c) the influence of the protein and lipid oxidation level on the beverage quality.

MATERIALS AND METHODS

Ripe fruits of *Coffea arabica* cultivars IAC Mundo Novo, harvested in 2008, and IAC Catuaí Vermelho and IAC Obatã Amarelo harvested in 2009, were collected in Campinas, São Paulo State, Brazil. All samples were processed by dry (natural coffee) and semi-dry (pulped natural coffee) methods and sun dried until about 10% moisture.

After finished the drying, the beans were hulled, ground and analyzed regarding the moisture (Brasil, 1992), thiobarbituric acid reactive substances (TBARS) (Heath and Packer, 1968), expressed as nmol MDA (malondialdehyde) g^{-1} dry matter, total lipid content (Speer and Kolling-Speer, 2006) expressed as g 100 g^{-1} **dry matter**), soluble protein content (Baú et al., 2001; Bradford. 1976) expressed as g 100 g^{-1} dry matter and concentration of carbonyl groups in protein (Levine et al., 1994), expressed as nmol mg^{-1} protein. All analyses were performed in triplicate.

RESULTS AND DISCUSSION

Results in Table 1 show that total lipids and soluble protein contents in just dried beans, were in agreement with the literature data (Speer and Kolling-Speer, 2006; Baú et al., 2001; Shimizu and Mazzafera, 2003). There was not influence of the processing method on the bean lipid content but there were clear differences between the genotypes. Green beans of IAC Mundo Novo cultivar harvested in 2008 presented higher lipid content than green beans of IAC Catuaí Vermelho cultivar, Green beans of IAC Catuaí Vermelho cultivar on its turn presented more lipid than IAC Obatã Amarelo cultivar, both of them harvested in 2009.

Table 1. Total lipids, TBARS, soluble protein and carbonyl groups in coffee Arabica.

Samples	TBARS (nmol MDA g^{-1} d.b)	Total lipids content (% d.b)	Carbonyl content (nmol mg^{-1} protein)	Soluble protein content (% d.b)
ACN	9.4 ^{c}	9.9 ^{bc}	2.5 ^{c}	7.2 ^{abc}
ACD	8.8 ^{c}	9.4 ^{c}	3.7 ^{b}	7.7 ^{ab}
VCN	10.3 ^{b}	10.3 ^{b}	5.0 ^{a}	7.6 ^{ab}
VCD	7.9 ^{d}	10.1^{b}	4.0 ^{b}	7.3 ^{abc}
MNCN	10.7 ^{b}	13.8 ^{a}	1.7 ^{d}	6,8 ^{bc}
MNCD	11.9 ^{a}	13.2 ^{a}	2.6 ^{c}	7.0 ^{bc}

ACN: IAC Obatã Amarelo natural coffee; ACD: IAC Obatã Amarelo pulped natural coffee; VCN: IAC Catuaí Vermelho natural coffee; VCD: IAC Catuaí Vermelho pulped natural coffee; MNCN: IAC Mundo Novo natural coffee; MNCD: IAC Mundo Novo pulped natural coffee. Mean values followed by the same letter in the same column do not differ significantly by Tukey test at 5% probability.

The values of TBARS revealed the presence of aldehyde in the beans just after the drying. Despite the difference in the values, it is not possible to say that TBARS number is influenced by the processing method and cultivar when considering the results showed in Table 1. Although TBARS number is normally used as an oxidation index of lipid oxidation and is

expressed as malondialdehyde concentration, other aldehyde, not derived from lipid oxidations, including sugars, for example, may be also quantified (Silva et al., 1999).

Some works show the relationship between the lipid oxidation and ROS generation in coffee with stressful situations, such as high doses of cadmium or low temperature (Queiroz et al., 1998; Gomes-Junior et al., 2006). It is possible that drying process also implies stress condition and ROS formation resulting lipid oxidation. Lipoxygenase and lipase activities could also be important for lipid oxidation (Patui et al., 2008).

TBARS analysis is often followed by the quantification of carbonyl groups in proteins, since reactive compounds formed during the lipid oxidation may induce the protein oxidation (Estevez et al., 2007; Huang et al., 2006).

The presence of carbonyl groups in the soluble fraction of the coffee protein suggested some degree of protein oxidation. However, it is also possible that the quantified carbonyl group is due to monosaccharides bonded to the protein molecule and not to the protein oxidation (Ludwing et al., 1995).

The difference between the protein content in Table 1 and that normally presented in the literature is due the methodology of analysis. In this work only soluble protein was quantified whereas normally the total nitrogen is measured.

In conclusion, the TBARS and carbonyl groups quantified in recently processed coffee suggested some degree of oxidation of the bean proteins and lipids even before the resting time, regardless the cultivar, processing method and harvest year.

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The Importance of the Resting Period in the Coffee Grain Aspect and Beverage Quality

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SUMMARY

There is an empirical knowledge that the resting period of green coffees is important for the improvement of the beverage overall quality through the reduction of the grass-like and tea flavors and through the reduction of the astringency. The empirical knowledge also indicates that the storage of non hulled beans is better than the storage of hulled beans for the color preservation. Moisture homogeneity of the coffee lot and higher uniformity of the color of the non hulled stored bean are also expected to occur during the resting period. However, it was not found any published research quantifying these changes.

In this work the changes in the green beans of Arabica coffees cv. IAC Catuaí Vermelho and IAC Obatã Amarelo during the resting period were evaluated. Fully ripe fruits were processed by both semi-dry and dry methods, dried to around 10% moisture and stored in a medium lighted room at 24-27 °C and at 63-66% R.H. Hulled and non hulled coffees rested for 3 months under these conditions. After the resting period, the whole and parchment coffees were hulled and evaluated regarding the color index, moisture, viability and beverage quality.

The overall beverage quality of the all coffee samples was improved on average 1.9 points in the course of the resting time. The viability of the hulled stored green beans decreased from 69 up to 89% in comparison with that measured at the end of the drying step. Furthermore, the color index decreased on average 0.3% and 47% in non hulled and hulled stored beans, respectively.

INTRODUCTION

Normally, in Brazil just after the end of the drying process, coffee fruits and parchment beans are stored in barns for the so-called “resting period”. It is empirically known that improvement in the beverage flavor and in the bean appearance regarding the color uniformity occurs during this time. Usually, only non hulled coffee beans are submitted to the resting period in barns, because it is known that the hull contributes for the bean quality.

The flavor of the coffee beverages prepared from just dried not rested coffee resembles to leaves or green tea, probably due to the presence of 2-methoxy-3-isopropyl pyrazine and 2-methoxy-3-isobutyl pyrazine (Holscher and Steinhart, 1995). Beverages from such products are also more adstringent than that from rested coffee, and typical sensory characteristics of the coffee beverage such as acidity, sweetness and bitterness, are not well perceived. The decrease of the bean viability which occurs during the resting period could be also related to the beverage quality (Selmar et al., 2008).

In this study the changes in the viability, beverage quality, moisture and color index of the beans were evaluated.

MATERIALS AND METHODS

Fully ripe fruits of *Coffea arabica* cultivars IAC Obatã Amarelo and IAC Catuaí Vermelho were collected in Campinas (São Paulo State), processed as dry and semi-dry methods and dried until moisture of about 10%. Half of the processed coffees were stored as non hulled beans and half was stored as hulled beans (Table 1). All samples were stored at 24-27 °C for 3 months (resting period) in small (100x150 mm) jute bags into a desiccator containing ammonium nitrate solution enough for 63-66% R.H.

Table 1. Description of the samples processed by dry (CN) and semi-dry (CD) method stored as hulled (SC) and non hulled (CC) beans.

Code	Arabica coffees
ACN-0	IAC Obatã Amarelo processed as natural coffee at the beginning of storage
ACNCC-3	IAC Obatã Amarelo processed as natural coffee after 3 months of storage – non hulled
ACNSC-3	IAC Obatã Amarelo processed as natural coffee after 3 months of storage – hulled
ACD-0	IAC Obatã Amarelo processed as pulped natural coffee at the beginning of storage
ACDCC-3	IAC Obatã Amarelo processed as pulped natural coffee after 3 months of storage – non hulled
ACDSC-3	IAC Obatã Amarelo processed as pulped natural coffee after 3 months of storage - hulled
VCN-0	IAC Catuaí Vermelho processed as natural coffee at the beginning of storage
VCNCC-3	IAC Catuaí Vermelho processed as natural coffee after 3 months of storage – non hulled
VCNSC-3	IAC Catuaí Vermelho processed as natural coffee after 3 months of storage – hulled
VCD-0	IAC Catuaí Vermelho processed as pulped natural coffee at the beginning of storage
VCDCC-3	IAC Catuaí Vermelho processed as pulped natural coffee after 3 months of storage–non hulled
VCDSC-3	IAC Catuaí Vermelho processed as pulped natural after 3 months of storage – hulled

All samples were analyzed immediately after the end of the drying step and at the end of the resting period regarding the moisture (Brasil, 1992), color index (Carvalho et al., 1994), viability (Dias and Da Silva, 1998) and overall quality of the beverage (ISO, 1991). The beverage quality was evaluated by 3 professional tasters using a scale of 8 points (“1” low quality, “4” medium quality and “8” excellent quality). All analyses were performed in triplicate.

RESULTS AND DISCUSSION

There were no significant difference in the moisture of hulled and non hulled samples at the end of storage considering a variety and a processing method (Table 2).

Only the color index of hulled bean decreased during the resting period. Similar results were also described by other researchers (Godinho et al., 2000; Nobre et al., 2007).

During the resting period significant loss of the viability occurred only in the hulled coffee beans, in which the embryos axis or the whole embryo acquired brown color. This result could be attributed to oxidative stress or lack of sufficient reducing power (Da Silvaey al., 2005). These results indicated that the dried peel and parchment would protect the embryo of the light, preventing the induction of the synthesis of gibberellins. Gibberellins on its turn would reduce the activity of antioxidant enzymes, with consequent death of the endosperm cells end embryo (Bethke et al., 2001; Valio, 1976).

Table 2. Moisture, color index, viability and beverage quality of the coffee beans processed by dry (CN) and semi-dry (CD) methods, at the end of drying step (0) and after 3 (3) month storage.

Sample Code	Moisture (%)	Color index	Viability (%)	Overall Quality Scores
ACN-0	10.4 [b]	1.2[a]	92 [a]	3.0 [b]
ACNCC-3	11.3 [a]	1.1[a]	85 [a]	4.3[a]
ACNSC-3	11.2 [a]	0.7 [b]	18 [b]	4.7[a]
ACD-0	9.9 [b]	1.2 [a]	90 [a]	4.0 [b]
ACDCC-3	11.3 [a]	1.0 [a]	86 [a]	5.7[a]
ACDSC-3	11.2 [a]	0.7 [b]	21[b]	6.3[a]
VCN-0	10.5 [b]	1.3[a]	83[a]	2.0 [b]
VCNCC-3	11.4 [a]	1.0 [a]	79 [a]	4.3 [a]
VCNSC-3	11.3 [a]	0.5 [b]	4 [b]	4.3 [a]
VCD-0	10.0 [b]	1.4 [a]	93 [a]	3.0 [b]
VCDCC-3	11.4[a]	1.0 [a]	89 [a]	4.7 [a]
VCDSC-3	11.3[a]	0.8 [b]	4.3 [b]	5.0 [a]

Mean values followed by the same letters in the same variety and column do not differ significantly by Tukey test at 5% probability.

According to the tasters, all beverages presented some adstringency and grass-like flavor, described as “green”, at the end of the drying step. The overall quality of coffee beverage was improved, on average around 1.9 points in a 8.0 points full scale, after 3 months of storage (Table 2), regardless the viability level of the green bean. According to the literature,

reduction of astringency, could be due to the decreasing of phenolic compounds content (Godinho et al., 2000; Leite et al., 1996).

Therefore, the results showed that during the three month resting period: 1- the beverage overall quality of all samples was improved, 2- the moisture of the hulled and non hulled beans was uniform and in equilibrium with the ambient R.H. and 3- the hulls helped the maintenance of the viability and of the initial color of the beans.

ACKNOWLEDGEMENTS

To Fapesp and CAPES for the financial support.

To the coffee tasters: R. Scotton, M. Bacceti, L. Ribeiro, F. Cabral and to Centro de Comércio de Café de Londrina-PR.- BR

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Morphological Response to Drought of *Coffea arabica* L.

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INTRODUCTION

The ongoing global climatic change will bring along prolonged periods of drought in various tropical regions. As a perennial, coffee may be severely and durably affected, as well as economies, and people who rely on it.

The limitations of classical breeding approach has led to increasing attention being paid to criteria of morphological and physiological resistance (integrated approach) (DaMatta and Ramalho, 2006). Morphological parameters together with physiological parameters may help phenotyping coffee genotypes regarding drought tolerance in order to understand underlying genetics and molecular physiology. However as a whole no single narrowly defined functional type is needed for tolerance to drought (Sack wt al., 2003). Thus, the aim of this study was to identify morphologic parameters that indicate different responses to drought between coffee genotypes.

Figure 1.

MATERIALS AND METHODS

A nursery trial was set up at IAPAR, Londrina Parana, Brazil, with two contrasting cultivars, ‘IAPAR 59’ and ‘Catuaí’. 'IAPAR59' is a Sarchimor variety, it originated from a *C. canephora* introgression through 'Hibrido de Timor'; 'Catuai' is derived from the cross ‘Caturra’ x ‘Mundo Novo’ (no introgression). Seeds were germinated in June 2007,

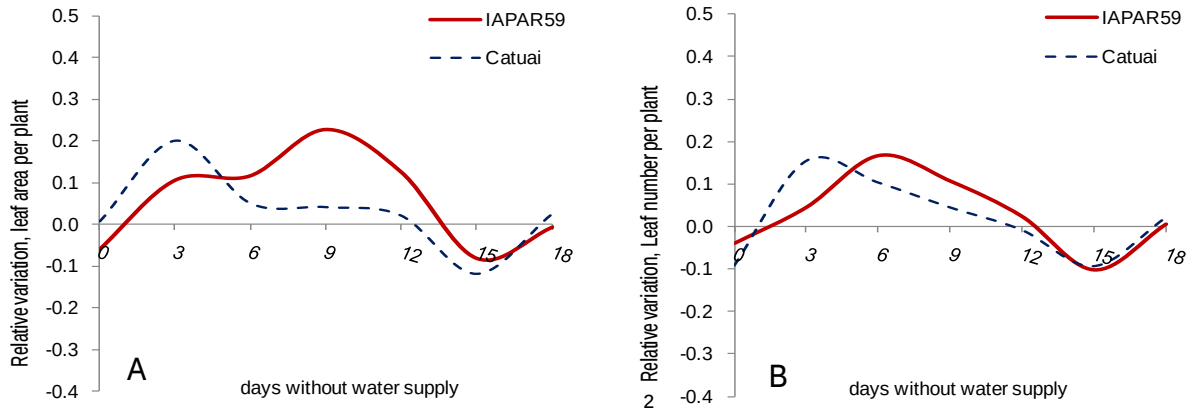
transferred into 5 liters ceramic pots in November 2008, and a total of 50 plants per cultivar were installed under a plastic tunnel in April 2009 (photo) to simulate abiotic water stress.

Detailed non destructive morphological measurements of each plant were made before the drought experiment (March 2009) namely 'size and number of leaves', 'height', 'number of orthotropic internodes'. The plants were distributed into 7 homogeneous lots (treatments = watering regimes) that were submitted to 0 - 3... 18 days without water supply (drought), followed by normal watering.

Above-ground and subterranean structures were examined by morphological and destructive methods after the trial (May 2009). 'Total leaf area' per plant was checked using a planimeter (LI 3100, LI-COR, Inc. Lincoln, NE, USA). Aerial parts (leaves, orthotropic and plagiotropic axes) and roots (primary, secondary, tertiary orders) were weighed and measured then dried separately at 53 °C for 48 hours.

RESULTS AND DISCUSSION

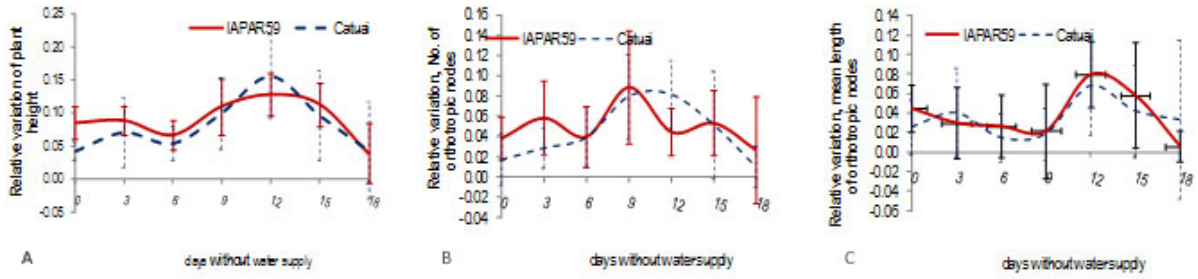
Both varieties were very different from the start of the trial, resulting in highly significant differences between their aerial parts at all stages of the trial. Indeed all growth parameters measured at the end of the trial show highly significant difference between both cultivars ($p<0.01$), whereas absolute difference between measurements before and after the trial only gave significant differences between the treatments ($p<0.05$). Therefore **relative evolution of growth parameters** was calculated for each plant (before and after the trial) except for roots.



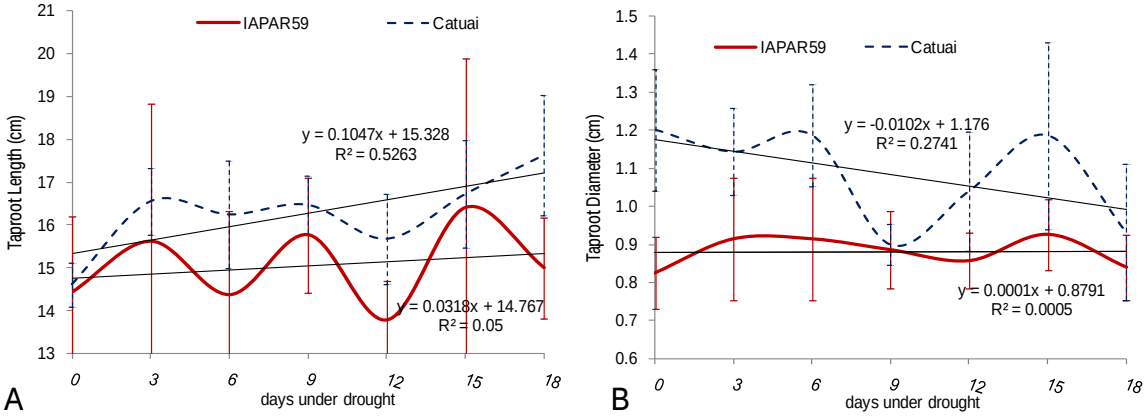
Figures 2. Relative evolution of leaf area and number of leaves per plant for cultivars 'Catuai' and 'IAPAR59' resulting from seven watering regimes, over two months including the 18 days trial (vertical bars: confidence interval at p=0.95, lines: linear trends).

'Catuai' responded significantly **earlier** to drought than 'IAPAR59' (3 days vs. 6-9 days), by increasing its '**relative leaf area**' **per plant** (Figure 2A) and '**No. of leaves',** while the apparition of new leaves hardly compensated leaf fall for both the control treatment and the longest dry periods (Figure 2B). Indeed leaf area' and 'number of leaves' are considered good indicators on other plants (Cordeiro et al., 2009) Other parameters such as 'relative plant height', 'relative number and mean length of orthotropic internodes' followed the same pattern for both varieties (Figure 3). 'Specific leaf area' might be more water dependant than 'carbon isotope discrimination' for differentiating genotypes (Monclus et al., 2006). However, we

found the same evolution of both cultivars in specific leaf area; it decreased from 146 to 125 $cm^2\ g^{-1}DW$ (-13%) from control to 18th day of drought.



Figures 3. Relative evolution of plant height, No. of orthotropic nodes, and mean length of orthotropic nodes over two months including the 18 days trial, for cultivars 'Catuai' and 'IAPAR59' under seven watering regimes (vertical bars: confidence interval at p=0.95).



Figures 4. Influence of seven watering regimes on taproot diameter and length for cultivars 'Catuaí' and 'IAPAR59' (vertical bars: confidence interval at p=0.95, lines: linear trends).

The relative stability of 'IAPAR59' compared to 'Catuai' appears through both 'taproot diameter' (Figure 4A) and 'taproot length' (Figure 4B), and not for taproot weight nor for total root weights.

'Catuaí' had on average twice more roots than 'IAPAR59' ('total weight', data not shown), with an increase in taproot length that may be useful for drought tolerance differentiation (Pinheiro et al., 2005), however this may be due to its initial development. Indeed, a tendency in lower 'Catuaí' taproot diameter was observed after prolonged drought (Figure 4A).

Future works based on results from this analysis, will benefit from the collaborative projects (Marraccini et al., 2009) established between Agropolis (Montpellier, France) and Embrapa (Brazil) within the frame of CIBA (International Consortium on Advanced Biology).

CONCLUSION

'Catuai' plants were stimulated by short-term drought (3 days), while 'IAPAR59' was more stimulated by midterm drought (6-9 days), regarding 'leaf number' and 'leaf area' per plant.

Temporal drought prorogation provoked the significant decrease in both above- and below-ground measured parameters. 'IAPAR 59' showed more morphogenetic stability to drought than 'Catuai'; i.e. retarded effects on growth stimulation and less intensive global growth decrease. Morphological traits, namely the evolution of number of leaves and of leaf area, together with root diameter and length, can be used along ecophysiological parameters, in order to differentiate varietal responses to drought. Responses to drought measured in nursery conditions may thus help differentiating genotypes, after short to midterm stress induction, paying attention to leaf development and shedding. Those findings shall have to be confirmed in field conditions.

ACKNOWLEDGEMENTS

This time consuming work would not have been possible without the availability of plant material, manpower and assistance of Agronomy Department of IAPAR (Ecophysiology Section, and Coffee Breeding Section - Dr. Tumoru Sera).

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Ecophysiological Differentiation for Drought Tolerance in *Coffea arabica* (L.) Genotypes

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SUMMARY

The aim of this study was to define easily recordable and accurate ecophysiological parameters for quick differentiation between genotypes of Arabica coffee (*Coffea arabica* L.). A nursery trail was set up with two contrasting varieties ('IAPAR 59' and 'Catuaí'). Twenty-one-months-old pot plants were submitted to 0-3-...18 days of drought followed by normal irrigation. The differentiation between stressed and controlled plants, in both varieties, was observed starting from 3rd day for stomatal conductance (g_s), plant relative weight, and transpiration (E); from 9th day of drought treatment for leaf water potentials. 'Catuaí' showed greater sensitivity than 'IAPAR 59', and differences in genotype responses were recordable from 9th to 15th day for E and g_s and from 9th to 12th day for water potential components. Therefore g_s, E or/and water potential component measurements can be used, after nine days of drought in nursery conditions, in classifying Arabica coffee genotypes for drought tolerance, thus allowing screening of great number of plants for association or mapping studies .

INTRODUCTION

A considerable advance in ecophysiology of *Coffea arabica* L. has been undertaken during the last two decades (Barros et al., 1995; Amaral et al., 2001), but the knowledge about stress physiology still remains questionable (Beining, 2007). Most experiments focused on a single stress treatment under controlled environmental conditions. The limited number of original plants from which most *C. arabica* cultivars were derived, its early domestication, artificial selection and intensive breeding severely eroded allelic variations in this species (Silvestrini et al., 2007), which thus can increase its sensitivity to environmental stresses.

Drought stress may be induced by multiple environmental variables, such as high temperature, solar radiation, saturated vapor pressure deficit, low rainfall amounts, a poor precipitation distribution, as well as increasing intensities of soil moisture deficits (DaMatta and Ramalho, 2006). Some adaptive mechanisms in plant drought response are possible, *e.g.* regulation of stomatal conductance and stomata opening or/and osmotic adjustments (DaMatta, 2004). The aim of this study was to define easily recordable and accurate ecophysiological responses for quick differentiation between genotypes of *C. arabica* when submitted to drought conditions.

MATERIALS AND METHODS

We compared architectural characteristics and drought tolerance of two *Coffea arabica* cultivars, namely 'Catuai' and 'IAPAR 59'. 'Catuai', being derived from a 'Caturra' x

‘Mundo Novo’ cross, is a "pure" *Coffea arabica* variety, whereas ‘IAPAR 59’ is derived from a ‘Caturra’ x ‘Hibrido de Timor’ cross, thus contains some *Coffea canephora* introgression.

Fifty seedlings per variety, germinated in June 2007, were transferred to 5 l ceramic pots in November 2008 (filled with 4kg sterile soil – 65% sand + 27% clay + 8% silt). The seedlings were grown in nursery under 50% shade.

Seven treatments were determined in April 2009 aiming at testing the drought impact: T1 - ten daily irrigated control plants and six groups of plants submitted to water regimes defined by number of days without water supply: T2 – three days (7 plants); T3 – six days (7 plants); T4 – nine days (7 plants); T5 – twelve days (7 plants); T6 – fifteen days (7 plants); T7 – eighteen days (5 plants). The plants of each cultivar were randomly distributed to each treatment, but ensuring that the average initial development was similar for all treatments. Drought was secured by placing the pots under a plastic tunnel, allowing air circulation between the plants. The plants under different drought treatments were disposed in a sequence and a physical separation was placed between irrigated and non irrigated treatments when watering.

At the end of each water regime treatment (3, 6....18 days) some ecophysiological parameters were collected in the morning (9:00-10:00 a.m.) from control and drought treatment plants. Stomatal conductance (g_s - mol $m^{-2}s^{-1}$) and transpiration (E – mmol $m^{-2}s^{-1}$) were measured by using the LI 1600 porometer. In order to evaluate maximum efficiency of PS II, the quantum efficiency or yield (*Fv/Fm*) was estimated by using not modulated device Handy PEA Pocket PEA from Hansatech Instruments.

In order to fit the measurements of g_s and E with local microclimate condition under the tunnel, they were corrected with vapor pressure deficit (VPD). The leaf tissue temperature (porometer output) was used to determine accurately the VPD, and calculated using VPD calculator: http://www.hydro.co.nz/1_information/1_vpd/info_vpd.html.

Leaf water potentials were measured using thermocouple psychrometer (C-30, Wescor, Inc., Logan, Utah, USA.) branched to one datalogger (CR-7, Campbell Scientific, Inc., Logan, Utah, USA). Potential measurements in drought treatments (day 3, 6 ... 18) were compared with data from the control at initial stage. The procedure consisted in exposure of leaf discs (2 cm^2) to psychrometers. The datalogger recorded the outputs at 10 min intervals , until the equilibrium of vapor pressure in chamber was reached. The system outputs (μV) were converted in water potential (MPa) as a function of previous sensor calibration in NaCl solutions. The total water potential (Ψ_t) was recorded firstly, followed by the sensor immersion in liquid nitrogen during 4 min, in order to obtain the osmotic potential (Ψ_s). The pressure potential (Ψ_p) was calculated from $\Psi_t - \Psi_s$.

The relative weight reduction (% transpiration loose) was calculated as the total pot weight difference relative to control plants. Pots were wrapped in aluminum paper, in order to eliminate evaporation.

Statistic analysis was performed using the one-way ANOVA from the free "R" software (version 2.11.1). Plant reactions under different water regimes were compared with those of control plants. In the figures variances are represented by Standard Errors of the Means.

RESULTS AND DISCUSSION

Non-irrigated plants quickly showed the reduced g_s values (Figure 1A), *E* (Figure 1B) and relative weight (Figure 3A), starting from the 3rd day with no water supply (Table 1). The response of leaf water potentials (Ψ_t, Ψ_s and Ψ_p) measured in plants submitted to drought treatments started from 9th day with no water supply (Figure 2 and Table 1). Significant alterations in quantum efficiency (*Fv/Fm*) were expressed only at the end of drought treatments, from 15th to 18th day (Figure 3B and Table 1).

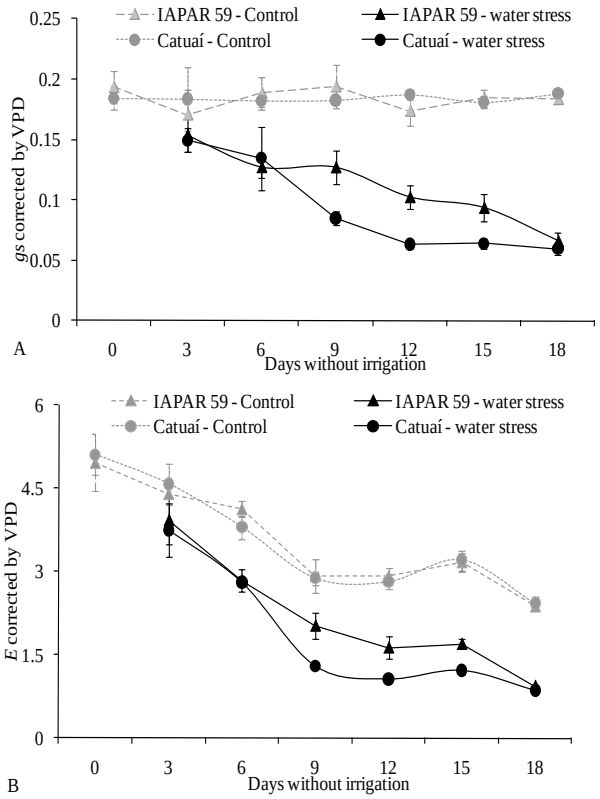

Figure 1. A/ Leaf stomatal conductance (g_s) and B/ leaf transpiration rate (*E*) corrected by water pressure deficit (VPD) in two Arabica coffee cultivars (IAPAR 59 and Catuaí) grown under water stress (no irrigation).

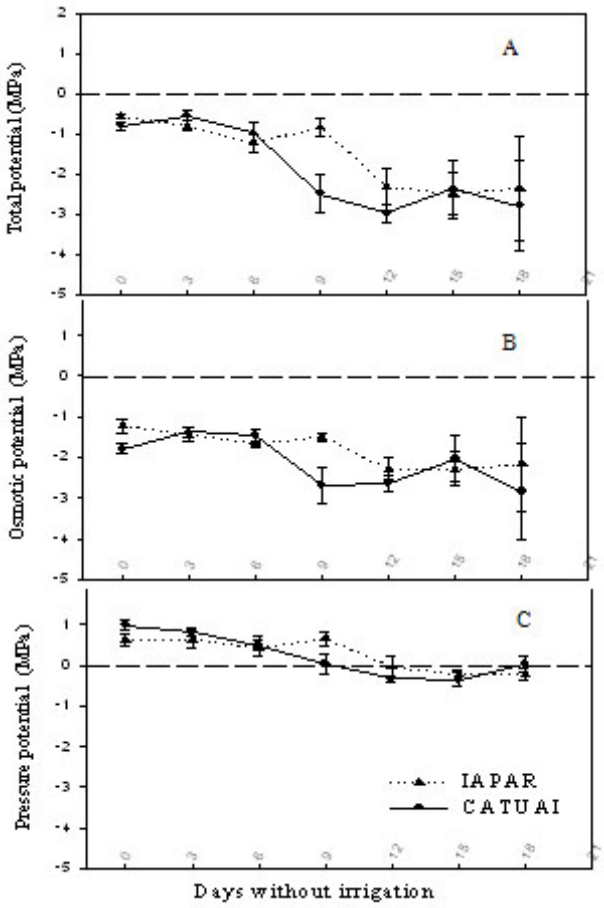

Figure 2. A/ Total water potential (Ψ_t), B/ osmotic potential (Ψ_s), and C/ pressure potential (Ψ_p) in two Arabica coffee cultivars (IAPAR 59 and Catuaí) grown under water stress (no irrigation).

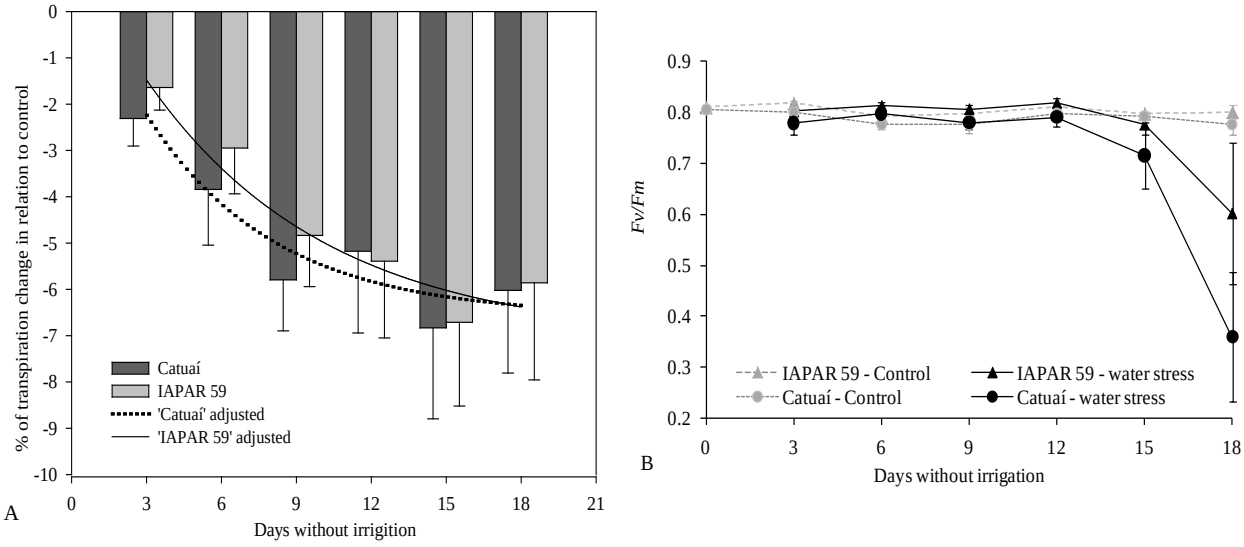

Figure 3. A/ % of transpiration change (loose) in relation to control and B/ quantum efficiency (*Fv/Fm*) in two Arabica coffee cultivars (IAPAR 59 and Catuaí) grown under water stress (no irrigation).

Both cultivars had the similar initial responsiveness and sensitivity to induced drought. In prolonged drought conditions, 'Catuaí' showed greater sensitivity than 'IAPAR 59'. The

differences in genotype responses were recordable at 9th day for g_s and *E* (Figure 1 and Table 1) and for water potential components (Figures 2A-C). The genotype specific response in relative weight reduction was not observed (Figure 3A) neither for *Fv/Fm* (Figure 3B, Table 1).

Table 1. The p-values for one-way ANOVA of leaf stomatal conductance (gs), leaf transpiration rate (E) and chlorophyll fluorescence (Fv/Fm) for A/ drought treatments compared to control and B/ two cultivars ('Catuaí' and 'IAPAR 59'). P<0.05 were considered significant and marked in bold.

A/ Drought treatments	**DF**	**T2 *vs* T1**	**T3 *vs* T1**	**T4 *vs* T1**	**T5 *vs* T1**	**T6 *vs* T1**	**T7 *vs* T1**
g_s	1	0.0558	**0.005**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
E	1	**0.0470**	**0.002**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Fv/Fm	1	0.1756	**0.0399**	0.6562	0.9265	0.0673	**0.0003**
B/ Cultivars under drought	**DF**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
g_s	1	0.8074	0.8449	**0.0191**	0.0680	0.0531	0.5154
E	1	0.6834	0.9716	**0.0096**	0.0513	0.0627	0.5477
Fv/Fm	1	0.4032	0.2982	0.0826	0.1665	0.3429	0.2826

A sequence in plant ecophysiological reactions was observed. The mechanism of stomatal regulation reacts firstly (Figure 1A), inducing the transpiration responses (Figure 1B and Figure 3A). The mechanism of osmotic regulation was longer efficient, until 9th day of induced water deficit (Figure 2). Quantum efficiency was the last parameter to respond, starting from 15th day and finishing at 18th day (Figure 3B) when no recovery in turgidity occurred for majority of treated plants. In induced drought related to duration of water deficit and light conditions (Beining, 2007), the coffee seedlings grown under full light conditions decreased in g_s only five days after imposition of stress, whereas shade grown seedlings maintained g_s at normal values during the first week despite reduced soil moisture availability.

We consider that at 9th day of drought (and morning evaluations due to diurnal rhythmicity) it could be possible to apply the g_s, *E* or/and water potential component measurements in phenotyping Arabica coffee genotypes' tolerance to drought in nursery conditions, thus allowing screening of great number of plants from association or segregating populations.

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Preliminary Results on Phenotypic Plasticity of Coffee (*Coffea arabica* Cv. Rubi and Iapar59) Plants in Response to Water Constraint Under Field Conditions

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SUMMARY

The effects of drought-stress on aerial and root architecture, ecophysiology, anatomy and molecular responses were investigated during 2 years using the Iapar59 (drought-tolerant) and Rubi (drought susceptible) cultivars of *Coffea arabica*. Plants were grown under three water treatments: I-I a non limited watering treatment (irrigated each year during the dry season), NI-NI a limited water treatment (non-irrigated during the dry seasons) and NI-I, a limited watering in year 1 and non-limited watering in year 2 ("recovery"). Six points of measurements were taken along the experiment. For all conditions, the phenotypic plasticity was followed by analyzing architecture, evolutions of biomass, $\Delta^{13}C$ and sap flow for example.

INTRODUCTION

In the context of climate changes, adaptation of perennial plantations to water constraint becomes a major concern of wood and fruit productivity. Adaptation depends on the level of genetic diversity of breeding and natural populations as well as their plasticity. This project plans to describe adaptive mechanisms under water constraint of three perennial plants of the temperate and tropical regions including *Pinus*, *Eucalyptus* and *Coffea* by combining analysis of plant architecture, physiology, anatomy and molecular responses to drought stress. The adaptive mechanisms of coffee under water stress were evaluated along two years (2008-09). We studied the effects of drought-stress on aerial and root architecture, ecophysiology, anatomy and molecular responses of two cultivars of *C. arabica*, Iapar59 (I59) and Rubi. For the six points of measurements taken along the two years of the experiment, the phenotypic plasticity was followed by measuring leaf, root areas and biomass and characterizing the belowground and aerial architecture of coffee plants.

MATERIALS AND METHODS

Plant materials

Field trials were conducted using the 2 years old plants of cultivars Iapar59 (I59) and Rubi MG1189 of *C. arabica*, the former being considered more tolerant to drought than the latter (M.A.G. Ferrão, personal communication), grown in field condition at the experimental station of the Embrapa Cerrados center (Planaltina-DF, Brazil, 15°35'43"S - 47°43'52"O).

Field experiment

The experiment was designed with three blocks (I-I, NI-I and NI-NI) subdivided into plots corresponding to points of analysis (noted "P" in Figure 1). Each plot consisted in three parallel lines of thirteen plants for each cultivar, the middle line containing utile plants and the two lateral ones being border lines. The distances of planting were 3m between rows and 0.7m within rows. Water supply was provided by sprinklers (1.5 m height) organized in the field to perform uniform irrigation. Soil moisture was controlled using PR2 profile probes (Delta-T Devices Ltd, Cambridge-UK). Irrigation was supplied (mainly during the winter season from June to September) when soil moisture reached 0.27cm^3 $H_2O.cm^{-3}$. Points of analysis were defined as follows: P1 (06/2008), P2 (08/2008), P3 (11/2008), P4 (05/2009), P5 (08/2009) and P6 (02/2010).P2 and P5 corresponded to the points of analysis during the dry season, respectively for the first and second year (Figure 1).

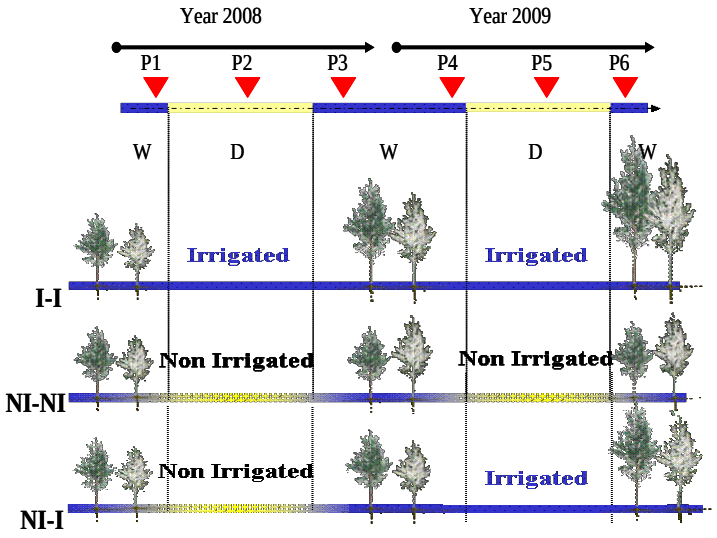

Figure 1. Schematic representation of water treatments applied to coffee plants. W: wet season (from October to April); D: dry season (from May to September). At each point of analysis (P1 to P6), plants were collected for a complete description of their architecture and their root system was excavated.

Measurements of leaf water potential

For P2 and P5 analysis points, the water stress was evaluated by measurements of predawn leaf water potential (Ψpd) with a Scholander-type pressure chamber using fully expanded leaves corresponding to those of the third node of plagiotropic branches (Figure 2).

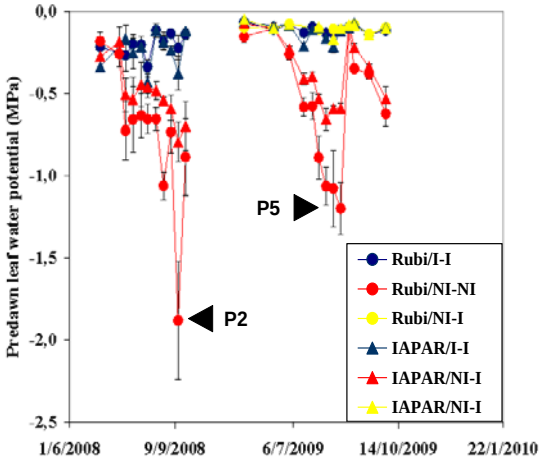

Figure 2. Leaf predawn water potential.

Sap flow

For each cultivars and each conditions (I-I vs. NI-NI), heat-dissipative (Granier) probes were installed on 5 coffee plants and data were stored during 9 months that overlapped P4 and P5 points of analysis (Figure 3).

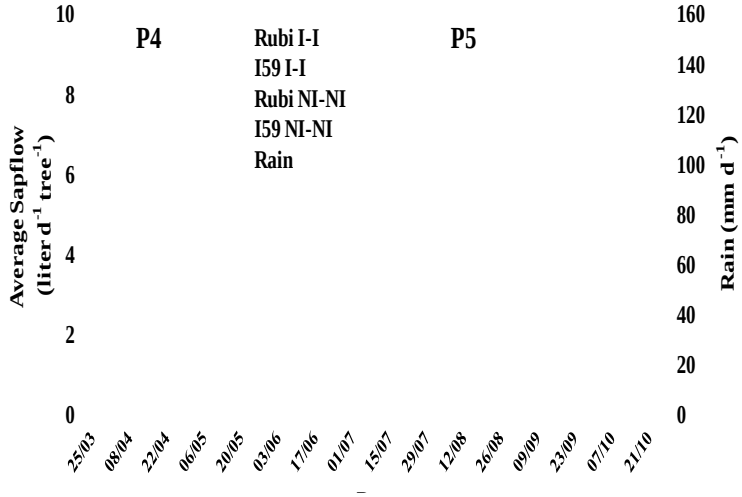

Figure 3. Sapflow and rainfall.

Analysis of $\Delta^{13}C$ analysis

Leaves (from P1 to P3) were dried and pulverized in liquid nitrogen before being analyzed for ^{13}C and ^{15}N composition (CENA Research center, Piracicaba-SP, Brazil) (Figure 4).

Plant biomass and morphological characteristics

For each cultivars and each conditions (I-I, NI-NI and NI-I), 10 to 7 plants were analyzed along the project (from P1 to P6) to evaluate all morphological characteristics like height, basal diameter as well as length internodes, leaf area index (LAI) for the trunk, primary and secondary ramifications. The same approach was also performed for the root system. In both case, all organs were dissected and dried separately to evaluate the dry biomass (Figure 5).

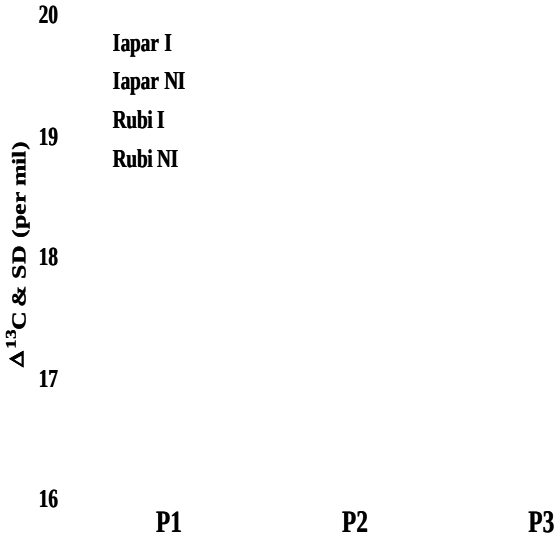

Figure 4. $\Delta^{13}C$ *vs.* treatments from P1 to P3.

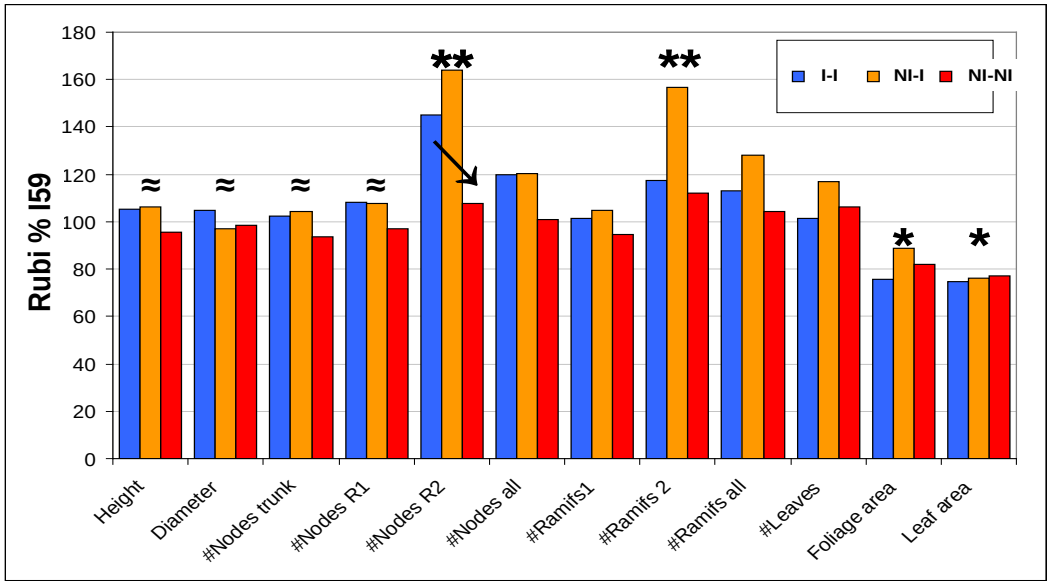

Figure 5. Comparison of plant variables of the two cultivars in P6. Values of Rubi are expressed in percents of I59 values.

RESULTS AND DISCUSSION

Under water stress conditions (NI condition), leaf predawn water potential (Ψpd) measured during the P2 and P5 dry seasons clearly showed less negative values for the cultivar I59 than for Rubi (Figure 2). At the same time, and for both cultivars, Ψpd of leaves collected in irrigated treatments ranged from -0.1 to -0.3 MPa, demonstrating the absence of water stress. Altogether, these results clearly indicated a better access to soil water for I59 than for Rubi cultivar.

Sapflows (in $L.d^{-1}.\ tree^{-1}$) were measured with Granier probes during the P4 and P5 points of analysis and compared to the amount of rain ($mm.d^{-1}$) during the same period (Figure 3).

Whatever the watering regime, data clearly showed higher values for Rubi than I59, indicating a higher transpiration rate (T) for Rubi with potentially a quicker soil dry-out.

Because drought is known to affect leaf ^{13}C composition through stomatal closure, isotopic discrimination ($\Delta^{13}C$) was also investigated in leaves from P1 to P3 (Figure 4). The analysis revealed higher $\Delta^{13}C$ values in I59 than in Rubi, indicating a lower intrinsic (leaf scale) water-use efficiency for I59 than for Rubi. At plant scale, WUE can be estimated by the ratio of biomass over transpiration (B:T). The biomass of the two cultivars being similar (data not shown) and T being lower in I59 than in Rubi, the WUE at plant scale was higher in I59 than in Rubi (Table 1). These results are to be confirmed by future $\Delta^{13}C$ measurements in leaves collected on plants from P4 to P6.

Table 1.

Traits	**159**	**Rubi**
Genetic origin	*Sarchimor*	*Catuai*
Tolerance to drought	**Higher**	**Lower**
Response to intense drought	Maintains its leaf	Loses its leafs
Leaf predawn water potential	Drops slowly during drought	Drops quickly during drought
Leaf area	20% higher	Unit
Number of leaves	Unit	5% higher
Canopy conductance + leaf area	Lower	Higher
Stomatal density	Similar	Similar
Transpiration	Lower	Higher
% biomass in shoots	Similar	Similar
% biomass in roots	Similar (decrease with age)	Similar (decrease with age)
% biomass in leaves	Similar	Similar
% biomass in reproduction	Similar	Similar
Total biomass	Similar	Similar
Root / Shoot ratio	Similar	Similar
Δ13C from leaves	Higher	Lower
WUE integrated at plant scale	Probably higher	Probably lower
WUE al leaf scale (A/g)	Probably lower	Probably higher

Regarding morphology, our data showed that, when irrigated, the I59 and Rubi cultivars had comparable (≈) developments of their trunk and primary branches in terms of length, diameter and number of nodes (Figure 5). However, they differed in their leaf size (the average area per leaf of Rubi being only 3/4 of the I59*) and their number of secondary branches (about 50% greater for Rubi than I59**). It is worth noting that water stress had a more pronounced depressive effect for Rubi than for I59, especially concerning the size of secondary branches

(↓). Even if the number of secondary branches was similarly reduced for both cultivars under water stress conditions (NI-NI), their apparition appeared to be delayed more for Rubi than for I59 as observed by the smaller nodes number for Rubi.

The main characteristics and differences observed between I59 and Rubi cultivars are given below. Further analyses are carried on to better understand the role of these differences in drought tolerance.

ACKNOWLEDGMENTS

This project was supported by the supported by CIRAD (ATP 2007/01) and Embrapa. Bruno Rapidel provided a coffee-specific calibration for the Granier probes.

Effects of Water Stress on Bean Biochemical Composition of *Coffea arabica* cv. Rubi

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INTRODUCTION

According to the last report of the IPCC (Intergovernmental Panel on Climate Change), the global temperature is supposed to increase in the Tropical zone that will modify agroclimatic suitability of *Coffea arabica* plantation areas, mainly in Brazil. Several research projects have now begun to analyze the effects of water stress on coffee plants either at the physiological and molecular levels. However, nothing is known about the effects of prolonged dry seasons on bean size, biochemical composition and consequently on coffee beverage quality. In order to initiate such studies, levels of some biochemical compounds of coffee fruits (caffeine, total lipids, sucrose and chlorogenic acids) were evaluated by the near-infra red spectroscopy (NIRS) in beans produced from adult plants of *Coffea arabica* cv. Rubi, cultivated under different hydric regimes (RH) under field conditions of Brazilian Cerrado.

In this region, the climate is characterized by a rainy season (from October till April) during summer and a long dry season (from May till September) during winter. In such conditions, coffee cultivation is only possible under irrigation to avoid deleterious effect of water stress on coffee plants. Recent findings also indicate that a controlled water stress also helps a better synchronization of coffee flowering after the dry season. In the experimental station of Embrapa Cerrados, four hydric regimes (RH1 to 4) were tested on the field: RH1 corresponding to continuous irrigation and RH2 to RH4 corresponding to plants submitted respectively to around 30, 60 and 90 days of water stress before the return of irrigation to stimulate flowering. As a control, RH0 plants corresponded to those without any irrigation. The aim of this work was to use the NIRS analyses to evaluate the effects of water stress on the biochemical composition of beans during three consecutive harvest periods (2007 up to 2009).

MATERIALS AND METHODS

Plant material and field experiments

Field trials were conducted using the 6 years old plants of Rubi cultivar of *Coffea arabica* grown in field condition. at the experimental station of the Embrapa Cerrados center (Planaltina-DF, Brazil, 15°35’43”S - 47°43’52”O) in full-sun condition under five hydric regimes (RH) characterized as follows: RH0, without irrigation (only subjected to natural rainfall); RH1, always irrigated during the year; RH2, without irrigation during 30 days of the

winter season; RH3, without irrigation during 60 days of the winter season and RH4, without irrigation during 90 days of the winter season. During the 3 years of this experiment, the date of the return of irrigation was fixed for the 4th of september for RH2 to 4, the flowering that occurred around 10 days after this date. Afterwards, from flowering up to the bean harvest, water soil moisture was controlled and regular irrigations are performed by a circular pivotal system to always maintain water level upper than 0,27cm^3 $H_2O.cm^{-1}$ even during the rainy period. Different fertilization conditions of N, P and K were also tested as follows: N Treatment (N1:0, N2: 100, N3: 250, N4*: 500 and N5: 800 kg $N.ha^{-1}$), P treatment (P1:0, P2: 50, P3: 100, P4*: 200 and P5: 400 Kg $P_2O_5.ha^{-1}$) and K treatment (K1:0, K2: 100, K3: 250, K4*: 500 and K5: 800 kg de $K_2O.ha^{-1}$). For each condition, 3 biological repetitions were used leading to 225 samples studied (3 NPK treatments x 5 doses NPK x 3 biological repetitions x 5 RH conditions). (*): fixed values of fertilizer used in combination of tests.

Fruit sampling and processing

Under the present conditions, and depending of year rainfalls, water stress accelerated fruit ripening by approximately one month, with a full ripening of fruits estimated at 210 DAF and 240-260 days after flowering (DAF) for RH0 and RH1 to 4 conditions, respectively. Fruits were harvested at maturity that corresponded to cherry fruits with red pericarp turning to purple and containing hard white endosperm (over-ripe cherries with a dried pericarp turning brown to black, were not considered). Fruits were sun-dried for 15-20 days until beans reached 10-12% humidity levels and then processed mechanically to remove dried pericarp and endocarp (parchment).

Chemical analysis

Chemical analyses were performed on green coffee beans by near infrared spectrometry (NIRS) by reflectance (Williams and Norris, 1990) of green coffee (50 g) using a NIR spectrometer system (NIRS model 6500, FOSS, Port Matilda, Pa.). Sucrose, total lipids, caffeine and chlorogenic acids (CGA) contents were measured using dry beans (30-50 g) that had been equilibrated (for 6 days at 60% humidity and 28 °C) prior to being frozen in liquid nitrogen and ground (<0.5 mm). A NIR spectrum was acquired in reflectance (R) mode in the 1,104- to 2,456-nm range (Downey and Boussion, 1996; Downey et al., 1994; Scanlon et al., 1999) and compared with previously established calibration curves (Decazy et al., 2003; Guyot et al., 1993) Data were treated by Winisi 1.5 (NIRS2 4.0) software (Intrasoft Int., Port Matilda, PA).

Statistical analysis

Within each RH condition, three randomized blocks of five plots were set up for each fertilizer N, P, and K, and each fertilizer was applied at five levels in the three blocks with a constant level of the two other fertilizers. Three distinct experiments were therefore considered and analysed separately. Data of each year were analysed separately because of very different climatic conditions between the three years. Data were analysed using a mixed model analysis of variance. Factors RH and fertilizer were considered fixed, and factor block within RH was considered random. For each fertilizer, the observed value Y_{ijk} corresponding to RH i, bloc j within RH i, and fertilizer level k, was modelled as follows:

$$Y_{ijk} = m + a_i + B_{ij} + c_k + (ac)_{ik} + E_{ijk}$$

where m (overall mean), a_i (fixed effect of RH i), B_{ij} (random effect of bloc j within RH i), c_k (fixed effect of fertilizer k), $(ac)_{ik}$ (interaction RH x fertilizer) and E_{ijk} (random residual error).
All the computations were performed using the statistical software package *R* (R Development Core Team, 2007), version 2.6.0. The analyses of variance were performed using the R package Nlme (Pinheiro et al., 2007).

RESULTS AND DISCUSSION

The results presented here were only focused on the effects of RH conditions, excluding interactions with fertilizer and RH (see also Vinecky et al., in the same issue).

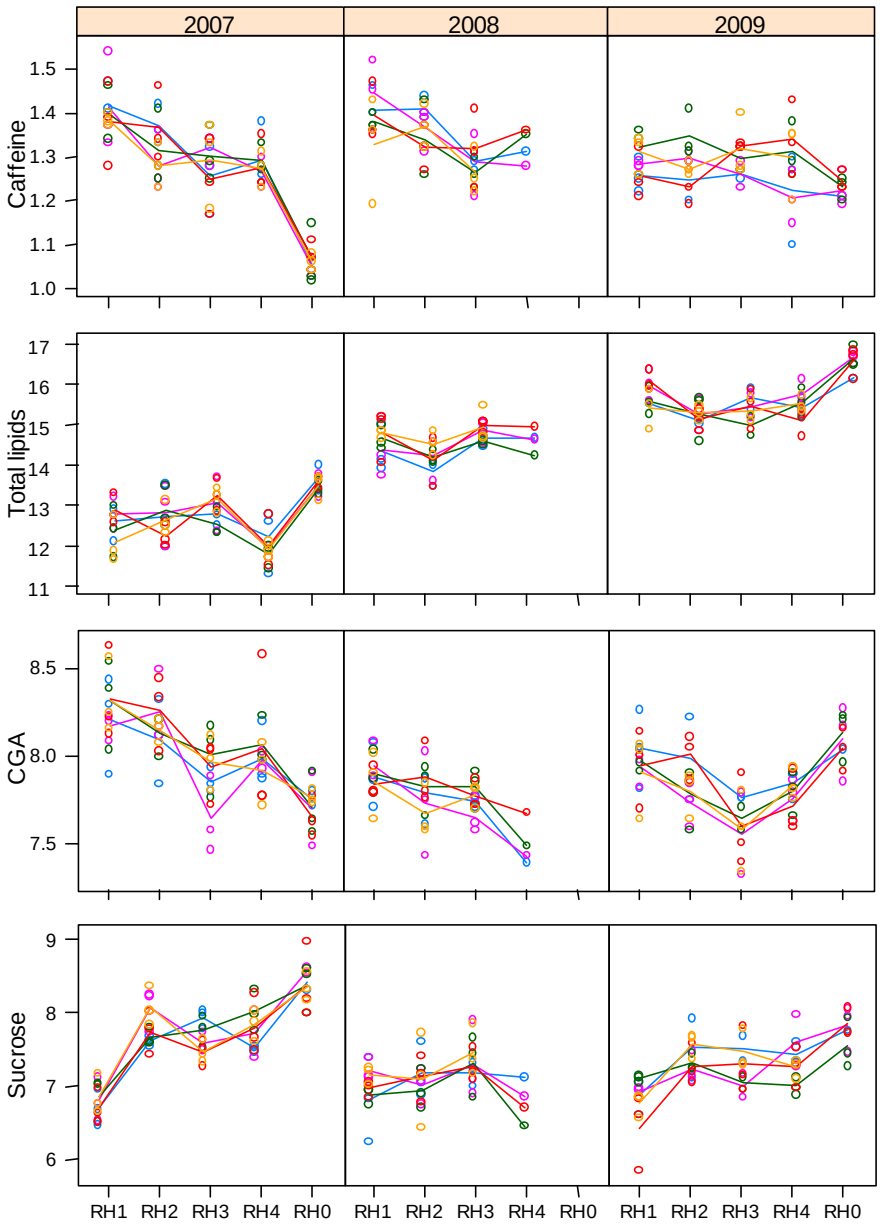

Figure 1. Evolution of biochemical contents of caffeine, total lipids, chlorogenic acids (CGA) and sucrose expressed in % of dry matter of mature beans with hydric regimes (irrigated: RH1 to RH4 and non-irrigated: RH0) and the year of harvest. Due to a severe drought period during in the winter 2007, RH0 fruits were not produced in 2008.

Values of caffeine, total lipids, chlorogenic acids (CGA) and sucrose were obtained from P treatment.

The main conclusions of this study are:

- contents of caffeine, chlorogenic acids [CGA] and sucrose were relatively similar during the 3 years of analysis,
- contents of total lipids were subjected to major fluctuations, ranging for 12-14% in 2007 to 15-16% in 2009,
- there is significant effect of water stress on caffeine and chlorogenic acids [CGA] (in 2007 and 2008) with decreasing contents of these biochemical compounds when increasing water stress during the winter season,
- there is significant effect of water stress on sucrose (in 2007 and 2009) and total lipids (in 2007, 2008 and 2009) with increasing contents of these biochemical compounds when increasing water stress during the winter season.

Except the RH0 treatment, it is worth noting that plants did not suffered of water stress during bean development period (plants under RH1 to RH4 were grown without water stress from the flowering to the harvesting period of mature beans). Therefore, the variations of bean biochemical composition presented here were more related to the water stress applied to the plants during the winter season (after the harvest and before the flowering). Even reduced during the winter season, these results suggest that the plant metabolism that occurred during this season influenced the final biochemical characteristics of coffee beans. The capacity of coffee plants to accumulate storage compounds (i.e. polysaccharides like starch) for example in stems and roots could be directly responsible of these variations.

ACKNOWLEDGMENTS

This work was supported by FINEP (Qualicafé), CNPq, CBP&D/Café, Cirad and FAPEMIG.

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High-Throughput Sequencing of cDNA Extracted from Meristems of Coffea arabica cv. Rubi and Iapar59 Submitted to Different Water Field Conditions

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SUMMARY

In order to study the molecular mechanisms underlying the response to drought stress in coffee plants, meristems of plagiotropic branches from Iapar59 (I59, drought tolerant) and Rubi (R, drought susceptible) cultivars of *Coffea arabica* grown in field-grown conditions and submitted (NI: non-irrigated) or not (I: irrigated) to water stress were collected at the end of the dry season and used to generate cDNAs that were sequenced using the GS-FLX Titanium strategy. The crude analysis of sequencing data revealed 282.213, 135.304, 345.751 and 230.064 reads obtained from I59-I, I59-NI, R-I and R-NI samples, respectively, totalizing more than 255Mb. For both experiments, most of the reads (>65%) had high quality and were assembled to generate around 24.000 contigs with an average length above 500pb. These data, which are the first one coffee meristems subjected to water stress, will be compared with all available transcriptome *Coffea* data, including the EST sequences from both *Coffea arabica* and *C canephora*. The results of these comparisons will be presented as well as the preliminary data of an electronic northern performed in order to identify differentially expressed genes between the two cultivars in both conditions (NI or I).

INTRODUCTION

It is well known that drought periods affect coffee plant development, leading to plant death and abortion of developing fruits in case of severe drought, or affecting flowering or bean development in case of moderate stress (DaMatta and Ramalho, 2006). In relation to coffee genetic diversity, several works reported the identification of plants of *C. canephora* var. Conilon susceptible or tolerant to drought (Ferrão et al., 200; Fonseca et al., 2004) which were analyzed at the physiological level (DaMatta et al., 2003; Lima et al., 2002; Pinheiro et al., 2004; 2005) and also used to identify candidate genes underlying stress responses (Vinecky et al., CD-rom). Even narrow, a genetic diversity for drought tolerance also exist in the species *C. arabica*. In addition to the identification of undiscovered transcripts, the recent development of low-cost, high throughput next-generation (NGS) sequencing technologies now opens the way to perform expression profiling and to identify gene presenting differential expression patterns by comparing the frequency of reads obtained after sequencing. In order to initiate such kind of approach in coffee, RNA of meristem tissues from Iapar59 (I59, drought tolerant) and Rubi (R, drought sensible) cultivars of *C. arabica* grown under field-grown with (I) or without (NI) irrigation were extracted and used to generate cDNA that were further sequenced. The preliminary results of this study are presented here.

MATERIALS AND METHODS

Plant materials

Field trials were conducted using the 2 years old plants of cultivars Iapar59 (I59) and Rubi MG1189 of *C. arabica*, the former being considered more tolerant to drought than the latter (M.A.G. Ferrão, personal communication), grown in field condition at the experimental station of the Embrapa Cerrados center (Planaltina-DF, Brazil, 15°35'43"S - 47°43'52"O).

Field experiment

For both cultivars, plants were cultivated with (I) and without (NI) irrigation during the dry season. Under the irrigated (I) condition, water was supplied by sprinklers (1,5m height) organized in the field to perform uniform irrigation. Water soil moisture was controlled using PR2 profile probes (Delta-T Devices Ltd, Cambridge-UK) and irrigation was supplied when soil moisture reach 0,27cm^3 $H_2O.cm^{-1}$.

Sampling procedures

For 454 analysis, apex (around 25 to 30) of plagiotropic branches were day collected (between 10:00 to 12:00 am) during the dry season from plants of I59 and Rubi field-grown under I and NI conditions. At the date of harvest (20/08/2009), the water stress was evaluated by measuring predawn leaf water potential (Ψ_{pd}) with a Scholander-type pressure chamber using fully expanded leaves corresponding to those of the third node of plagiotropic branches (Table 1). Values obtained were -0,12 ± 0,00, -0,11 ± 0,00, -0,59 ± 0,03 and -1,20 ± 0,16 Mpa respectively for I59-I, R-I, I59-NI and R-NI. After cuttings, apex were incubated for 5 min. in the washing buffer (66% chloroform, 33% methanol, 1% HCl) (Lécolier et al., 2009) and further incubated twice (30min.) under vacuum in the fixation buffer (25% acetic acid, 75% ethanol RNAse-free) cooled at 4 °C, changing the buffer between each incubation. Samples were stored in 75% ethanol RNAse-free and then dissected under binocular to separate the apex.

RNA extraction

Total RNA was extracted from the apex as described previously (Geromel et ali., 2006).

cDNA synthesis and sequencing

Apexes were separated under binocular microscope dissection from plagiotropic buds from three different plants for each condition and then ground to powder in liquid nitrogen using a mortar and pestle. Total RNAs were extracted using the Nucleospin RNA Plant kit (Macherey-Nagel), including a DNAse-I treatment. Quality and quantity of RNAs were checked with a Bioanalyzer (2100, RNA Nano 6000 Agilent). The 1st strand cDNA synthesis was performed using 1µg total RNA and following the SMARTer cDNA Double-stranded cDNA was then produced using 22 to 25 independent 2nd strand synthesis reactions and quantify with by Bioanalyzer (Agilent).

cDNA sequencing

cDNA sequences were then sequenced performing 2 runs (1 library per cDNA sample x 2) using GS-FLX Titanium (Beckman Coulter Genomics SA, Grenoble, France). This generated around one million of reads corresponding to more than 255 Mb.

Bioinformatics analysis of sequencing data

All dataset were inspected for low quality reads as well as 454 adaptors that were identified by SSAHA2 software (Ning et al., 2001). A reference full trancriptome was then built using *C. arabica* reads coming from the present project as well from the Brazilian Coffee Genome Project ESTs (Vieira et al., 2006) and available in GenBank public database, assembling all sequences (454 and sanger reads) using MIRA software (Chevreux et al., 2004). This full transcriptome reference was further used to map (using MIRA) all 454 libraries individually. The number of sequences anchored in each contig was analyzed using DEGseq (Wang et al., 2010), this information was used to normalize the data and identify the differential expressed genes between the libraries. *Coffea* transcriptomes were then annotated using Blast2go software (Conesa et al., 2005). The same program was also used to group by biological process datasets in Gene Ontology the following datasets: i) all genes from total transcriptoma; ii) differentially expressed genes in I59 looking between irrigated and non irrigated; iii) differentially expressed genes in Rubi looking between irrigated and non irrigated; iv) differentially expressed genes in irrigated library looking between Rubi and I59; v) differentially expressed genes in non-irrigated library looking between Rubi and I59.

RESULTS AND DISCUSSION

The reference assembly was formed by a total of 55578 clusters corresponding to 54628 contigs and 950 singlets. From the total raw 454 data, around 20% were discarded, mainly by low quality. These reads have, in average, 236bp (after adapter removal) and more than 85% of them were mapped against the reference (Table 1).

Table 1. Statistics of all reads mapped against the Core Assembly (reference assembly).Cultivars (R: Rubi and I59: Iapar59) of *C. arabica* and field conditions (I: irrigated and NI: non-irrigated) are indicated. Reads: total (T), cleaned (C), average read length in bp (A), mapped (M) and non- mapped (NM). RA: reference assembly.

Library	T reads	C reads	A reads	T clusters	Contigs	Singlets	M reads	NM reads
RA	1166084	876503	-	55578	54628	950	-	-
I59-NI	135304	109005	260	28408	14817	12353	77777	13118
I59-I	282213	207516	211	34941	22701	12240	132962	29615
R-NI	230064	173693	233	37679	25499	12180	117827	23404
R-I	345751	250413	241	33703	20984	12719	166476	36490
Total	993332	740627	-	134731	84001	49492	495042	102627

We found many enriched GO terms between the differentially expressed genes in these libraries and the total transcriptome (p-value <0.05). A hypergeometric statistic (Martin et al.,

2004) was used to calculate a *p*-value for libraries against the reference transcriptome and identify the enriched GO terms. For example, genes involved with stress response appeared over-represented in the drought-susceptible Rubi cultivar in comparison with I59 (Figure 1).

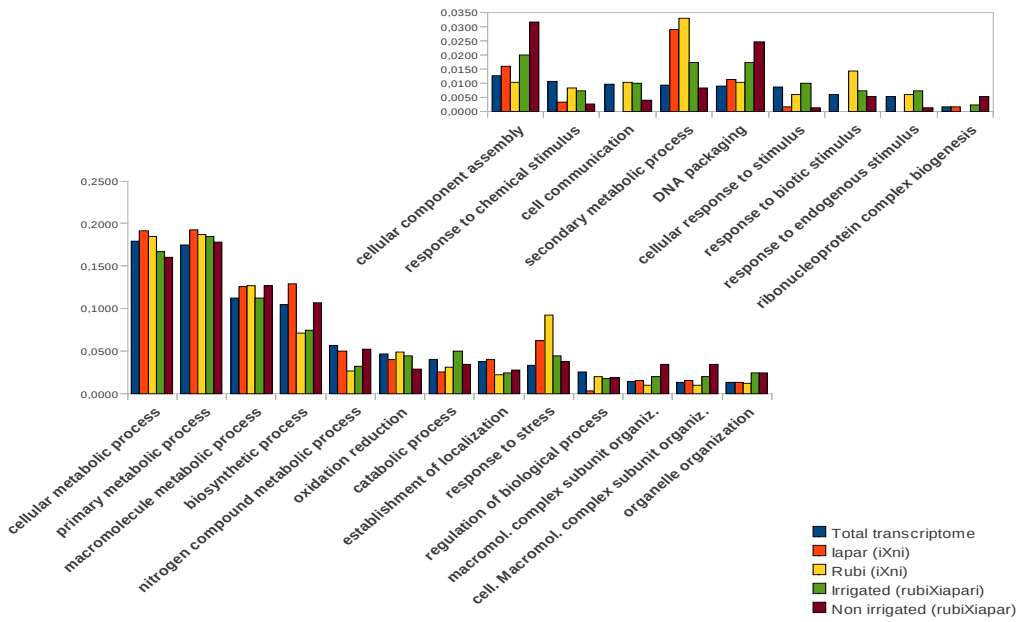

Figure 1. Gene ontology (GO) terms enriched for any library in comparison with the total transcriptome. The Y axis indicate the number of genes normalized by the total of mapped genes from each library.

This could be related with the fact that I59 presented higher Ψ_{pd} than Rubi at the time of harvest, demonstrating that the former cultivar as higher water use efficiency than the second. It is worth noting the number of genes up-regulated in Rubi non irrigated (NI) and involved with drought stress response, like *RD22*, *PDIR10* (dirigent-like protein), *MYB* and caffeine synthase for example, while these genes were not detected under this stress in I59 . In other hand, many drought genes related to drought-stress tolerance were detected in the I59 cultivar involved in biosynthetic process such as sugar transporters, some proteins related with abiotic stress tolerance (i.e. osmotins), water channel protein, LEA proteins (implicated in detoxification and alleviation of cellular damage during dehydration) and heat shock proteins (HSPs). In the I59 cultivar, genes coding for enzymes involved in the ABA synthesis (isopentenyl diphosphate isomerase, geranylgeranyl reductase), which causes stomatal closure, precursor and enzymes, were also highly expressed.

ACKNOWLEDGMENTS

This project was supported by the supported by CIRAD (ATP 2007/01), INRA and Embrapa.

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Current Status and Management of Coffee Leaf Rust Disease in Rwanda

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SUMMARY

Coffee is the most important cash and export crop which is a source of income to many small-scale farmers in Rwanda, and earns the country much needed foreign currency. However, the crop suffers heavy yield losses due to a wide range of factors including pest and diseases, especially coffee leaf-rust (CLR), caused by the fungus *Hemileia vastatrix*. Coffee leaf rust causes yield losses averaging 40%, but can be as high as 100 % in severe cases. Studies were initiated under a collaborative project which is funded by the Common Fund for Commodities, executed by CABI, and supervised by the International Coffee Organization. Objectives of the studies were to assess the incidence, distribution, severity and management of CLR in Rwanda. After establishing incidence and severity of CLR through biological surveys, a series of experiments (screening of fungicides and varieties) were implemented to come up with the most effective and environmentally sound control measure(s). A number of contact and systemic fungicides provided up to 50% CLR control. Cyproconazole (Alto) resulted in significantly ($P \leq 0.05$) better control than the control treatment. A botanical pesticide, ground pawpaw leaves did not control CLR. The results are discussed in light with other CLR management options in Rwanda.

INTRODUCTION

Coffee is the most important cash and export crop, which is a source of income to many small-scale farmers in Rwanda, and earns the country much needed foreign currency. However, the crop suffers heavy yield losses due to a wide range of factors including pests and diseases, especially the coffee leaf-rust (CLR), caused by the fungus *Hemileia vastatrix*. Coffee leaf rust causes yield losses averaging 40%, but can be as high as 100 % in severe cases.

For the management of CLR a number of studies have been initiated under a collaborative project, which is funded by the Common Fund for Commodities, executed by CABI, and supervised by the International Coffee Organization. Objectives of the studies were to assess the incidence, distribution, severity and evaluate control practice that is used to control the disease in Rwanda; to screen fungicides and varieties for the most effective control measure of the disease.

MATERIALS AND METHODS

Biological surveys were carried out in different coffee production areas in all five provinces in Rwanda. Coffee farmers were randomly selected for interview and field diagnostic was also made. Before each farm visit a small discussion with the farm owner was held to explain about the survey and discuss the current status of the farm to be surveyed. In each farm, 30

trees were randomly selected on a diagonal transect and assessed for diseases and general plant health. Coffee leaf rust incidence was assessed using a scale of 1 to 4 (1: no CLR, 2 = <10% diseased leaves; 3 = 10-30% diseased leaves; 4 = >30% diseased leaves), the severity was assessed by estimating the number of pustules per leaf. A total number of 303 farms were surveyed.

For screening fungicides and varieties, on-farm and on-station trials were established. Six fungicides (Alto, Benlate, Copper oxycloride, Dacobre, Kocide and papaya extract) were tested. Alto and Benlate are systemic fungicides, Copper oxycloride, Dacobre, Kocide are contact fungicides while papaya extract is botanical fungicide. Fungicide trials consisted of 7 treatments including control. For on-station trials a randomised complete block design (RCBD) was used with four replications. Seven on-farm trials were established in different regions, one farm being a replicate. Fungicides were applied at recommended rate for field application. Copper oxychloride, Dacobre and Kocide were applied by adding 50 mg the knapsack sprayer containing 15 litres of water, mixing thoroughly and spraying onto coffee leaves to runoff (*ca*. 2 kg of the fungicide per ha assuming an application volume of 600 L/ha). Benlate, alto and papaya extract spray solutions were prepared by mixing 5 g, 10 ml and 300ml respectively to 15l of water in a knapsack sprayer. Initial application of the fungicides was made in October 2009 and subsequent sprays were done every month except Alto and benlate, which were applied once. Data on incidence and severity of CLR were also recorded per month prior to spray application.

For screening varieties, two clones (5A and 6) were received from India and were tested under nursery conditions together with existing varieties (Harrar, Jackson 2/1257 and BM 139) for their resistance to CLR. The incidence and severity of CLR in the trials were assessed every month for three months and data were combined for analysis. For the incidence, a scoring scale of 1 to 4 was used (1: no CLR, 2 = <10% diseased leaves; 3 = 10-30% diseased leaves; 4 = >30% diseased leaves) and the number of pustules per leaf was estimated for severity assessment. These varieties are also being tested under field conditions.

RESULTS AND DISCUSSIONS

Survey studies

The survey demonstrated that CLR was the most damaging coffee disease in all areas. A total of 85% of sampled farms were infected by rust in Rwanda. Another alarming issue is that all released varieties were susceptible to Coffee Leaf Rust. The results also showed that CLR is a problem in the middle and low altitude areas of the country.

Most surveyed farms were infested by CLR (Figure 1), since only 15% of the farms had no CLR. The farms had CLR incidence of between 10 and more than 30% (Figure 1 and 2), implying that CLR is a very important disease in Rwanda. The result has provided a detailed information on prevalence of CLR in Rwanda and will help in targeting efforts to managing this important disease of coffee. During the surveying period, there was no CBD. Regarding the control of CLR, the survey indicated that only 20% of farmers use copper based fungicides while the remaining percentage does not apply any control technique. Based on these results a series of farmer field schools were established in the different infestation zones to train farmers on the management of coffee plantations in general and coffee pests and diseases in particulars.

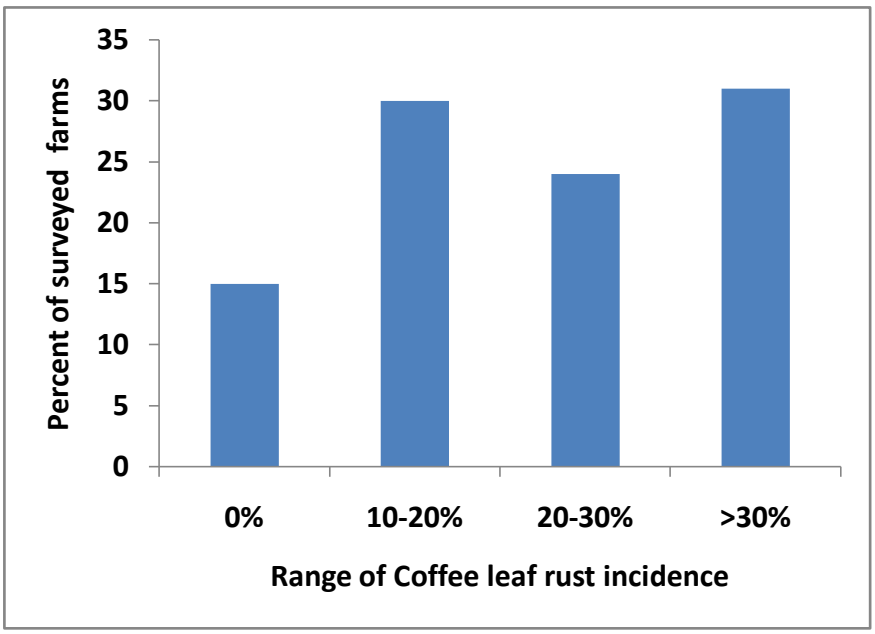

Figure 1. Percentage of farms under each Coffee leaf rust incidence range in Rwanda.

Figure 2. Infected coffee farm with CLR in Eastern Province (Kayonza).

On-station and on-farm fungicide trials

Among the six tested fungicides in Research station field trial, Alto, Benlate and Copper oxychloride were promising in controlling CLR while the other three remaining fungicides were not significantly different from the control (unsprayed plots) ($p>0.05$ for all the assessment times) in most of the assessment periods (Figure 3). The best fungicide was Alto in term of CLR incidence and severity reduction followed by benlate and copper oxycloride

(Figure 3 and 4). However, these results are still preliminary and need to be confirmed at the end of the trials in December 2012.

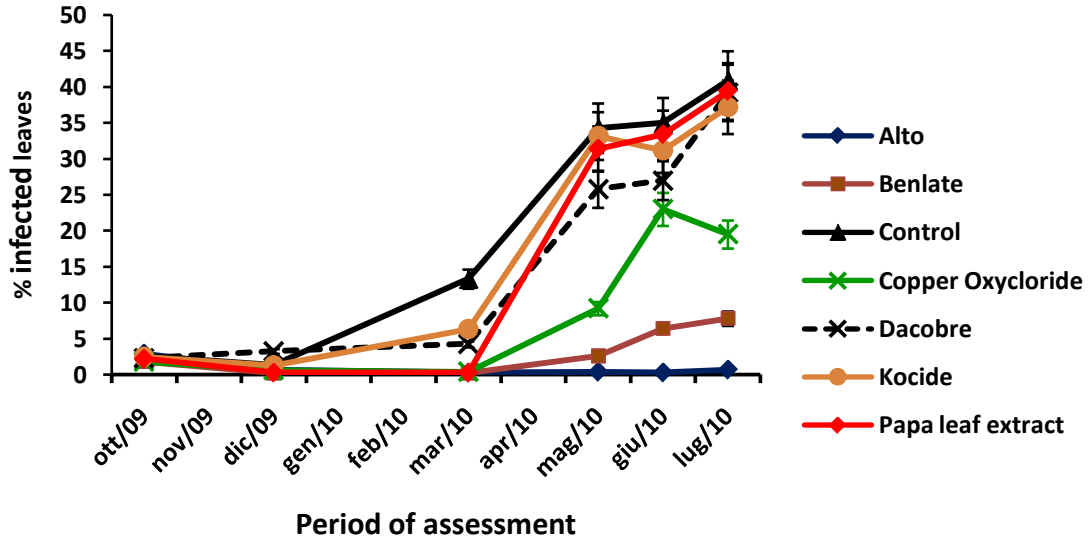

Figure 3. Assessment of Coffee leaf rust incidence in field trial on-station (Rubona). Bars represent the standard errors of means.

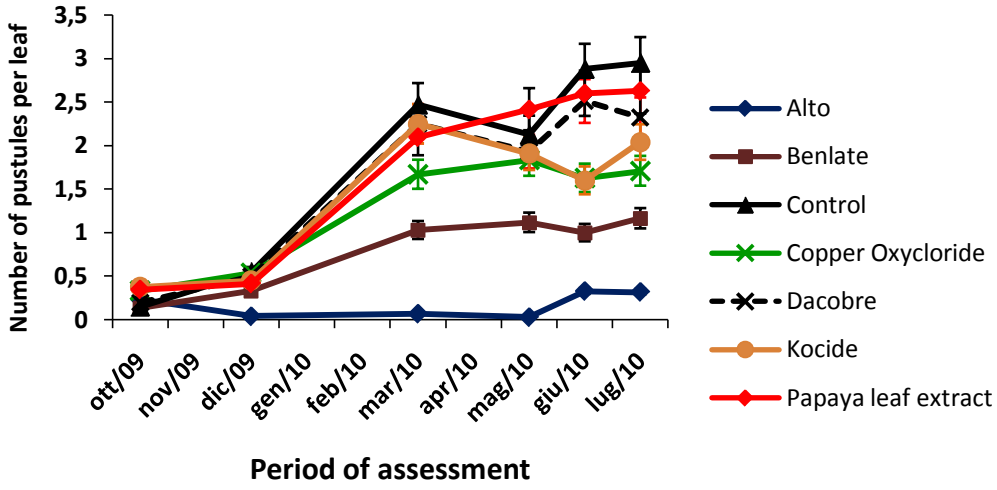

Figure 4. Assessment of Coffee leaf rust severity in field trial on-station (Rubona). Bars represent the standard errors of means.

In the trials, the incidence was generally high from May to July in the different treatments. During this period no chemical pesticides were sprayed as it corresponded to the harvesting period of coffee berries. The high rainfall of April and May could also have favoured the development of the disease in coffee plantations (Bock, 1962; Pedro Jr, 1983).

Copper based fungicides have been relied upon for the control of CLR in Rwanda (OCIR Café, 2008). However, these are not always effective. Reason for the lack of success with these contact fungicides would include the poor coverage of leaves and poor timing of the initial application; often coffee growers wait until they see plant damage before making the first application, by this time application of proctectant fungicides does not provide an efficient control.

The incidence and severity observed in on farm trials due to fungicide application varied much between the sites and not much difference were observed between treatments within the same site. However Alto, was the most effective and consistent for all trials. Gicumbi and Rusizi trials were less affected by CLR compared to the other sites probably because of the high altitude in Gicumbi and the type of coffee variety (Pop) grown in Rusizi.

Variety trials

Among the varieties tested under nursery conditions for their resistance to CLR, the two clones from India did not show any attack by the fungus suggesting that there were more resistant compared to existing tested varieties (Figure 5). However, the races to which these are resistant need to be determined. The variety Jackson, commonly planted in Rwanda was the most susceptible to CLR followed by BM 139. These varieties are being tested under field conditions and data taken for analysis.

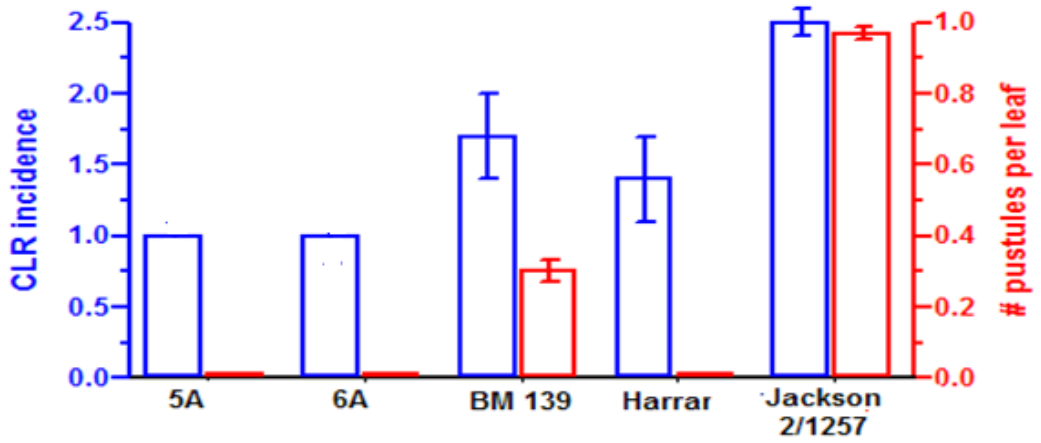

Figure 5. Incidence and severity of CLR on coffee varieties under nursery conditions. The incidence was scored from 1 to five 5 rating scale (1: no CLR, 2 = <10% diseased leaves; 3 = 10-30% diseased leaves; 4 = >30% diseased leaves).

CONCLUSIONS

Coffee leaf rust is the most damaging disease of coffee in Rwanda. This disease is more severe in low and middle altitude regions in the country. Farmers do not know how to control this disease, thus Farmer Field Schools were established to help these farmers in coffee management system, and pest and disease management.

Of the six fungicides tested against CLR, the systemic fungicide Alto was the most promising. However, these results are still preliminary and need to be confirmed. After confirmation, Alto should be recommended for application to control CLR in alternation with the copper oxychloride to reduce the risk of developing resistance.

The clones 5A and 6 were resistant to CLR under nursery conditions, and field trials are being carried out to confirm these findings.

ACKNOWLEDGEMENT

We acknowledge ICO and CFC for sponsoring the Coffee Rust Project, CABI-Africa and ISAR for the execution and implementation of the CLR-Project.

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Evaluation of Coffee Varieties Derived from Diverse Genetic Sources of Resistance for Prospective Exploitation. An International Cooperative Effort

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SUMMARY

Coffee leaf rust (CLR) caused by the obligate parasitic fungus *Hemileia vastatrix* Berk & Br. and Coffee berry disease (CBD) caused by *Colletotrichum kahawe* are the two most important diseases of coffee (*Coffea arabica* L.) causing considerable economic damage. While CLR is widely distributed in coffee growing countries round the globe, the CBD is restricted to African countries. Although coffee farmers are mainly relying on copper based prophylactic fungicidal sprays for management of these diseases, timely chemical sprays are quite often constrained with resource crunch. Development of disease tolerant cultivars ensures sustainable coffee production especially for the small and medium farm holdings. In order to address the problem of CLR and CBD, there is an urgent need to focus research efforts on searching for new sources of resistance, exploitation of the available genetic resources for development of new varieties and to provide resistant and resilient coffee cultivars to farmers. Nevertheless, it is imperative to test the potential varieties under realistic conditions to assess their performance.

The present paper deals with some important leads of an international initiative involving India and four African countries, Kenya, Uganda, Rwanda and Zimbabwe to address CLR and CBD problems through an CFC supported programme. Two Indian varieties (Sln.5A, a derivative of the cross Devamachy x Rume Sudan collection and Sln.6, a Robusta x Arabica hybrid) were selected as potential sources of resistance to the two diseases and shared with the participating countries for field evaluation under different disease pressures. Among the varieties evaluated in on-farm and On-station trials, Sln.6 recorded superior performance with respect to seedling growth and juvenile vigour. The two varieties manifested high field tolerance to rust both in India and Kenya. Laboratory screening of the two varieties against CBD at Coffee Research Foundation (CRF), Ruiru, Kenya revealed high levels of tolerance in Sln.5A. Monitoring pathogen diversity in India resulted in isolation of three new races, two from Sln.6 and one from Sln.5A. Analysis of the seedling progenies with DNA marker approaches enabled nine cultivar specific markers, useful for marker assisted selection and breeding. The potential aspects of these two Indian varieties as new sources of resistance to the African countries, application of DNA markers for tracking the homogeneity of seedling populations and marker assisted breeding programmes for integrating the CBD and CLR resistance are presented.

INTRODUCTION

Coffee is a commodity of interest worldwide and one of the most economically important crops in the tropics and sub-tropics. Coffee exports constitute an important source of foreign exchange earnings for over 50 countries involved in its cultivation. More importantly, majority of the coffee farmers in the producing countries are small growers who primarily depend on coffee for their livelihood. On the other hand, because of high reliance of majority of the producing countries on export markets, the prices are quite often influenced by supply-demand mismatches. In this realm, the coffee farmers especially the small grower segments are always vulnerable to the price crisis forcing to abandon the routine farm operations. Further, the pest and diseases continue to be the principal production constraint in many countries, especially the small holdings because of insufficient resources to take up timely control measures. Among the diseases affecting coffee production, coffee leaf rust (CLR) caused by the obligate parasitic fungus *Hemileia vastatrix* and coffee berry disease (CBD) caused by *Colletotrichum kahawe* are the most serious ones. Asia and Africa are the two regions mostly affected by CLR and CBD respectively, leading to higher production costs compared to other parts of the world. In recent years, the gradual climate changes leading to warmer conditions and erratic rainfall patterns in many coffee growing regions, creating ideal conditions for flaring up of diseases and pests causing additional stress on coffee cultivation. Considering the economics of disease managements and also to discourage the chemical control measures, development of disease tolerant cultivars is the most effective and viable option for sustainable coffee cultivation. Although systematic breeding programmes undertaken across the continents resulted in development of resistant varieties, breakdown in resistance has been commonly observed due to the evolution of new virulent races of CLR pathogen especially in countries like India where the climatic conditions are favourable for rust disease build up (Prakash et al., 2005). Nevertheless, inspite of the occurrence of large number of virulent races of the rust fungus, some of the coffee (*Coffea arabica* L.) varieties developed by the Indian Coffee Board manifest high levels of field tolerance to leaf rust. In Africa on the other hand, most of the varieties now being grown are susceptible to both CLR and CBD. Hence, the coffee research community should come together and pool all available resources in order to address the challenge of evolving resistant and resilient cultivars for the coffee farming sector.

The paper is an outcome of one such international cooperative effort where India and four African countries, Kenya, Uganda, Rwanda and Zimbabwe joined together to tackle the problems of CLR and CBD through an CFC supported programme (CFC/ICO/40) coordinated by CABI, Africa. Two Indian varieties (Sln.5A and Sln.6) were shared with the participating countries for evaluation and to test their potential under different disease pressures. Field trials have been established both on-farm and on station, covering a wide range of climatic and agronomic conditions, with the two selected Indian materials along with local coffee varieties. In India too, systematic field trials have been established with selected Indian varieties across the major growing zones, in order to test their potential under changing climate and disease pressures due to increased CLR pathogen diversity. In this communication, we present the preliminary findings pertaining to the field as well as laboratory experiments conducted on the two Indian varieties with respect to vegetative vigour, disease resistance, isolation and characterization of new rust races in Indian conditions, usefulness of the DNA markers in tracking the genetic purity of seed plots and also for marker assisted selection. The potential implications of the findings in developing disease tolerant varieties are discussed.

MATERIALS AND METHODS

Plant materials

Two Indian materials manifesting high field tolerance to leaf rust under Indian conditions (Sln.5A, derivative of Devamachy (spontaneous Robusta x Arabica hybrid) x Rume Sudan (wild coffee collection introduced from Kenya) and Sln.6 (derivative of the cross S.274 x Kents followed by recurrent back crossings to Kents) were selected to share with the African counterparts. Both Sln.5A and Sln.6 were registered under National Plant germplasm (No. INGR No.02009 and INGR 01042, respectively) of India. In order to study the field performance with respect to major diseases, CLR and CBD, selfed progenies of the two selected materials have been established in all the participating countries during 2008-2009 seasons. Laboratory screening of the materials against CBD was carried out at Coffee Research foundation, Ruiru, Kenya (Kenyan isolates) and at Coffee Rusts Research centre (CIFC), Portugal against all available isolates of CBD.

Field planting and data collection

In India, a total of 13 field trial plots (seven on-farm and six on-station) were established by 2009 planting season. The standard Indian variety, S.795 was planted as control. Only in nine trial plots, a new variety `Chandragiri has been included as second control to study the relative field performance. All the genotypes were planted in compact plots at a spacing of 1.8 x 1.8 m^2, under a mixed canopy of shade and a total of 600 to 1000 plants per genotype were established as a single block. The plants were trained on single stem system and standard agronomic practices were adopted. Observations were recorded on vegetative growth characters such as stem girth, length of primary branches, number of nodes per primary, inter-nodal length, during June 2010. For data collection, each plot was divided to five sub-plots of 50 to 100 plants each and observations were recorded from 10 plants per sub-plot at random, in total 50 plants per each variety covering 5 sub plots. In order to study the seedling vigour separate on-station nurseries were raised in five different locations during January to June 2010 and growth parameters (seedling height, girth, number of leaf pairs, leaf tip colour, tap root length, fresh and dry weights of shoot and root biomass) were recorded on 25 seedlings per variety (6 months old seedlings in the basket nursery). The data was subjected to the analysis of variance.

Monitoring CLR incidence in nucleus plots of target selections & characterization of rust

Observations on disease build up were recorded at monthly intervals at Central Coffee Research Institute (CCRI) and presented as mean percent incidence. Uredospores were collected from single pustule of the plants showing very mild susceptibility and characterized by cross inoculations on a set of differential plants as detailed by Rodrigues et al. (1965).

Screening tests against CBD

Screening experiments against CBD were performed at Coffee Research Foundation, Ruiru, Kenya (against a general inoculum of Kenyan isolates) and at Coffee Rusts Research centre (CIFC), Portugal (using Q2 isolate from Kenya and Ca1 from Cameroon). Inoculation was done on 100 six-week old hypocotyl seedlings using the method developed by Van der Vossen et al. (1976). The seedlings were scored individually, based on lesions developed on

the hypocotyl stems at 3 weeks after inoculation, on a scale of 1 to 12 (Van der Vossen et al., 1976) to assess the levels of susceptibility/resistance.

DNA marker analysis

DNA was isolated from the fresh leaves following the protocol of Krizman et al. (2000). Molecular marker analysis has been carried out using a wide array DNA markers viz., Sequence related amplified polymorphism (SRAP) and Random amplified polymorphic DNA (RAPD) to identify cultivar specific markers (Sln.5A, Sln.6, S.795 and Chandragiri). The SRAP and RAPD analysis were carried out following the procedures Li and Quiros (2001) and Williams et al. (1990), respectively with slight modifications suitable to coffee. A small random population of 8 plants per selection was used for initial screening and a total of 50 SRAP primer combinations and 100 RAPD primers were used. The identified cultivar specific primers were validated on a random population of 25 plants of each variety in first phase to check the homogeneity of the populations. Two sequence-characterized DNA markers closely linked to S_H3 gene, Sat244 and BA-124-12K-f (Mahe et al., 2008) were tested in S.795 variety a hybrid derivative involving *C. liberica* introgressed line (Prakash et al., 2002). The PCR assays using specific primer pairs (SCAR or SSR) and electrophoresis conditions were followed as described by Combes et al. (2000) and Mahe et al. (2008).

RESULTS AND DISCUSSION

Analysis of variance of seedling parameters, based on average values for each character revealed that the varietal differences were not significant ($p>0.05$) for most of the 8 characters studied, except for plant height. Location differences were significant ($p<0.05$) for most of the characters except for root fresh weight. This shows that genetic differences are not yet expressed in these varieties at this stage. Among the locations studied, the Technology Evaluation Centre (TEC) Mudigere appeared to be the best location. Observations on field trial plots revealed that the initial establishment and growth is good in all the varieties under evaluation. Analysis of data on juvenile vigour showed highest mean values for all the 4 characters namely length of primary (55.44 cm), internodal length (6.6 cm), nodes on primaries (7.86) and stem girth (19.51 mm) in Sln.6, compared to other three varieties. Varietal mean squares were significant ($p<0.05$) for length of primaries, internodal length and nodes on primaries and non-significant ($p>0.05$) for stem girth based on 4 varieties at 4 locations. Varietal mean squares were significant ($p<0.05$) only for internodal length and nodes on primary in case of 3 varieties (talls) at 6 locations. This shows that adding Chandragiri (being semi-dwarf) for evaluation has upgraded the genetic variance for the characters. Heritability estimates were high (87% when 4 varieties were considered and 66 % when only 3 varieties were considered while heritability was low (16 and 22%, respectively) for stem girth. Location-wise, CDF Yercaud appeared to show the best environment for the growth of all the 4 varieties as revealed by higher mean values for all characters at this location. However, this needs confirmation in later years.

Monitoring of leaf rust build up in nucleus plots of the target varieties revealed the mean percent incidence was 5.25 in Sln.5A, 1.51 in Sln.5B, 2.72 in Sln.6 and 4.78 in Chandragiri, indicating high field tolerance of these varieties to CLR. Characterization of rust samples collected from these varieties indicated the build up of two new races with gene combinations $V_{1,2,5,6,8}$ and $V_{2,5,6,8}$ from Sln.6 and race 37 ($V_{2,5,6,7,9}$), race 40 ($V_{1,2,5,6}$) and a new race ($V_{1,2,4,5,6}$) from Sln.5A. In general, the populations of Sln.6 and Sln.5A manifest high field tolerance to rust except few susceptible segregants in the expected frequency. The nature of resistance in these genotypes has still to be confirmed, but might be similar to that of Icatu

cultivars from Brazil of which some selections appear to have durable resistance to rust based on major as well as minor genes (Carvalho et al., 1989; Fazuoli et al., 1999). Earlier reports on periodic sampling of the rust samples on Sln.6 revealed the occurrence of rust races XXIV ($V_{2,4,5,}$) and XXV ($V_{2,5,6}$) (Ramachandran et al., 1979). Identification of new races with new virulent gene combinations on Robusta x Arabica hybrids, in the present study reflects the adaptive ability of *H. vasatarix* depending on host prevalence.

Screening against CBD indicated that the Indian materials Sln.5A manifested high tolerance while Sln.6 showed relative tolerance. The mean CBD score from three replications was 2.77 in Sln.5A and 6.30 in Sln.6 as against 4.15 and 10.36 in Ruiru 11 and SL28 respectively. Although some segregation was observed among individual genotypes in the population, there exists a vast potential for further selection for CBD resistance in Sln.5A. Interestingly, Sln.6 manifested field tolerance to Bacterial blight of coffee (BBC) as recorded in Kisii sub-station of CRF**,** Kenya. The mean per cent incidence of BBC from three replications was 3.33 in Sln.6 and 12.38 in Sln.5A as against 13.47 and 32.22 in Ruiru 11 and SL28, respectively (Gichimu, 2010 un published data). Similarly, screening of S.795, against CBD (Q2 isolate from Kenya) at CIFC, Portugal indicated low percentage of susceptibility (85.4%) as against 99.6% susceptibility in control (Catimor) after three weeks of inoculation. These preliminary leads demonstrated the great potential of Indian varieties as sources of resistance to major coffee diseases.

DNA marker analysis involving all the four selections included in varietal trials revealed very useful information. Out of 50 SRAP and 100 RAPD primers screened, nine cultivar specific SRAP primers (one for Sln.5A, five for Sln.6, two for S.795 and one for Chandragiri) and four cultivar specific RAPD primers (two for 5A, one for S.795 and one for Chandragiri) were identified. Validation of these cultivar specific markers on a random population of 25 plants confirmed that the markers could be effectively used to check the homogeneity of the seedling progenies. Validation of SCAR markers for S_H3 gene in S.795 variety established that the marker Sat 244 is more informative as it could distinguish the plants that are homozygous (++) and heterozygous (+ -) for S_H3 gene and highly suitable for MAS in coffee.

CONCLUSIONS AND PERSPECTIVES

- Preliminary data generated in the project so far, clearly demonstrated the potential of the Indian varieties, Sln.5A and Sln.6 as prospective sources of resistance to the major coffee diseases i.e. CLR, CBD and BBC and there exists a vast scope for exercising selection.
- Detection of new rust races on these Indian varieties indicates the selection pressure operating on pathogen in India, which might have implications in durability of resistance.
- The study demonstrated the utility of DNA marker approaches for maintaining the homogeneity of seed plots and MAS in coffee.
- The broad spectrum of disease resistance in Indian materials provides a scope for further improvement with respect to CLR by integration of S_H3 gene through MAS.

Lastly it can be inferred that the leads accomplished within a short period, bears testimony to the international co-operative spirit of this multi-country project.

ACKNOWLEDGEMENTS

Special thanks are due to Dr. C. S. Srinivasan, Joint Director of Research (Rtd), Coffee Board of India for the timely help in data analysis. Financial support from the Common Fund for Commodities (CFC), Amsterdam and International Coffee Organization (ICO), London for the ongoing multi country project (CFC/ICO/40), is gratefully acknowledged.

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Fourteen Years of Coffee Breeding in Ghana: Achievements and Prospects

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SUMMARY

Increasing coffee production and productivity are the main objectives of most coffee producing countries including Ghana. To meet these objectives a coffee breeding program was initiated in 1977, with the introduction of *Coffea canephora* germplasm from Côte d'Ivoire. This material, comprising seed-lots of five half-sib families were evaluated alongside two half-sib families developed locally and found to be a rich source of genetic variation for improvement of the crop. Initial breeding process was slow with the tentative release of seven clones with average yields of 2-3 tons clean coffee per hectare over two 5-year cycles of production without fertiliser application in 1992, through adaptive breeding. Multisite testing of selected high yielding genotypes began in 1996. Subsequently, breeding efforts were directed towards generative breeding alongside clone selection. In this presentation, the breeding strategies adopted from 1996 to 2010 are presented and their results discussed. In particular, accounts are given of the choice of parents with emphasis on yield and agronomic characters of interest. Projections for future breeding work are highlighted.

INTRODUCTION

Robusta coffee cultivation in Ghana started around the same time as cocoa in the high forest zone. As coffee was regarded as financially less attractive and more labour intensive, it became a minor crop and was therefore planted to the poorer soils. Small holdings were therefore scattered throughout the cocoa areas. The total area under cultivation was 3 000 hectares as at 1985 (Anon, 1996). Among the major factors identified for the low production of the crop was poor yields ranging between 100 and 200 kg/ha clean coffee which was attributed to unselected planting material of unknown origin. A selection programme was started at the Afosu sub-station of the Cocoa Research Institute of Ghana (GRIG) in 1977 with the introduction of open pollinated half-sib seed from Cote d`Ivoire and subsequently clones from Togo, resulting in the selection and distribution of nine clones to growers by 1992.

Various breeding and selection programmes were initiated from 1996, including testing of selected clones for adaptability, development of seed planting material to augment clonal planting material, development of planting material with compact growth habit for high density planting, and improvement of green bean quality for growers. The main achievements and the direction along which current research is being conducted are outlined in this report.

SELECTION OF GENOTYPES FOR ADAPTABILITY

Water deficit stress is a main factor limiting coffee yields (DaMatta and Ramalho, 2006). In Ghana, coffee is cultivated under varying climatic conditions. Over-cropping and poor land-use practices have resulted in soil fertility loss, with new plantings shifting from old

farmlands to new areas which are much drier. It is therefore important that, outstanding genotypes selected on the experimental station are assessed in different geographical areas with varying climatic conditions, to select genotypes with a wider range of adaptability and tolerance to drought stress. It is also important to identify plant characters associated with adaptability to aid future breeding and selection.

Eighteen genotypes, comprising 12 clones among the top seven percent of introductions from Côte d'Ivoire in 1977, together with six clones introduced from Togo in 1990, were planted in three locations (Tafo, Fumso and Bechem). Tafo has optimum conditions for Robusta coffee cultivation, and therefore considered a non-drought stress area. Bechem is a relatively drier area and drought-stress, whilst Fumso is intermediate. Thirty-two plants each of the 18 clones were planted per location in 1996 in a randomised complete block design with four replications and eight plants per plot. Yield was recorded on each tree for seven production years from October to January each year for the period 1998/99 to 2004/05. Data collected on vegetative and reproductive traits were previously described (Anim-kwapong and Adu-Ampomah, 2001). To select adaptable genotypes to drought stress, the drought susceptibility index (DSI) was calculated according to Fischer and Maurer (1978) for each genotype as:

$$DSI = (1 - Yds / Yns) / (1 - Xds / Xns),$$

where Yds is the clean coffee yield under drought; Yns is the clean coffee yield under non-stress conditions; Xds and Xns are the average clean coffee yields of all genotypes under drought and non-drought stress conditions respectively. Adaptable genotypes were selected based on yields under drought stress and non-stress conditions and DSI by clustering.

Six generally adaptable genotypes (E138, E90, E139, E152, 126 and 149) with average clean coffee yields of 1 786 to 2 178 kg/ka across the three locations have been selected for growers in Ghana. They also have clean coffee yields ranging from 880 to 1 263 kg/ha at Bechem, under drought stress and 2 126 to 2 857 kg/ha at Tafo under optimum conditions. They have very low to average environmental sensitivity (0.65 to 1.06), high outturn (21.3 to 25.7%) and bean weight (13.5 to 15.7g per 100 beans). They also have, on the average, higher mean values for span, number of primary branches, fruit-set, number of fruits per node and drought tolerance scores. Two genotypes (A129 and B36), with specific adaptation to non-drought stress conditions (clean coffee yields of 726 to 769 kg/ha at Bechem and 2 219 to 2 246 kg/ha at Tafo) with good outturn (21.6 and 23.4%) and bean weight (13.8 and 14.7) have also been released to farmers in less drought-prone areas. All eight genotypes have been tested for cross compatibility and found to be cross-compatible (Anon, 2008).

DEVELOPMENT OF SEED PLANTING MATERIAL OF ROBUSTA COFFEE

The high cost and cumbersome procedure of clonal planting material production and its low multiplication rate necessitates the use of hybrid varieties alongside clonal ones. In the Cameroon, Côte d'Ivoire, Madagascar, Indonesia and most Robusta producing countries, the planting material recommended to farmers` consists of clonal and hybrid varieties (Ravohitrarivo, 1980; Bouharmont et al., 1986; Leroy et al., 1993,1997; Montagnon et al., 2003). In Ghana, a programme has been initiated to produce planting material through generative reproduction with the aim of: (i) reducing the cost of production and transportation of planting material; (ii) supplying the farmer with planting material which will be available in large quantities and easier to distribute.

Trial 1

In this programme, parents were selected based on average yields in two production cycles from introductions of half-sib families (A, B, C, D, E) from Cote d`Ivoire, and clone 181 selected from introductions from Togo after yield evaluations for three years. For each individual, the letter beginning the name indicates the family from which it was selected. Six female parents (A129, A213, B170, B96, E138, E139) and five male parents (A115, A101, 181, E186, E174) crossed using the North Carolina 2 crossing design resulted in thirty progeny families. Evaluation of these families, from May 1998 to December 2005, led to the selection of seven full-sib families. They have five-year average annual clean coffee yield ranging from 1760 to 2030 kg/ha and bean weights of 12.5 to 14.0 g/100beans, as against a plot mean yield of 1570 kg/ha and bean weight of 12.7 g/100beans. The top 5% of the plot made up of 51 individual plants were selected based on yield and bean weight. They have been cloned and planted in the field in a completely randomised design to encourage random mating to provide seed for the next generation of recurrent selection. The selected plants have a 5-year mean annual yield of 2,980 kg/ha and bean weight of 14.8g/100beans. The progeny is expected to have a mean minimum bean yield of 1670 kg/ha and bean weight of 12.83 g/100 beans. Seed obtained from random-mating the selected individuals will be distributed to farmers as interim planting material, whilst the seven selected full-sib families will undergo further testing for adaptability.

It is also the aim of this programme to partly sib-mate the parents to increase homozygosity for hybrid vigour in subsequent crossing. Sib-mating resulted in the selection of six individuals from the A-Family and six from the E-Family with annual yields ranging from 2237 to 4672 kg/ha and bean weights from 12.3 to 20.4 g/100 beans. They have been crossed randomly in a cross classified manner to explore hybrid vigour. The progeny comprising 36 full-sib families are being evaluated in the field.

Trial 2

In this trial planted in June 2005, parents were selected based on yield and stability in adaptation trials and on yield in comparative clone trials established in 1996. Six clones (E90, E138, E139, E152, A101, 197) with seven year average annual yields of 1760 to 2850kg/ha were crossed as females to six clones as males (PA193, PA286, PB443, PB372, 149, PB413) with average yields of 1880 to 3130 kg/ha, using the North Carolina Model 2 Design. Fourteen full-sib families have been selected for adaptation trial based on two year average yields, fruit set, number of fruits per node, girth, number and diameter of primary branches, which are traits observed to be positively related to overall 7-year average yields of Robusta coffee (Anim-kwapong and Adomako, 2010).

Trial 3

The breeder`s stock at CRIG consists of individual plants from the introductions from Cote d`Ivoire, local selections, introductions from Togo and Cameroon, all tested in comparative clone trials and selected based on yield, bean weight, outturn, plant architecture and drought tolerance, among other agronomic traits of interest. Sixty clones from the breeders stock were crossed pair-wise to produce thirty full-sib families or bi-parental progenies. They were planted in the field in June 2009 for evaluation. It is the objective of the trial to select progeny families with good specific combining ability and mean value for yield and other traits of agronomic importance. Further testing for adaptation will be done and adaptable genotypes distributed to growers.

SELECTION AND BREEDING OF ROBUSTA COFFEE GENOTYPES FOR HIGH DENSITY PLANTING

Investigation into high density planting in coffee has been undertaken for Arabica coffee (Mitchell, 1976; Pavan et al., 1999; Kufa et al., 2001), but there is limited information in this direction for Robusta coffee (Carvalho, 1988; Anim-Kwapong et al., 2010). The available information is mainly on spacing with hardly any information on the breeding for compact growth habit. The spreading growth form of most Robusta clones does not allow for high density planting for high productivity. A programme was therefore initiated with the objective to produce high yielding genotypes with compact growth habit amenable to high density planting. Two field trials have been planted for evaluation.

Trial 1

Yield data analysis of Robusta coffee genotypes from introductions from Côte d'Ivoire planted in 1978 has resulted in the selection of high yielding plants. Eight female parents with canopy radius of 0.91-1.28 m were crossed to three male parents with canopy radius of 0.83-0.89 m by the North Carolina Design 2 crossing model. Twenty-four progeny families resulting from the cross were planted together with two standard clones in May 1998. Planting was at a spacing of 2 x 3m in a randomised complete block design with 4 replicates and a total of 32 plants per family. Six full-sib progeny families were selected for yield, with five-year average clean coffee yields ranging from 1 520-1780. They also have good Specific Combining Ability (SCA) for bean/berry physical characteristics. Three of these full-sib families also have low values and SCA for height, span, and inter-node length on primary branches and main stem, hence suitable for high density planting.

Trial 2

In the second trial planted in 2005, eight clones with outstanding yields were crossed as females to four clones with canopy radius as in Trial 1. Selection among the progeny was based on first two years yield, fruit set, span, girth, diameter and number of primary branches, and inter-node length on primary branches and main stem. Nine families were selected with two-year annual clean coffee yields of 1 200 to 1900 kg/ha. The selected families in both trials will undergo further testing for spacing and adaptability.

QUALITY IMPROVEMENT OF ROBUSTA COFFEE

Characteristics determining the quality of green coffee such as bean size, proportion of defective beans, and physical characteristics (Leroy et al., 2006), which are considered for the improvement of green coffee are of major importance in the breeding activities of Ghana. Bean weight of high yielding clones, distributed to farmers, range from 11.4 to 16.6 g/100 beans. Analyses of the coffee germplasm at CRIG gave individual plant range of 9.7 to 25.0 g/100 beans. This observed variability is being exploited to combine the good characteristics of the genotypes. A programme with the objective of producing coffee genotypes with good bean characteristics among other factors for commercial cultivation in Ghana was therefore initiated. Ten clones with bean weights of 11.4 to 16.6 g/100beans and yields of 1 500 to 2 850 under non-drought-stress conditions at Tafo were crossed as females to four moderately high yielding clones (with bean weights of 17.3 to 25 g/100 beans), as male parents, using the North Carolina Design 2 model. Forty progeny families resulting from the crossing were planted in June 2003 and evaluated for yield, bean/berry physical characteristics, vegetative and reproductive traits. Seven families with yields between 1580 to 2240 kg/ha and bean

weights of 13.5 to 15.8 g/100beans were selected for further testing for adaptability. Two of them, E139 x C134 and E138 x C193 with yields of 2240 and 1980 and bean weights of 15.1 and 14.5 respectively have their parents planted in seed gardens in isolated plots to produce seed, as interim planting material, for distribution to farmers` from October 2011.

TESTING OF SEED PLANTING MATERIAL FOR ADAPTABILITY

It is important that selected progenies from the above trials, seeking to produce seed planting material for growers are tested for adaptation. The selected progenies comprising 44 full-sib families have been reproduced in specific crosses during flowering time in February this year. Seed from these crosses will be nursed and planted in May/June 2011 in environments varying for agro-climatic factors, to select adaptable genotypes for farmers.

COLLECTION, CHARACTERISATION AND EVALUATION OF LOCAL *COFFEA CANEPHORA* GERMPLASM

Germplasm is the basic material for plant breeding and crop improvement. The sustenance of long term advances in breeding and selection of improved coffee varieties depends on availability of sufficient genetic diversity in the working population or the breeders` stock. Maintenance of adequate genetic variability through germplasm collection is therefore essential for sustainable coffee production.

Realising the importance of germplasm in breeding in the light of the narrow genetic base of the crop at CRIG, the collection of coffee germplasm was embarked upon between September and December, 2009. The divested coffee plantations, established under the Ghana Cocobod Plantations, were planted mainly with seed introduced from Uganda, Tanzania, Cameroon and Cote d`Ivoire (personal communication). Germplasm in the form of seed and stem cuttings was collected at seven plantations in localities, namely: Bodi, Bibiani, Kenyasi, Duayaw Nkwanta, Bepong, Manso Mim and Adansi-Brofoyedru. Two hundred and twenty-four accessions were collected in the form of cuttings, of which 188 were also in the form of seeds. The accessions were planted in the field at two locations (Tafo and Afosu) in July this year for characterisation and evaluation.

THE WAY FORWARD

Past breeding and selection at CRIG was towards clone selection. Although adaptation trials resulted in high yielding and generally adaptable genotypes, multiplication rate and distribution of clonal planting material is slow, hence low availability to growers. In the quest to develop seed planting material, various crossing programs were undertaken and selection made for specific combining parents for yield, bean weight and other plant characters of interest. Best performing individuals and genotypes with good general combining ability in these programs are selected for population improvement under recurrent selection. They are also being tested as clones in comparative trials. It is expected that in the near future coffee breeding at CRIG will yield populations, clone hybrids and clones with varying characteristics, including compact growth habit for high density planting, and erect stems for ease of field management. It is also expected that, such planting material will combine high yields, resistance/tolerance to disease and pest, good berry and bean attributes and adaptability among other desirable agronomic traits. With the collection, characterisation and evaluation of new germplasm, it is projected that, the genetic base of material available for breeding will be wide enough for continuous progress in the set breeding objectives.

Identification of traits that aid early selection should help shorten the breeding cycle thus enabling the achievement of our objectives within the next few years.

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Quality Assessment of Coffee Genotypes from Brazil

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SUMMARY

Coffee quality is linked to physical and chemical characteristics of the beans. In order to better understand this relationship, several physical and chemical analyses were carried out on green coffee, processed by the wet method. The evaluation included parchment percentage; olfactory and visual examination, bean mass, apparent density, ratio between flat, pea and shell beans, percentage of defects and foreign matter, size analysis, colour evaluation, moisture content and caffeine content. For this study, five genotypes were selected, kindly supplied by Instituto Agronómico de Campinas (Brazil). The overall colour difference showed a promising discriminatory capability, at least for these studied samples.

INTRODUCTION

Coffee represents one of the most important crops of the world. As its volume of sales depends on coffee quality, much attention is paid to quality improvement and maintenance. As an example, when green coffee is stored for a prolonged time, its quality decreases distinctively. As a consequence, the provenience-specific characteristic features, especially those of top quality coffees, gradually diminish during the course of elongated storage (Selmar et al., 2008). In fact, coffee production still faces several problems, some of them related with quality and safety. In order to face this demand and to assure public wealth, a characterisation of five genotypes, of wet-processed parchment coffee from Brazil, was carried out, trying to find possible indicators of quality and safety of green coffee.

MATERIAL AND METHODS

Material

Coffee beans of five genotypes processed by the wet method were analysed: *C. canephora* cv. Apoatã, *C. dewevrei*, *C. arabica* cv. Catuaí, Icatu (hybrid of *C. canephora* x *C. arabica*) and Obatã (hybrid of Villa Sarchi x Híbrido de Timor).

Methods

Parchment percentage: determination on coffee manually husked. *Olfactory and visual examination*: according to Norma Portuguesa NP-1795 (1989). *Bean mass*: mass of 1000 beans, according to Esteves and Oliveira (1970). *Apparent density*: evaluated according to Norma Portuguesa NP-2285 (1991). *Ratio between flat, pea and shell beans*: determination on a sample of 30 g. *Percentage of defects and foreign matter*: according to Norma Portuguesa NP-1521 (1985). *Size analysis*: determined following Norma Portuguesa I-1636 (1981).

Colour evaluation: determination on ground coffee (ASTM 35 sieve, 500 μm mesh) using a colorimeter Minolta CR-300 (Japan) and a white standard (L* = 97.46; a* = -0.02; b* = 1.72). *Moisture content*: evaluated according to Norma Portuguesa NP-1794 (1988). *Caffeine content*: determined following Norma Portuguesa NP-1840 (1986).

RESULTS AND DISCUSSION

Bean mass, apparent density and caffeine content – Results are presented in Table 1. *C. dewevrei* had the heaviest beans and Apoatã beans revealed to have the highest apparent density. As it was expected, *C. canephora* (Apoatã) showed the highest caffeine content, almost twice the content of *C. arabica* (Catuaí) or a hybrid that is now considered very close to *C. arabica* (Icatu) and almost three times the content of Obatã and *C. dewevrei*.

Table 1. Bean mass, apparent density and caffeine content of the 5 genotypes under analysis.

Cultivar	Bean mass (g)	Apparent density (g cm^{-3})	Caffeine (%)
Apoatã	139.3 ± 0.6^{r}	0.751 ± 0.002^{r}	2.85 ± 0.02^{r}
C. dewevrei	163.2 ± 1.0^{s}	0.723 ± 0.001^{r}	1.12 ± 0.01^{s}
Catuaí	117.4 ± 0.7^{t}	0.628 ± 0.008^{s}	1.51 ± 0.01^{t}
Icatu	117.9 ± 0.6^{t}	0.649 ± 0.004^{s}	1.67 ± 0.03^{t}
Obatã	134.0 ± 0.7^{r}	0.649 ± 0.005^{s}	1.10 ± 0.10^{s}

Different letters (r, s, t) mean significant differences amongst cultivars (n = 3).

Olfactory and visual examination

All cultivars possessed a normal aroma, and the dominant colour was greenish, although not uniform. Table 2 shows the results of the complementary visual examination carried out on whole and ground beans.

Table 2. Complementary visual examination of colour.

Cultivar	Whole bean colour	Ground bean colour
Apoatã	- green, golden green and brownish green - silver skin strongly adherent	brownish green
C. dewevrei	- from green pea to whitish green	green
Catuaí	- green lead - silver skin adherent	grayish green
Icatu	- green lead - silver skin adherent	grayish green
Obatã	- green - silver skin adherent	whitish green

***Colour evaluation* (ground coffee)**

The values obtained for colour evaluation (L*, a*, b* coordinates) showed that *C. dewevrei* possesses a colour significantly different from the others (Table 3).

Table 3. L* a* b* coordinates from a colour analysis of green beans of the studied genotypes.

Cultivar	L*	a*	b*
Apoatã	72.70 ± 0.12 [r]	-0.65 ± 0.02 [r]	16.90 ± 0.15 [r]
C. dewevrei	72.42 ± 0.20 [r]	-3.35 ± 0.05 [s]	21.01 ± 0.08 [s]
Catuaí	67.57 ± 0.07 [s]	-0.06 ± 0.02 [t]	14.05 ± 0.08 [t]
Icatu	67.54 ± 0.09 [s]	-0.64 ± 0.03 [r]	14.29 ± 0.08 [t]
Obatã	72.59 ± 0.08 [r]	0.11 ± 0.02 [t]	13.93 ± 0.03 [t]

Different letters (r, s, t) mean significant differences amongst cultivars (n = 10).

To better assess colour differences amongst cultivars, overall colour difference (ΔE) was evaluated (Table 4), according to Chervin et al. (1996). The highest ΔE was obtained between *C. dewevrei* and Catuaí, and the lowest between Catuaí and Icatu (these last two have similar values of L* and b*), as should be expected due to the genetic proximity of the later two. Thus, ΔE seems to constitute a useful discriminator for coffee genotypes, confirming earlier results also involving the colour index (CI) (Cavaco-Bicho et al., 2008).

Moisture content (%)

All cultivars had similar moisture contents: 10.2 (Apoatã); 10.4 (Icatu); 10.5 (Obatã); 10.7 (Catuaí and *C. dewevrei*), in good conditions for trade and storage (between 8 and 12.5%, according to ICO recommendations (Leroy et al., 2006)).

Table 4. Overall colour difference (ΔE) amongst green beans of the studied genotypes.

Cultivars relationship	ΔE *
C. dewevrei - Catuaí	9.1
C. dewevrei - Icatu	8.7
C. dewevrei - Obatã	7.9
Apoatã - Catuaí	5.9
Apoatã - Icatu	5.8
Icatu - Obatã	5.1
Catuaí - Obatã	5.0
Apoatã - *C. dewevrei*	4.9
Apoatã - Obatã	3.1
Catuaí - Icatu	0.6

* $\Delta E = [(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$

Parchment percentage

The lowest value was found in Apoatã (good green coffee producer) and the highest in *C. dewevrei*: 12.5 (Apoatã); 18.7 (Obatã); 19.3 (Catuaí); 20.6 (Icatu); 25.0 (*C. dewevrei*).

Ratio between flat, pea and shell beans

As it was expected, flat beans represent the highest percentage of three types of beans in all cultivars, with Apoatã showing the highest value. Icatu presented the highest percentage of pea beans and Catuaí the highest percentage of shell beans (Table 5).

Table 5. Ratio between flat, pea and shell beans of the studied genotypes.

Cultivar	Type of bean (mass %)		
	flat	pea	shell
Apoatã	95.1	4.9	0.0
C. dewevrei	83.3	12.1	4.6
Catuaí	82.6	2.0	15.4
Icatu	79.2	13.2	7.6
Obatã	74.7	11.5	13.8

Percentage of defects and foreign matter (mass %)

C. dewevrei showed the lowest percentage of defects (6.6) and Icatu the highest (17.1). The other genotypes showed 10.6 (Catuaí); 12.6 (Apoatã) and 14.7 (Obatã), values under maximum allowed for Arabica and Robusta (Leroy et al., 2006). Defects that were detected: shell, withered, insect damaged, black, green, dark brown, malformed and quaker beans. No foreign matter was found in any sample.

Size analysis

Medium beans predominate in all cultivars; however Obatã showed to be the best producer of coarse beans (56.3%), Apoatã of medium beans (64.5%) and Icatu of small beans (13.2%), (Figure 1). *C. dewevrei* presented more commercial homogeneity (maximum percentage of beans retained by two successive sieves) than the others. Bean size is an important factor since price is related to the coffee grade (Leroy et al., 2006).

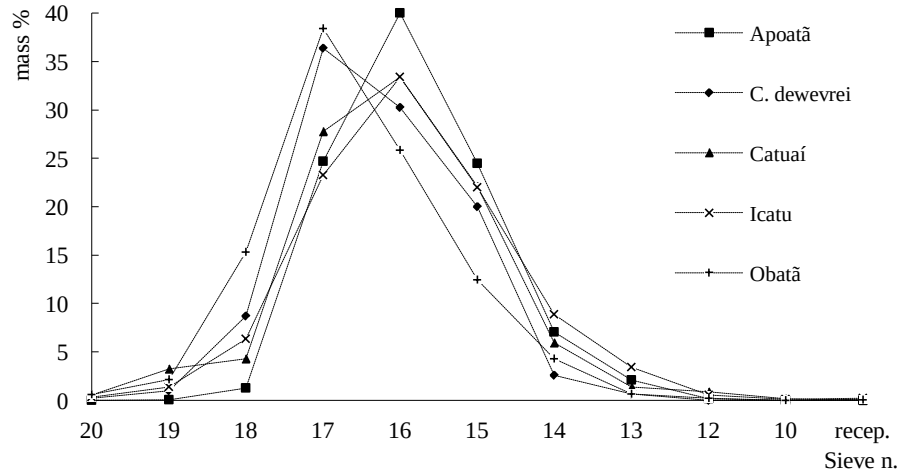

Figure 1. Fractionation of bean mass by sieve (ASTM) of the 5 genotypes under analysis.

CONCLUSION

Concerning the analyzed samples, it was possible to separate the several genotypes through this physical-chemical analysis and to conclude that Apoatã and *C. dewevrei* were the best processed samples, in terms of implication in the commercial value. Amongst bean parameters, the overall colour difference (ΔE) showed a promising discriminatory capability, at least for this small number of samples. Apoatã is the best producer of green coffee (lowest parchment percentage) and has the highest caffeine content. *C. dewevrei* has the heaviest beans, presents more commercial homogeneity and contains fewer defects than all the others.

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Analysis of Population Genetic Diversity and Differentiation in *Hemileia vastatrix* by Molecular Markers

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SUMMARY

The present study intends to assess *H. vastatrix* population genetic diversity and differentiation, migration/dispersal patterns and gene flow among populations using a comprehensive and across-time coverage of isolates from different geographical origins, as well as to get some insights on the pathogen evolution. Here we report the initial stage of this study with the analysis of a first set of 31 *H. vastatrix* isolates from 12 coffee-growing countries using AFLPs and gene sequences. Several loci, including the universally used rDNA-ITS region, *β-tubulin* 1, *TEF*1 and candidate genes, were tested for marker informative value. Some level of variability could be detected in ITS and *β-tubulin* 1 loci providing some insights on the discrimination among *H. vastatrix* populations and a first step to better understand the underlying population structure. On the other hand, among a set of AFLP selective primers tested for sample screening, two AFLP primer combinations generated distinctive fragment patterns among different isolates. Based on both datasets, population structure and diversity parameters will be discussed, as well as inferences on the spatial distribution of the genetic variability. These first results open the way to unravel the molecular differentiation and the dynamics of *H. vastarix* populations.

INTRODUCTION

Coffee leaf rust (CLR) caused by the biotrophic fungus *Hemileia vastatrix* Berk. & Br. has long gained a world-class status, reaching almost all coffee growing countries with severe economical damages. Breeding for rust resistance has proven successful over the years to control the disease, but the highly adaptable nature of the fungus shaped by the dynamic system of host-pathogen co-evolution has shown to be a critical limitation for achieving durable CLR resistance (Várzea and Marques, 2005). As a consequence, gradual breakdowns of resistance have been observed in many of the improved varieties in several countries (Várzea and Marques, 2005; Prakash et al., 2005). In fact, the emergence of new evolving pathotypes under a strong selective pressure and the potential for these new races to become epidemically spread on a continental scale is a serious and constant threat. Thus, a better understanding of the genetic variation of *H. vastatrix* populations across large geographic areas and periods of time, and their phylogenetic relationships is a priority. High molecular diversity among rust isolates has been documented using RAPD markers (Gouveia et al.,

2005; Nunes et al., 2005), but patterns of differential population genetic structure has not been yet demonstrated. It is most probable that increasing the resolution of the molecular analyses with a more comprehensive rust sampling and higher informative markers will bring detailed information about the genetic variation observed among isolates of *H. vastatrix* and the pathogen evolution.

MATERIALS AND METHODS

Rust isolates and DNA extraction

A group of 31 isolates of *H. vastatrix* from the collection of CIFC/IICT were analyzed, comprising 12 geographical origins, different collection years and different virulence profiles. DNA was extracted as described by Kolmer et al. (1995).

Gene sequence data

Four nuclear gene regions were amplified in this study: the internal transcribed spacer (ITS) region (ITS1-5.8S ribosomal gene-ITS2), using primers ITS1Ext and ITS4Ext (Brown et al., 1996); *β-tubulin* 1; translation elongation factor 1-α (*TEF*1) and a hexose transporter (*HXTp*1) from a *H. vastatrix* haustoria-rich cDNA library (Talhinhas et al., 2010). For these latter loci, primers were designed using PerlPrimer v1.1.17. Sequencing reactions were performed in both directions with BigDye v3.1 (Applied Biosystems) and run on an ABI Prism 310 automated sequencer. Since clean sequences could not be obtained for the ITS region, cloning was performed for 10 isolates using the Fermentas CloneJET PCR cloning kit according to manufacturer's instructions. Multiple alignment of sequence data was performed using MAFFT v6.717b (Katoh et al., 2009). The phylogenetic tree was generated using the Maximum Likelihood (ML) method in PAUP* v4.0d99 (Swofford, 2002) with the best fit model of nucleotide evolution, under the Akaike Information Criterion (AIC) from ModelTest (Posada and Crandall, 1998). Heuristic searches with 100 random addition replicates were executed. Nonparametric bootstraping was also conducted using 100 pseudoreplicates with 10 random additions.

Amplified Fragment Length Polymorphism (AFLP) data

A subset of 28 rust isolates was used in this study. AFLP was performed according to Vos et al. (1995) modified and adapted to the ABI system, using *Pst*I and *Mse*I restriction enzymes, a preamplification step with no selective nucleotide and a specific amplification step with +2/+3 selective nucleotides. A prescreening test was carried out using 22 *Pst*I/*Mse*I combinations. Two informative AFLP combinations were selected and used for further characterization of all studied isolates: P-TG/M-CTA (PM105) and P-TG /M-AG (PM109). Selective PCR products were separated by capillary electrophoresis using an ABI Prism 310 automated sequencer. Factorial Correspondence Analysis (Benzécri, 1973) of the individual multilocus scores, as implemented in the program GENETIX v. 4.01 (Belkhir et al., 2004), was used as an exploratory tool to assess the similarity/dissimilarity between isolates. The original dataset is converted into a new three state matrix for each isolate in each allele of each locus (1 for absence and 2 for presence). The algorithm finds independent eigenvectors of the matrix and determines the factorial axes. New coordinates of each individual are recalculated in each factor and can then be plotted.

RESULTS AND DISCUSSION

From the DNA sequence datasets, nucleotide diversity among isolates was only found on ITS and *β-tubulin* 1 loci. Analysis of ITS sequences revealed the presence of at least two different copies in all the isolates investigated, as confirmed by inference from the double peak pattern in chromatograms as described by Sousa-Santos et al. (2005) in comparison with cloned sequences. However, all isolates shared the same pattern of heterozygous indels, except for one isolate which differed in one nucleotide site. Also some cloned genotypes suggested the existence of more than two ITS copies. Multiple ITS copy sequences is not a rare phenomenon in fungi and have been reported in other rusts (Morin et al., 2009). Moreover, detection and analysis of different copies from unlinked nuclear gene regions can uncover events of hybridization, which is now believed to be an important mechanism for rapid evolution and speciation in fungi (Morin et al., 2009). Further investigation is required to ascertain the number and type of ITS copies present in *H. vastatrix* populations, representing a promising tool to explore and understand the extant genetic variability. The *β-tubulin* 1 loci dataset presented seven neutral polymorphic sites, with a nucleotide diversity (Pi) of 0.00149, revealing four diverging genotypes. The slight genetic structure revealed in the present phylogeographical frame shows no apparent correlation with geographical location or virulence profile (Figure 1).

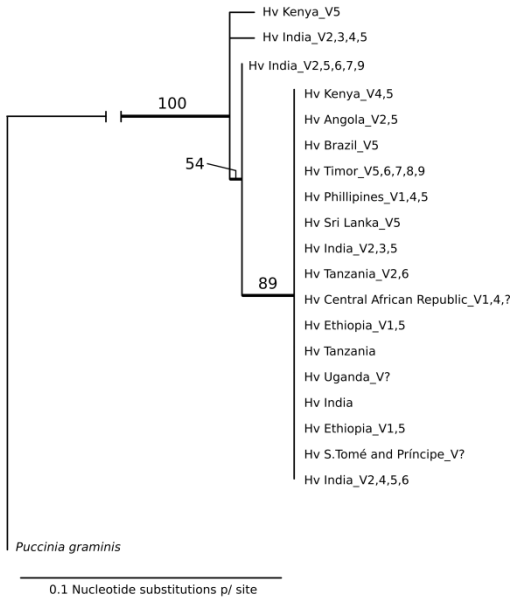

Figure 1. Maximum likelihood tree obtained for the *β-tubulin* 1 dataset following 100 replicates. The tree was rooted using *Puccinia graminis* as outgroup taxa.

In contrast, AFLP markers revealed to be highly polymorphic and informative. A very preliminary factorial analysis based on the AFLP data generated by 2 selective primer combinations clearly clustered the *H. vastatrix* isolates studied in three distinctive groups (G1, G2 and G3, Figure 2). In these data projection, where the first four axes explain 55% of total variation, in particular the first axe, these groups are very well separated and delimited. A higher similarity of G2 with G3 is suggested, being the former only constituted by 3 isolates which seem genotypically more distinct. On the other hand, this apparent structuring seems tendentiously correlated with geographical origin since in a total of 14 African isolates, 12 are clustered together between G2 and G3. Although the AFLP data is still very limited and these results can only be interpreted as potential real relationships, it shows the existence of

underlying genotypic affinities and population structure whose significance we hope to unravel in the future. Together with the higher resolution power of AFLP markers for genome coverage and ability to discriminate small differences and identify potential relationships, searching for additional polymorphic gene loci is on progress aiming to improve information on *H. vastatrix* phylogeographical patterns. In particular, ITS region seemed to constitute a source of variability worth exploring for the reconstruction of a more complete scenario towards a possible evolutionary history of *H. vastatrix*.

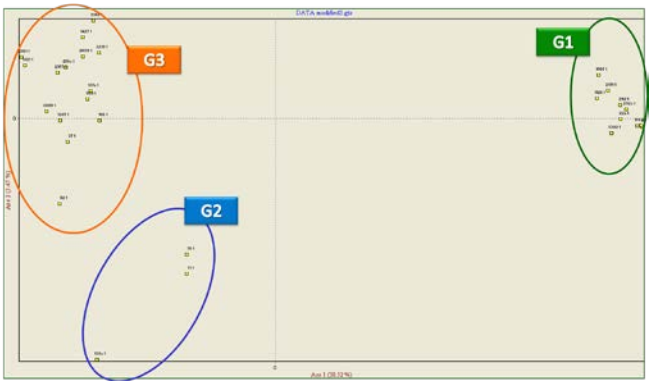

Figure 2. Factorial correspondence analysis of 28 *H. vastatrix* isolates based on the polymorphic AFLP loci generated by primer combination PM105 and PM109. G1, G2 and G3: Groups distinguished by the present analysis.

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Chlorogenic Acid Content in Coffee Leaves: Possible Role in Coffee Leaf Rust Resistance

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SUMMARY

The resistance of *Coffea arabica* S4 Agaro to *Hemileia vastatrix* is characterized by a rapid localized plant cell death (hypersensitive reaction-HR), associated with the restriction of fungal growth, early increase in phenylalanine ammonia-lyase (PAL) activity and accumulation of phenolic compounds. This work aims to understand the involvement of chlorogenic acids (CGAs) in coffee resistance particularly during HR. *C. arabica* S4 Agaro leaves in the first stages of the infection process of *H. vastatrix* (compatible and incompatible interactions) were collected, grounded and extracted with a methanol-water mixture. Extracts were treated with Carrez reagent, filtered and than analysed by HPLC-DAD and LC-MS. For CGAs identification MS data were compared with the ones from the literature as for most compounds standards are not commercially available. The main caffeoylquinic acids (CQA), dicaffeoylquinic acids (diCQA) and feruloylquinic acids (FQA) detected in the coffee leaves were quantified using chlorogenic acid as standard. Preliminary results showed a high accumulation of CGAs in young coffee leaves. The possible role of CGAs in the resistance response of coffee against *H. vastatrix* is discussed.

INTRODUCTION

Chlorogenic acids (CGA) are products of phenylpropanoid metabolism, i.e. one branch of the phenolic pathway. They are a family of esters formed between certain hydroxycinnamic acids and quinic acid (Farah and Donangelo, 2006). Although 30 different species of CGAs have been identified in green coffee beans, the great majority of these compounds are: caffeoylquinic acids (3-, 4- and 5-CQA); dicaffeoylquinic acids (3,4-, 3,5- and 4,5-diCQA); feruloylquinic acids (3-, 4- and 5-FQA); *p*-coumaroylquinic acids (3-, 4- and 5- *p*CoQA) and mixed diesters of caffeoylferuloyl-quinic acids (CFAQ) (Clifford, 2003; Clifford et al., 2006). Some of these compounds were also detected in leaves of different coffee species (Mondolot et al., 2006; Bertrand et al., 2003). Due to their antioxidant and antibiotic properties, CGAs are involved in numerous biological plant functions such as pest and disease resistance (Mondolot et al., 2006). In the current study, CGAs contents were evaluated in *C. arabica* leaves during the time course of the infection process of *H. vastatrix* (incompatible and compatible interactions) by HPLC-DAD-MS.

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 XIV ($v_{2,3,4,5}$) (D'Oliveira, 1954-1957) establishing an incompatible and a compatible interaction, respectively.

CGAs extraction.

Sample preparation was made according to the method described by Correia et al. (1995). Briefly, fresh leaf tissues were ground in liquid N_2, and homogenised in 10 ml of methanol 40% (v/v). After 30 minutes of agitation (125 rpm) at 4 °C, samples were centrifuged at 10000 g for 5 minutes at 4 °C. Supernatant was collected and transferred to 50 ml flask. The extraction of the pellet was repeated three more times and the supernadants were collected and treated with Carrez reagent. The volume of the 50 ml flask was completed with methanol 40%. After a 15 minutes rest the extracts were filtered and analyzed by HPLC.

HPLC-DAD and MS analysis

HPLC-DAD was performed in a Thermo Finning equipment (Surveyor model). The chlorogenic acids separation was achieved in a RP-18 (5 µm) 250 × 4 mm Lichrocart ® (Merck) column and detection was performed at 325 nm. A gradient of eluents was used for analysis (eluent A: water:phosphoric acid (999:1 v/v) and eluent B: water:acetonitrile: phosphoric acid (599:400:1 v/v)) at a flow rate of 700 µL/min. For LC-MS analysis a system (Alliance, Waters 2695 Separation Module) with a photodiode array detector (Waters 2996) in tandem with a TQ mass spectrometer (Micromass Quattro Micro API) and an ESI source operating in negative mode was used.

RESULTS AND DISCUSSION

The HPLC analysis of coffee leaf extracts produced chromatograms with more than 40 peaks (detected at 325 nm) and 9 of them were identified as CGAs (Figure 1 and Table 1). Chlorogenic acid was used as standard to determined CGAs content of leaf extracts. CQAs were the most abundant compounds followed by diCQAs and by FQAs.

No differences were observed when the overall CGAs content of healthy leaves, incompatible and compatible interactions were compared. However 4,5-diCQA increased significantly 30 hours after inoculation in the incompatible interaction and this was not observed in the compatible nor in the control (Figure 2). Similar results were obtained for peak 1 and 7 (not identified compounds).

CGAs can be substrate of peroxidase (POD), leading to polymerization products such as insoluble brown pigments and lignin that contribute to the plant defense mechanisms (Farah and Donangelo, 2006). Previous results have shown that the resistance of *Coffea arabica* S4 Agaro to *Hemileia vastatrix* is characterized by a rapid localized plant cell death (hypersensitive reaction-HR), monitored by cell autofluorescence and/or browning, which indicate accumulation of phenolic compounds. HR is also associated with early increase in PAL and POD activities and host cell wall lignification (Silva et al., 2002; 2008). Thus, the differential accumulation of 4,5-DiCQA during the time course of the experience, may

suggests the possible involvement of this compound in the resistance of coffee against *H. vastatrix*. The hystochemical localization of CGAs in healthy and infected leaves as well as the identification of peaks 1 and 7 is currently under study.

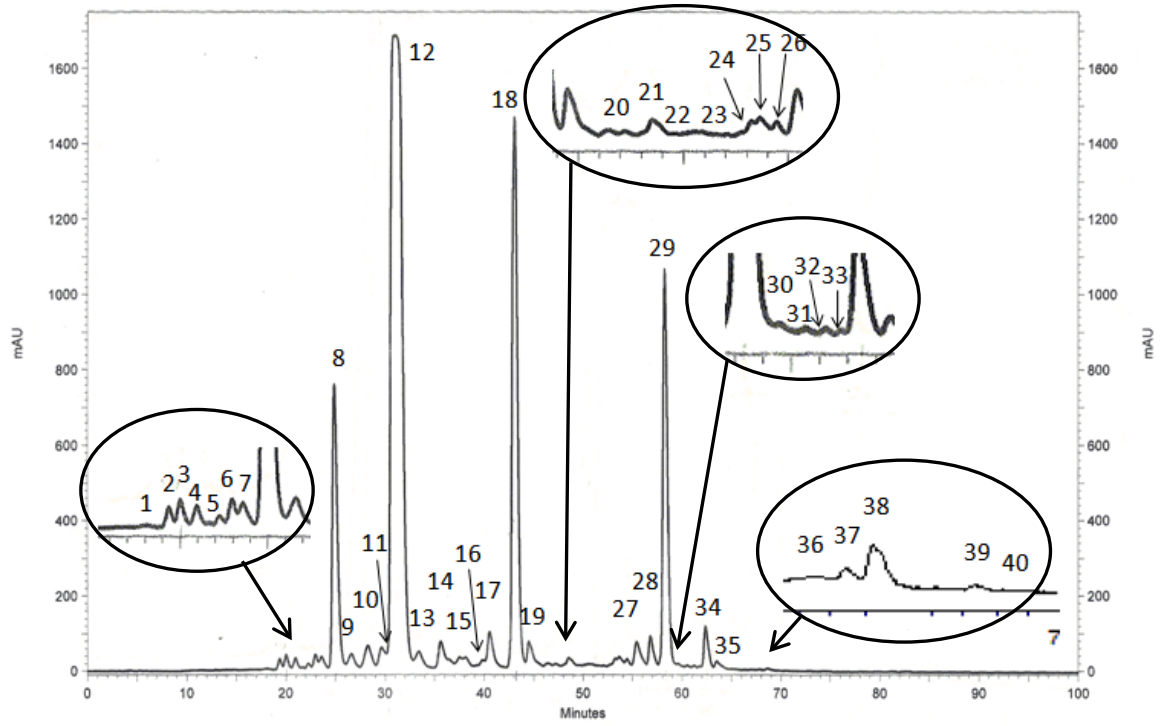

Figure 1. Chromatogram of coffee leaf extracts recorded at 325 nm (peak identification in Table 1).

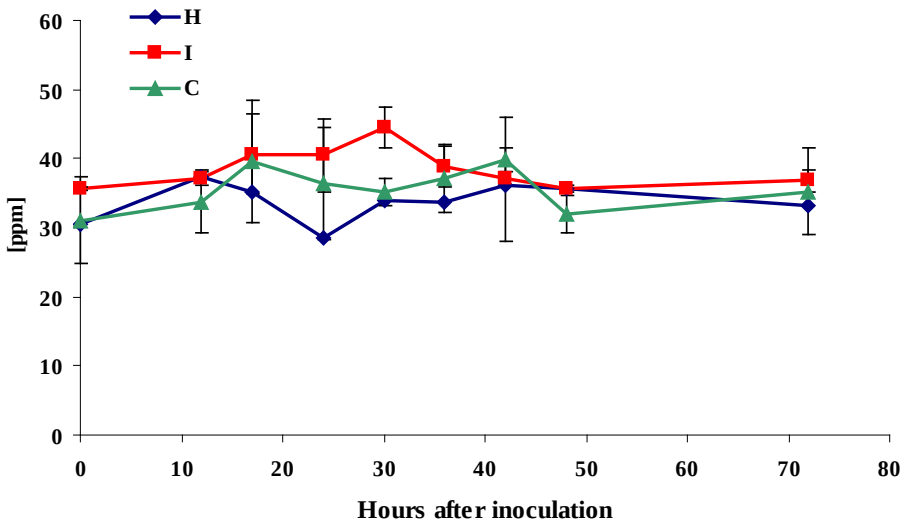

Figure 2. 4,5-diCQA (peak 29) content in healthy (H) coffee leaves and in incompatible (I) and compatible (C) interactions, at different hours after inoculation.

Table 1. Identification of some compounds found in coffee leaf extracts' chromatogram.

Peak number	RT (min)	m/z	λ max (nm)	Compound*
1	17.52	191	**230**, 275, 308	Not identified
3	19.49	353	**325**, 290	3-CQA (Clifford, 2003; Clifford et al., 2006)
7	22.85	**475**, 407	**230,** 280, 310	Not identified
12	29.91	**353**, 191	**325**, 300	5-CQA (Clifford, 2003; Clifford et al., 2006)
18	41.92	**289**, **353**	**230**, 255, 320, 365	Epicatechin (Tomás-Barberán et al., 2001), 4-CQA (Clifford, 2003; Clifford et al., 2006; 2006)
19	43.48	337	**215**, 270, 330	*p*-coumaroyl-quinic acid (*p*CoQA) (Clifford, 2003; Clifford et al., 2006)
20	45.96	**367**	280	5-FQA
27	54.28	515	**325,** 285	3,4-diCQA
28	55.68	515	**325**, 290	3,5-diCQA
29	57.11	515	**325**, 280	4,5-diCQA
38	67.33	499	**325, 240**	*p*-coumaroyl-caffeoylquinic acid (Clifford et al., 2006; 2006)

*RT = retention time, λ max = maximum wave length; m/z = mass-to-charge ratio (bold number represents the predominant one); *the identification was made by comparison with data from literature.*

ACKNOWLEDGEMENTS

This work was funded by Fundação para a Ciência e Tecnologia (FCT): PTDC/AGR-AAM/71866/2006 and REDE/1518/REM/2005 for the LC-MS/MS equipment used at Faculdade de Farmácia da Universidade de Lisboa, Portugal.

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Cellular and Molecular Responses in Host and Nonhost Coffee-Rust Interactions (*Hemileia vastatrix* and *Uromyces vignae*)

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SUMMARY

Coffee plants are seriously affected by leaf rust (*Hemileia vastatrix*) but are naturally immune to other rusts. In fact, non-host resistance (NHR) is among the most durable forms of disease resistance, making the study of non-host interactions of great importance to understand the mechanisms of disease resistance and to improve its durability. This work addressed coffee resistance to *Uromyces vignae* (cow pea rust) as a non-host resistance model in comparison with the resistance of HDT 832/2, and its derivative Sarchimor, to *H. vastatrix* race II, with the broader purpose of better understanding HDT 832/2 resistance to *H. vastatrix*. Comparisons between coffee non-host and host resistance were performed along the infection process at the cytological and molecular levels (bright field and fluorescence microscopy techniques; RT-qPCR expression analysis of 8 genes involved in recognition, signalling and defence). In both coffee lines, *H. vastatrix* ceased growth more frequently at the penetration hypha stage, with <10% infections reaching haustoria formation. *H. vastatrix* infection induced hypersensitive reaction (HR), phenol accumulation and haustorium encasement with callose. *U. vignae* growth was more restricted in HDT 832/2 than in Sarchimor, but failed to form haustoria in both lines. Resistance responses to *U. vignae* infection included HR induction and phenol accumulation. Most genes were activated in the host interaction (HDT 832/2–*H. vastatrix*). Genes *rlk*, *wrky1*, *pal*, *13-lox*, *pr1b*, *pr10* and *gt* presented two activation peaks, the first in a period when appressoria differentiation and penetration hypha formation occurred, and the plant responses at cellular level begun (6 to 12 hours after inoculation - hai) and the second when anchors and haustoria mother cells differentiate and plant responses were recorded in 50% of infection zones (21-24 hai). In the non-host interaction (HDT 832/2–*U. vignae*) most genes were not regulated or poorly activated in the first hours after inoculation (3-6 hai), being activated when no new fungal infection structures were differentiated, and plant responses were detected in over 50% of infection zones (9-12 hai). Both in the host and non-host interactions the expression profile of *rlk* and *wrky1* suggested that the recognition of the fungus and signalling occurred during the formation of appressoria and the differentiation of the first post-penetration infection stages. *13-lox* was moderately activated in both interactions, with the first activation peak coinciding with the onset of the detection of cell death (HR). *pr1b* exhibited high expression levels in the host interaction, being poorly activated in the non-host interaction. *13-lox* and *pr1b* are markers respectively of the jasmonate and the salicylic acid (SA) pathways. Results suggest that both pathways may coexist, although the SA pathway seams to prevail in the host interaction. The pre-haustorial resistance of HDT 832/2 and Sarchimor to *H. vastatrix* race II is an exception to the more common post-haustorial resistance in host-rust interactions. Pre-haustorial resistance, closer to

non-host resistance, has proved more difficult to be broken, which may explain the longer durability of resistance found in HDT 832/2 and Sarchimor, as compared to other genotypes in which resistance has been broken.

INTRODUCTION

Leaf rust, caused by the fungus *H. vastatrix* Berk & Br., is considered the main disease of Arabica coffee. Coffee-rust interactions are governed by the gene-for-gene relationship, being the resistance of coffee plants conditioned at least by nine major dominant genes ($S_H1 - S_H9$) singly or associated (Rodrigues and Bettencourt, 1975; Bettencourt and Rodrigues, 1988). Breeding resistant coffee varieties, such as Sarchimor, has been the most efficacious strategy against this disease, namely using descendents of the Timor Hybrid (HDT) as sources of resistance against all known races of *H. vastatrix*.

Nonhost resistance is the most durable form of plant disease resistance, making the study of nonhost interactions of great importance to understand the mechanisms of host resistance and to improve its durability (Heath, 2001; Niks and Marcel, 2009). The objective of this work was the characterisation, at cellular and molecular levels, of resistance to *H. vastatrix* in coffee lines HDT 832/2 and Sarchimor, using the non-host resistance model coffee – *U. vignae* Barclay for comparison.

MATERIAL AND METHODS

Coffee plants and rust fungus

The lower surface of young coffee leaves from plants of Timor Hybrid (HDT 832/2) and the commercial variety Sarchimor (HDT 832/2 x Villa Sarchi), bearing the resistance genes $S_H5,6,7,8,9,?$, were inoculated with fresh urediospores of *H. vastatrix* (isolate 1065, from India, race II, virulence gene v_5) and *U. vignae* (isolate CPR-1, race I) (Heath, 1971; Silva et al., 1999).

Light microscope observations of fresh tissues

Pre-penetration fungal growth stages (germinated urediospores and appressoria formation over stomata) were visualized on leaf pieces, as described (Silva et al., 1999). For time course studies of fungal growth and plant cell responses, cross sections of infected leaf fragments, made with a freezing microtome, were submitted to blue lactophenol staining, epifluorescence and aniline blue tests (Silva et al., 1999; 2002). Observations were made with a microscope Leitz Dialux 20 microscope equipped with a mercury bulb HB 100W, u.v. light and blue light (Silva et al., 2002).

Molecular studies

RNA was extracted from inoculated and control plants and cDNA synthesised as described (Azinheira et al., 2010). RT-qPCR was performed on an iQ5 thermocycler (BioRad) using the iQ SYBR Green Supermix (BioRad) according to the manufacturer's recommendations, with primers for receptor-like kinase (*rlk*), *wrky1*, phenylalanine ammonia-lyase (*pal*), chalcone synthase (*chs*), 13-lipoxygenase (*13-lox*), glycosyltransferase (*gt*), pathogen-related (*pr1b*, *pr10*), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) and *ubiquitin* genes (the latter two as control genes) (Ramiro, 2009) and data analysed following the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

RESULTS AND DISCUSSION

Host resistance to rust fungi is commonly expressed after the formation of the first haustorium (post-haustorial resistance) (Heath, 2000; Mellersh and Heath, 2003). In contrast, nonhost resistance sometimes involves pre-penetration events, such as poor location and recognition of nonhost stomata and invariably is associated with restricted growth if the fungus enters the stomatal cavity. In such cases, fungal growth may be inhibited before haustorium formation (Azinheira et al., 2010; Mellersh and Heath, 2003). Our microscopic analyses have shown that *U. vignae* uredospores presented lower ability to germinate on coffee leaves surface, than *H. vastatrix* (data not shown). The same happened with the appressoria differentiated over stomata on Sarchimor leaves, but on HDT 832/2 no significant differences were found (data not shown). The *H. vastatrix* growth inside HDT 832/2 and Sarchimor (Figure 1A) leaf tissues, from 24-96h after inoculation (hai), was similar. In both coffee lines, the fungus ceased growth more frequently at the penetration hypha stage, with less than 10% of infection sites reaching haustoria formation. *U. vignae* stopped its growth in more advanced stages of the infection in Sarchimor (Figure 1B) than in HDT 832/2, but failed to form haustoria in both coffee plants. The pre-haustorial resistance of coffee plants to *H. vastatrix* was associated with the hypersensitive cell death (HR), accumulation of phenolic-like compounds and haustorium encasement with callose (Figures 2A, 2C). At 24 hai HR was detected in the stomatal cells in 50% and 55% of infection sites, of HDT 832/2 and Sarchimor, respectively. At 72 hai, cell death began to be observed in the mesophyll cells invaded by haustoria. In both host plants, haustorium encasement was observed from 48 hai. *U. vignae* induced similar responses in the coffee plants, such as HR and accumulation of phenolic-like compounds (Figure 2B). At 24 hai, HR was detected in the stomatal cells in 65% and 83% of infection sites, of Sarchimor and HDT 832/2, respectively. Cell death of mesophyll cells began to be observed at 48 hai in HDT 832/2, and at 96 hai in Sarchimor, in a very low percentage of infection sites (maximum 2%).

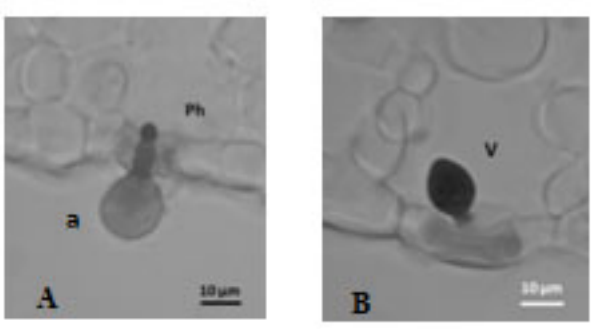


Figure 1. Colonization of Sarchimor leaf tissues by two rust fungi and responses induced in the plant - light microscope observations. Cotton blue lactophenol staining: infection sites showing a penetration hypha (arrow) of *H. vastatrix*, 48 hai (A) and a substomatal vesicle of *U. vignae*, 24 hai (B).

The present study showed that the pre-haustorial resistance of coffee to *H. vastatrix* and *U. vignae* was associated with HR and accumulation of phenolic compounds, particularly in the stomatal cells. HR is the most common expression of incompatibility of gene-for-gene interactions, but it can also occur during nonhost resistance (Azinheira et al., 2010; Heath, 2000; Christopher-Kozjan and Heath, 2003; Prats et al., 2007). However, evidences suggest that processes leading to cell death are not necessarily the same in host and nonhost interactions (Christopher-Kozjan and Heath, 2003; Prats et al., 2007). On the other hand, several studies agree that accumulation

of phenols may be associated with cell death in both types of resistance (Silva et al., 2022; Mellersh and Heath, 2003; Prats et al., 2007; Tiburzy and Reisener, 1990).

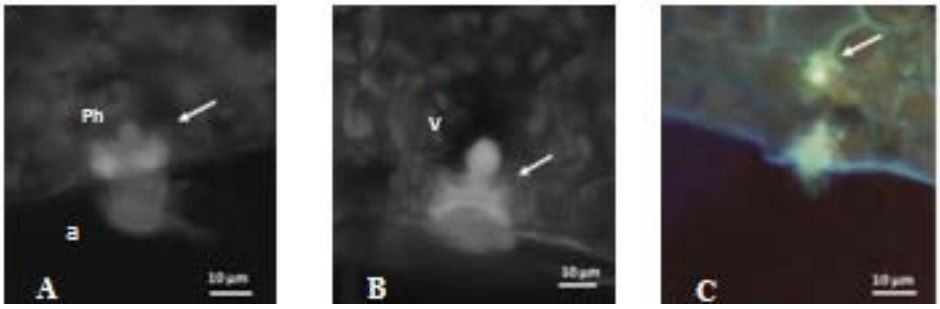
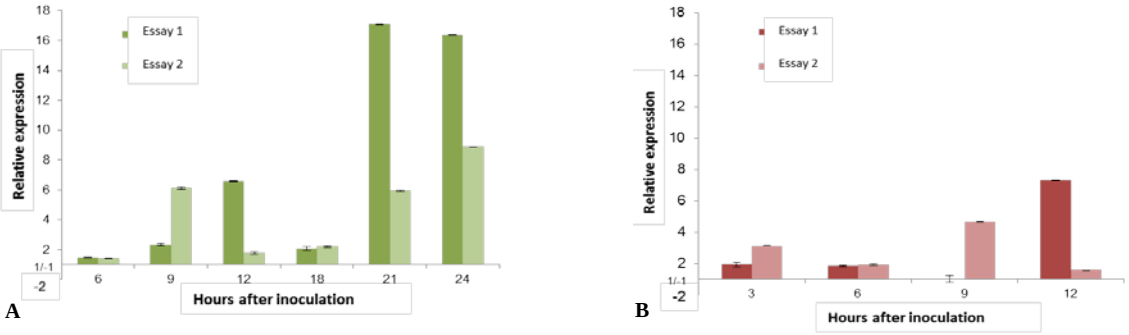


Figure 2. Colonization of Sarchimor leaf tissues by two rust fungi and responses induced in the plant - light microscope observations. A, B: Epifluorescence test (blue light): infection sites showing a penetration hypha of *H. vastatrix*, 48 hai (A), a substomatal vesicle of *U. vignae*, 96 hai (B) associated with autofluorescence of guard and subsidiary cells (arrow) (A, B). Cell autofluorescence under blue light indicated plant cell death (HR) and accumulation of phenolic compounds. C: Aniline blue fluorescence test (u.v. light): infection site of *H. vastatrix* with callose (arrow) around a haustorium within a mesophyll cell, 48hai. A= appressorium, ph = penetration hypha, v= substomatal vesicle.



Figre 3. Relative gene expression of *wrky1* along the first 24 and 12h after inoculation in host (A) and non-host (B) interactions, respectively, using *gapdh* and *ubiquitin* as control genes (results from two independent experiments).

This work showed analogy on resistance to *H. vastatrix* between the two genotypes but *U. vignae* had a more restricted growth in HDT 832/2, therefore chosen for molecular studies. In the host interaction, genes *rlk*, *wrky1*, *pal*, *13-lox*, *pr1b*, *pr10* and *gt* presented an expression profile of two activation peaks. The first peak (6-12 hai) was coincident with appressoria differentiation and penetration hypha formation and also with the beginning of the plant responses at cellular level. The second peak (21-24 hai) was coincident with differentiation of anchors and haustoria mother cells and with plant responses in 50% of infection sites. In the nonhost interaction most genes were not regulated or poorly activated during uredospore germination, appressoria formation, beginning of penetration and differentiation of substomatal vesicles and infection hyphae (3-6 hai), being activated at 9-12 hai (no new fungal structures differentiated; plant responses in >50% infection sites). The *rlk* and *13-lox* genes were exceptions as they were activated at 3-6hai.

In both host and nonhost interactions the expression profile of *rlk* and *wrky1*, previously reported (Ramiro, 2009; Fernandez et al., 2004), suggested that the recognition of the fungus

and signalling occurred during the formation of appressoria and the differentiation of the first post-penetration infection stages.

Although the accumulation of phenols was observed in the cells at the infection sites (from 12 hai in the host interaction and from 6 hai in the nonhost interaction) and contrary to previous results (Ramiro, 2009), *pal* was only moderately activated in the host interaction (inconclusive results in the nonhost interaction), while *chs* was not regulated (data not shown). This accumulation of phenols may arise from post-translational modifications in Pal or from the expression of other genes (Dorey et al., 1997). *13-lox* was moderately activated in both interactions, with the first peak coinciding with the onset of the detection of cell death (HR) and *pr1b* exhibited high expression levels in the host interaction, being poorly activated in the nonhost interaction. *13-lox* and *pr1b* are markers respectively of the jasmonate (JA) and the salicylic acid (SA) pathways (Wasternack, 2007; Thomma et al., 2001). Although the SA pathways seem to prevail in the host interaction, our results suggest that both SA and JA pathways may coexist in the incompatible coffee-rust interaction, contrary to previous results (Ramiro et al., 2009).

ACKNOWLEDGMENTS

This work was supported by FCT, Portugal (Project PTDC/AGR-AAM/71866/2006).

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Hemileia vastatrix Gene Expression during the Infection Process of Coffee Leaves

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SUMMARY

Leaf rust (*Hemileia vastatrix*), the main disease of Arabica coffee, causes up to 30% yield losses in susceptible plants. Although resistant genotypes are identified against all known rust races, loss of resistance to emerging races is a current threat. A thorough knowledge of the plant resistance mechanisms requires a deep understanding of the mechanisms of pathogenicity, particularly because the *Coffea* spp. – *H. vastatrix* interaction follows the gene for gene theory, enabling the inference of virulence genes in the pathogen and the identification of susceptibility genes in the plant from resistance/susceptibility phenotypes. However, very little is known on the molecular mechanisms governing the fungal infection process. Addressing *Coffea* spp. - *H. vastatrix* interactions from the pathogen's perspective, this work aims to study *H. vastatrix* genes expressed during the infection process in coffee leaves.

For such, an *in vitro* system was used to obtain the first steps of *H. vastatrix* differentiation process, while the post-penetration infection stages were obtained *in planta*. Uredospores germination and appressoria differentiation were monitored by light microscopy and no significant differences were observed between artificial membranes and leaves. Nuclear condition of spores, germlings and appressoria observed *in vivo* and *in vitro*, studied by DAPI detection, was identical. Infection stages from 24 hours (formation of infection hyphae) to 21 days after inoculation (profuse host tissue colonization) were collected *in planta* and monitored by light microscopy.

Genes described in the literature involved in the differentiation and pathogenicity of other rust fungi were compared with a *H. vastatrix* EST database, enabling the selection of homologue *H. vastatrix* candidate genes for differential expression analysis by RT-qPCR during differentiation and infection. These included genes involved in signalling (mitogen-activated protein kinase and Gα protein subunit), transport (aminoacid and sugar), carbohydrate metabolism (manitol dehydrogenase, invertase and chitin deacetylase) and maintenance of the biotrophic interaction (RTP1).

The characterisation of the expression patterns of genes involved in the infection process will enhance our comprehension on the genetic basis of pathogenicity in *H. vastatrix*. Together with the information on the genetics of *Coffea* spp. resistance to *H. vastatrix*, this knowledge will improve our understanding on gene for gene interaction in the *H. vastatrix – Coffea* spp.

pathosystem, leading to a more informed deployment of resistant varieties in coffee crops in order to attain a sustainable agriculture.

INTRODUCTION

Coffee leaf rust is the most serious disease of Arabica coffee (*Coffea arabica*) and cause serious epidemics throughout the world. The disease is caused by *Hemileia vastatrix* which is responsible for up to 40% yield losses if no control measures are applied (Silva et al., 2006).

H. vastatrix and *C. arabica* have evolved a complex molecular interaction that led to a high degree of host specificity. *H. vastatrix* is a biotrophic fungi depending on living host tissues for growth and reproduction. During the infection process, it develops complex structures, such as haustoria, responsible for the uptake of nutrients and regulation of the interaction (Silva et al., 2006; Voegele, 2006). Additionally, haustoria must not be recognized by the host, in order to avoid defense reactions (Voegele, 2006). In 1997, the first haustorium-specific cDNA library was developed for *Uromyces fabae*, allowing the identification of genes preferentially expressed in haustoria (PIGs) (Hahn and Mendgen, 1997). Since then, several studies focused on the identification and characterization of these genes in rust fungi (Voegele, 2006). Moreover, an effort was made to identify genes expressed in early stages of fungal development, in order to understand the mechanisms responsible for avoiding plant recognition and involved in fungal development (Voegele, 2006; Deising et al., 2000). However, none of these genes was studied in *H. vastatrix*, therefore molecular studies are needed aiming to better understand this interaction. The purpose of this work was to study the expression profile of genes involved in the infection process and establishment of biotrophy, like chitin deacetylases, aspartate aminotransferase (Asp_AT), Gα protein and MAP kinase, and some PIGs.

MATERIAL AND METHODS

Fungal Material

In vitro growth: Germinating uredospores and appressoria from H. vastatrix isolate CIFC 1065 (race II) were obtained as described (Talhinhas et al., 2010). In vivo growth: Leaves of *C. arabica* (Caturra) were inoculated with H. vastatrix isolate 1065 as described (Azinheira et al., 2010). Leaves were collected at different times after inoculation (24 h, 48 h, 72 h, 7 D, 14 D and 21 D) in order to represent the progression of infection and host tissue colonization (Silva et al., 2006).

Fungal and plant material was immediately frozen in liquid N_2 and stored at -80 °C. Successful uredospore germination, appressoria formation and fungal growth in host tissues were confirmed by light microscopy after staining with cotton blue lactophenol.

Gene Expression Analysis

RNA was extracted from inoculated plants and cDNA synthesized as described (Azinheira et al., 2010). RT-qPCR was performed on a iQ5 thermocycler (BioRad) using the EvaGreen® Supermix (BioRad) as recommended, with primers designed using PerlPrimer v1.1.17. Control genes: *Cytochrome oxidase subunit III*, *GADPH* and *40S*. The primer efficiency was tested with the LinRegPCR program (Ramakers et al., 2003) and data analyzed as: fold change = $E_{target}^{\Delta Ct\ target\ (control-\ target)}$ / $E_{NF}^{\Delta Ct\ Nf\ (control-\ target)}$ (Pfaffl, 2001). The mean and

standard deviation of biological replicates were calculated using mathematical methods previously described (Willems et al., 2008).

RESULTS AND DISCUSSION

In order to establish a biotrophic interaction, rusts need to suppress or evade host defense reactions (Voegele, 2006). Chitin deacetylases, Gα-protein, and MAP Kinase have a key role in this process. The two chitin deacetylases (CH1, CH2) had a higher gene expression in the early stages of *H. vastatrix* development (24 h) (Figure 1A) as expected, since this enzyme is responsible for the conversion of chitin to chitosan during the intercellular growth. By this strategy the pathogen may protect itself, avoiding recognition by the plant and hyphal lysis by extracellular plant chitinases, enabling further colonization of the host (Voegele, 2006). Gα protein also plays an important role in this process, as it is involved in signaling pathways, including a MAP Kinase cascade (Deising et al., 2000). In our study, Gα protein gene was more expressed in germinated uredospores than in appressoria, illustrating its relevance in appressoria formation (Deising et al., 2000). Unexpectedly, *MAP Kinase* expression was very low in the *in vitro* phases, perhaps due to the absence of host signals. In the *in vivo* system, both *MAP Kinase* and *Gα protein* were expressed in the early stages of *H. vastatrix* development (24-48h) but not afterwards, suggesting that that they are more involved in the onset of biotrophy, rather than in its maintenance (Figure 1B). *Asp_AT*, a gene involved in the transfer of an amino group (Botton and Dell 1994), was more expressed in *H. vastatrix* germinated uredospores, being highly suppressed thereafter (Figure 1C). However, functional studies are required to better understand the role of this enzyme.

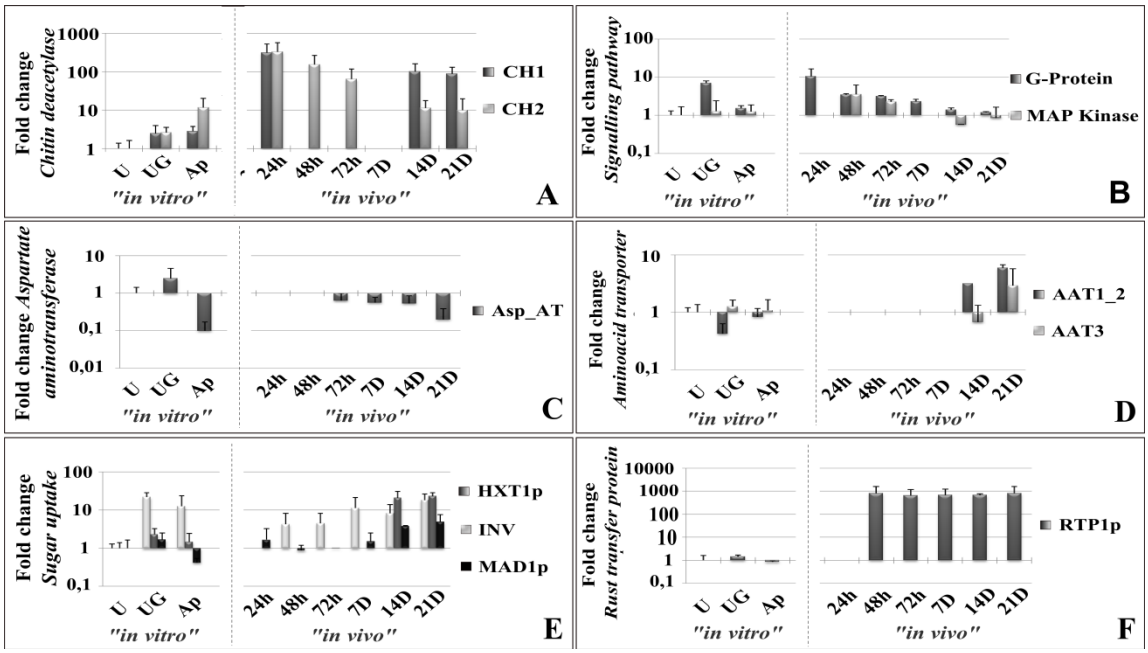

Figure 1. Gene expression (fold changes) in H. vastatrix development compared to resting uredospores: A, Chitin deacetylases; B, Genes involved in signalling pathways; C, Aspartate aminotransferase; D, Amino acids transporters; E, Enzymes involved in sugar uptake; F, Rust transfer protein.

The role of haustoria in nutrient uptake and influence on host metabolism is well established (Voegele, 2006). Three putative secondary amino acids transporters (*AAT1*, *AAT2* and *AAT3*) are known in *U. fabae* (Voegele, 2006). In a *H. vastatrix* haustoria-rich cDNA library

(Talhinhas et al., 2010), two genes showed homology to those amino acids transporters. The first (*AAT1_2*) is homologous to *AAT1* and *AAT2* and the second to *AAT3*, both being preferentially expressed in later stages (Figure 1D). However, in *U. fabae*, *AAT3* is constitutively expressed, while *AAT1* and *AAT2* are preferentially expressed in haustoria.

The sugar uptake in rusts is a complex process that involves different enzymes (Voegele, 2006). In this study, we analyzed the expression of homologues to *U. fabae* hexose transporter (*HXTp1*), invertase (*INV*) and malate dehydrogenase (*MADp1*). *INV*, responsible for the cleavage of sucrose (Voegele, 2006), was expressed in all phases of *H. vastatrix* development, preferentially in later ones (Figure 1E), similar to the profile in *U. fabae*. Sugar uptake is deeply associated with *HXT1p* (Voegele, 2006), which is preferentially expressed in late infection stages (14D and 21D; Figure 1E). Mannitol dehydrogenase (MADp1) is responsible for the reversible conversion of mannitol to D-fructose and, in *H. vastatrix* (Figure 1E), was preferentially expressed in late stages and in germinating spores. In *U. fabae*, *MADp1* is preferentially expressed in haustoria (formation of mannitol from D-fructose, on onset of sporogenesis) and in spores (mannitol utilization for germination), contributing to avoid the oxidative stress resulted from defense reactions (Voegele, 2006).

Rust fungi can suppress host recognition by transferring proteins from haustoria into plant cells. *RTP1p* (from *U. fabae*) was the first gene detected in the cytoplasm of host cells and maybe involved in the maintenance of biotrophy (Voegele, 2006). The *RTP1p*-homolog in *H. vastatrix* seems to be highly expressed *in planta* (48h-21D after the inoculation) but not in the *in vitro* phases, suggesting similar functions in the *H. vastatrix* - *C. arabica* pathosystem (Figure 1F).

This study is the first *H. vastatrix* gene expression analysis during the colonization process in *C. arabica*, contributing for a detailed view of the molecular basis of this interaction, which may lead to better understand plant susceptibility/resistance factors.

ACKNOWLEDGMENTS

Fundação para a Ciência e a Tecnologia (FCT), Portugal (PTDC/AGR-AAM/71866/2006 and SFRH/BPD/47008/2008); Ministère des Affaires Étrangères et Européennes, France, and FCT (Partenariat Hubert Curien PHC-Pessoa 14700TF); Genoscope-Centre National de Séquençage, France.

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A Preliminar Scan at Brazilian *Coffea* Genome Searching for MicroRNAs

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SUMMARY

This work was addressed to study the phenomenon of the RNA interference (RNAi) mediated by microRNAs in coffee plants. This is an important route for the stability and regulation of messengers RNAs, and it influences greatly the dynamics of the cells and the expressions of their genes. Based on the information generated by the "Brazilian Coffee Genome Project", it was possible to identify 22 putative microRNAs (miRNAs) potentially involved in the post-transcriptional gene silencing process in the species *Coffea arabica* and *Coffea canephora*.

INTRODUCTION

The knowledge about the nature and the content of genetic information, as well as the technologies available for the genome sequencing in broad scale, have made possible not only to discover thousands of new genes, but also to develop new technologies. With the transcriptome sequencing of coffee (Vieira et al., 2006), many opportunities have been opened, bringing the chance to learn deeply a lot about biological processes. Among the several options, the understanding of the post-transcriptional gene silencing (PTGS) is considered to be a very attractive issue by acting in several metabolic pathways, most of them crucial for the organism development and for the genome integrity maintenance.

The interference, or silencing, promoted by RNAs is a phenomenon that naturally occurs in eukaryotic organisms and seems to play a relevant role in the elimination of anomalous messengers RNAs and in the organism defense against viruses and transposons (Ketting et al., 1999). In plants, this machinery of post-transcriptional regulation is mediated mainly by recently discovered molecules known as microRNA (miRNAs).

The miRNAs are non-coding endogenous RNAs (20-24 nucleotides), which play an important role in processes such as proliferation, apoptosis, signaling and cell differentiation (Bartel, 2004). These RNAs are directly involved in the regulation of genes related to plant development processes, including meristematic cell identity, morphogenesis, leaf organs polarity, differentiation and flower development (Xie et al., 2007), besides being also involved directly in plant reactions to biotic and environmental stresses (Liu et al., 2008).

The transcriptome modeling process by miRNAs is, in general, very conservative. The silencing routes in plants, fungi and animals share a set of related proteins, suggesting that the common aspects of the routes are old (Barbosa and Lin, 2004). Relying on the high degree of conservation in the sequences and the structure of the miRNAs molecules, it is possible to

predict the existence of orthologous and/or homologous miRNAs among plant species (Yan-du et al., 2009). In this study, bioinformatics approaches have been used based on homology to characterize possible miRNAs in two species of the genus *Coffea*.

Here we report some of their sequences, structures, and possible roles in the process of modeling trancriptoma of the main species of the genus *Coffea* or (*Coffea*'s species)

MATERIALS AND METHODS

The approach used to characterize possible miRNAs was described by Zhang et al. in 2006, and has been broadly applied to characterize small homologous RNAs in plant species. Briefly, miRNAs known in other plant species are selected, repeated sequences are removed and the remaining ones are defined as a reference set of miRNAs. A similarity analysis from the software BLAST (*Basic Local Alignment Search Tool*) is made using that set of data as query sequences. Specifically for this study, this analysis was performed on the database CAFEST. Sequences with until 3 mismatches were accepted among the sequences of the known and the possible coffee miRNAs.

A total of 1549 mature miRNAs belonging to species, *Arabidopsis thaliana, Glycine max, Medicago truncatula, Orisa sativa, Physcomitrella patens, Populus trichocarpa, Saccharum officinarum, Sorghum bicolor* e *Zea mays,* were used to characterize the potential miRNAs contained in the coffee genome database. The sequences of these RNAs were taken from the platform of miRNAs, the miRBase (*Release* 13.0, March, 2009, http://www.mirbase.org/).

The prediction of secundary structure of pre-miRNAs was performed using the software online MFOLD (Zuker, 2003). The following steps were used for screening the candidates of potential miRNAs or pre-miRNAs: (1) Only 0-3 nucleotide mismatches between predicted mature miRNAs and previously known plant mature miRNAs were allowed. (2) miRNA had 30-70% contents of A + U. (3) The secondary structure of pre-miRNA sequence must exhibit an appropriate hairpin structure which contains the mature miRNA sequence within one arm. (4) The secondary structure of pre-miRNA had higher negative adjusted minimal free energies (AMFEs) and minimal free energy index (MFEIs), MFEIs were not less than 0.85. (5) miRNA had less than six mismatches with the opposite miRNA* sequence in the other arm. (6) No loop or break in miRNA sequences was allowed, (reviewed in Yan-du et al., 2009).

RESULTS AND DISCUSSION

After searching the EST database and comparing with characteristics of previously known miRNAs of others plant species, a total of 22 putative miRNAs were identified in *Coffea arabica* and *Coffea canephora*. These miRNAs belong to 8 different miRNA families' called mir167, mir171, mir172, mir319, mir390, mir414, mir417 and mir482.The homologues miRNAs families described by our *in silico* research are correlated with various biological processes in plant species such as *Arabidopsis thaliana, Oryza sativa, Brassica napus* and cotton. This processes, include growth and developmental patterning, metabolic processes, hormone responses, protein degradation, mature mRNA formation, stress and defense signaling.

One of the mir families characterized was mir167 (Figure 1). This family is associated to mRNAs that encode auxins response factors (ARF transcription factors) in both *Arabidopsis thaliana* and *Oriza sativa* (Rhoades et al., 2002). The regulation of these transcript levels is

important to the development of several plant responses to auxin signals, including for example, cell elongation, and roots and shoots division and differentiation. Transgenic plants phenotypes (35S: MIR167b) have abnormally short filaments, deformed anthers and pollen grains with difficulty to germinate, suggesting that there is an important connection between the regulatory process of gene expression mediated by miRNAs and auxin signaling network which promotes the reproductive development of plants (Ru et al., 2006).

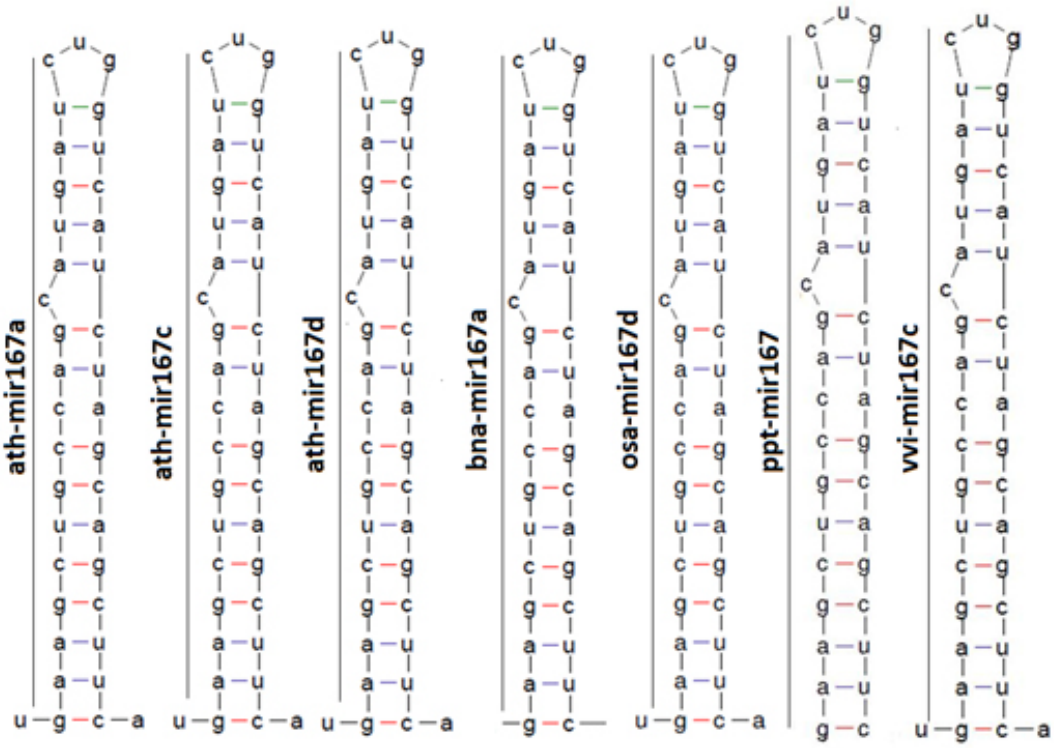

Figure 1. Secondary structure prediction of some microRNAs performed using MFOLD software.

CONCLUSIONS

In summary, in this study, we presented predictions of conserved coffee miRNAs. A total of 22 potential miRNAs, belonging to 8 miRNA families, and a survey of potential target genes by these microRNAs were identified.

The assessment of the role of microRNAs combined with characterization of the main transcription factors represents a key step to understanding the processes that act by controlling the development of the coffee crop.

The next steps, therefore, are to experimentally analyze the functional categories suggested by our computational approach, determine the analogous molecular functions among divergent plant species and further elucidate any significant relation between the miRNAs and their target.

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Shine Gene Improving the Drought Tolerance of Chimeric *Arabidopsis Thaliana* and *Coffea Arabica*

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SUMMARY

The main objective of this work was to express the *SHINE* gene of *Arabidopsis thaliana* in transgenic hairy roots of *Arabidopsis thaliana* and *Coffea arabica* after *Agrobacterium rhizogenes*-mediated root transformation in order to improve the drought tolerance of such chimeric plants. The chimeric plants, transformed with DsRED or GUS (control plants) and 35S::*SHN* were subjected to drought for 10 and 11 days (for *Arabidopsis thaliana*) and 15 days (*Coffea arabica*) by withholding water, and subsequently rehydrated to observe the recovery after 1 week. After only 12 days of treatment the 35S:*SHN* chimeric plants started to wither and at 15 days, the transgenic plants were completely withered but at that point the DsRED (control) chimeric plants had dried leaves. Whereas DsRED (control) chimeric plants did not recover from the 15-day dehydration treatment and had completely dried out, all the 35S:*SHN* chimeric plants recovered, becoming greener and stronger.

INTRODUCTION

Drought is the most serious environmental factor limiting the worldwide productivity of agricultural crops such as coffee, with devastating economical and sociological impact (Grisi et al., 2008). For example, in Minas Gerais, the largest coffee region in Brazil, short drought periods can result in 20 to 30% loss in yield at harvest (Rivero et al., 2007). Warmer global temperatures are expected to cause an intensification of the hydrologic cycle, with increased evaporation over both land and water. The higher evaporation rates will lead to greater drying of soils and vegetation, especially during the warm season. Climate models also project changes in the distribution and timing of rainfall. The combination of a decrease in summer rainfall and increased evaporation will lead to more severe and longer-lasting droughts in some areas (Union of Concerned Scientists, 2009).

Plant genetic transformation can be a shortcut for introducing a new trait and developing new cultivars without losing the genetic background of the original transformed cultivar (Ribas et al., 2006). Dehydration-response transcription factors, which are involved in the hydric, cold and salt stress response, have been used to produce transgenic plants with better stress tolerance (Kasuga et al., 1999). A number of genes that respond to desiccation and low temperature at the transcriptional level have been described, and their gene products are thought to be involved in stress response and tolerance (Hughes and Dunn, 1996).

The *SHINE* gene encodes an AP2/EREBP transcription factor suggesting that this clade of genes acts in the regulation of lipid biosynthesis required for plant environmental protection, including organ separation processes and wounding (Aharoni et al., 2004). The cuticular layer, comprising cutin and waxes, plays multiple roles in plants, including the regulation of epidermal permeability and nonstomatal water loss and protection against insects, pathogens,

UV light, and frost (Sieber et al., 2000). Overexpression of *SHINE* can induce the expression of these drought stress-related genes under normal growth conditions in transgenic plants and confer improved tolerance to drought.

The aim of this work was to express the *SHINE* gene of *Arabidopsis thaliana* in transgenic hairy roots of *Coffea arabica* after *Agrobacterium rhizogenes*-mediated root transformation in order to improve the drought tolerance of such chimeric plants.

MATERIALS AND METHODS

The plasmids used at the transformation of *Arabidopsis thaliana* and *Coffea arabica* were: pMOG22-35S::*SHN2* containing the *SHINE2* cDNA, under control of the constitutive CaMV35S promoter and 35S::HPTII gene construct which confers hygromycin resistance when expressed in plants (Aharoni et al., 2004).

The plasmids pMOG22-35S::*SHN2,* pCAMBIA1301 containing Gus and pREDRoot marker were introduced on *Agrobacterium rhizogenes* and used on the transformation.

After the transformation and selection, the chimeric plants, transformed with 35S::*SHN2*, DsRED or GUS (control plants) were subjected to drought for 10 and 11 days (for *Arabidopsis thaliana*) or for 15 days (coffee) by withholding water, and subsequently rehydrated to observe the recovery after 1 week. On the control treatment the transformants 35S::*SHN2* and DsRED or GUS (control plants) were maintained at 85% field capacity during the experiment. Four plants per treatment were used. The survival was scored, the fresh biomass was collected and the collected plants were oven-dried at 60 °C for 5 days and the dry weight was measured.

RESULTS AND DISCUSSION

On the drought tolerance experiment of chimeric *Arabidopsis thaliana* plants expressing 35S::*SHN2* in roots was possible to observe the 35S::GUS plants started wilting after 6 days without water, while the 35S::*SHN2* started wilting just after 8 days. As result of this about 50% of the 35S::*SHN2* chimeric transgenic plants exposed to 10 days withholding water and rewatered for one week recovered the drought treatment, while none of the 35S::GUS chimeric control plants recovered. When water was withheld for 11 or 12 days, none of the chimaeric 35S::*SHN2* and 35S::GUS plants survived. The dry weight of the 35S::*SHN2* plants, did not differ significantly from the well-watered plants, and was significantly higher when compared with the drought-treated control plants.

On the drought tolerance experiment of chimeric of *Coffea arabica* the drought tolerance experiment was set up with 8-month-old coffee plants transformed with either 35S::*SHN2* or DsRED (control). DsRED (control). The chimeric plants started to wither after 8 days without water while the 35S:*SHN2* chimeric plants still did not show strong effect related to the water deficiency. The 35S:*SHN2* chimeric plants started to wither only after 12 days and at 15 days the transgenic plants were completely withered but at this point the DsRED (control) chimeric plants had dried leaves.

Whereas DsRED (control) chimeric plants did not recover from the 15-day dehydration treatment and completely dried out, all the 35S:*SHN2* chimeric plants recovered, becoming greener and stronger. This showed that the 35S::*SHN2* chimeric plants were superior to the

DsRED (control), because they maintained leaf water potential during drought, even though they were partially wilted.

During the experiment the plants overexpressing 35S::*SHN2* showed growth retardation under normal growth conditions. The expression of *35S::AtDREB1A* and in transgenic Arabidopsis led to an enhanced dehydration tolerance and also severe growth retardation under normal growth conditions (Liu et al., 1998; Kasuga et al., 1999; Dubouzet et al., 2003). Transgenic *Hipericum perforatum* plants regenerated from hairy roots transformed with *A. rhizogenes* strain A4M70GUS showed normal phenotype, but also differences in several growth parameters (Koperdakóva et al., 2009).

These results indicate that expression of *SHN2* in roots alone is already sufficient to protect plants against drought, however, the window in which root expression of *SHN2* protects the plants from drought appears to be relatively narrow. Although the shortest period of withholding water causing 35S::GUS plants to die was not determined, only a day longer withholding water was sufficient to make the difference between full recovery and complete loss of 35S::*SHN2* plants. This means the *SHN2* transcription factor possibly can induce the earliest reply to drought activating stress-responsive mechanism and this can happen in the root inducing the expression of gene involved on signal sensing and/or the transcription factor and their induced genes can be transported to the shoots where can induce the other transcription factors responsible for drought and the stress-responsive mechanisms.

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In Silico Characterization of Putative Members of the Coffee (*Coffea arabica*) Ethylene Signaling Pathway

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SUMMARY

The plant hormone ethylene is involved in several developmental and physiological processes in plants and the control of its sensitivity is a key factor on limiting its responses at target cells. We report the results of our search in the Coffee expressed sequence tag (CAFEST) database for expressed sequence tags (ESTs) related to the ETHYLENE RESPONSE FACTORS (ERFs). Multiple alignments comprising the sequences found in the CAFEST database and other species characterized ERFs were performed and the phylogeny between them was assessed by a phylogenetic tree constructed by the MEGA4 software. The expression profile was assessed by *in silico* Northern performed by the Cluster and visualized by the TreeView program. The CAFEST database showed a high number of sequences related to ERFs and the motif and phylogenetic analysis allowed the classification of the sequences found into the four ERF classes previously described. The electronic Northern detected the expression of these putative coffee ERFs in different tissues, development stages and stress conditions.

INTRODUCTION

The plant hormone ethylene plays an important hole in such diverse physiological and development processes including organ senescence, seed germination, stem elongation, fruit ripening, as well as biotic and abiotic stress responses.

Once produced, being a gaseous hormone, ethylene easily defuses through the intercellular spaces and adjacent tissues. Without the possibility of having a transport regulation mechanism, the controlling of its sensitivity is a key factor on limiting its responses at target cells (Alonso and Ecker, 2001). Ethylene is perceived by a family of five membrane bound receptors, ETHYLENE RECEPTOR1 (ETR1), ETR2, ETHYLENE SENSOR1 (ERS1), ERS2 and ETHYLENE INSENSITIVE4 (EIN4), that along with CONSTITUTIVE TRIPLE RESPONSE (CTR1), a Raf protein kinase, act as negative regulators. Downstream CTR1, the integral membrane protein ETHYLENE INSENSITIVE2 (EIN2), the ETHYLENE INSENSITIVE3 (EIN3) transcriptional factors and the ETHYLENE RESPONSE FACTORS (ERFs) have been identified as positive regulators since their loss of function or overexpression can lead to ethylene insensitivity or a constitutive ethylene response in air, respectively.

The ERFs are uniquely present in plant kingdom and belong to the AP2/ERF superfamily of transcriptional factors (Nakano et al., 2006). These transcriptional factors regulates the expression of genes involved in many biological process related to plant growth and development, as well as environmental stimuli responses.

In this study, we have investigated the CAFEST genome database, employing bioinformatics tools and *in silico* expression analyses, to characterize putative coffee *(Coffea arabica)* ERFs.

MATERIAL AND METHODS

Database searches and alignments

In order to identify homologs of functionally characterized ERF genes involved in the ethylene responses, data mining in the CAFEST database (http://bioinfo04.ibi.unicamp.br), composed by 214.964 EST sequences obtained from 37 libraries (Vieira et al., 2006), were carried out using plant gene (BLASTn) and protein (tBLASTn) sequences as bait, as well as keyword searches. The sequences with significant similarity (e-value>10^{-4}) were selected and sent to the sequence manager and manipulation system, the *GeneProject*, and submitted to clustering by using the CAP3 program (Huang and Madan, 1999), which is integrated to the system, forming the EST contigs and singlets.

The *Coffea arabica* EST-contigs (CaC) and EST-singlets (CaS) obtained were manually annotated and data validation was performed by local tBLASTx and tBLASTn searches of the retrieved sequences against the GenBank database. Then, the selected sequences were used as bait in another search against the CAFEST database, aiming at finding new reads, as well as to remount incomplete clusters. This process was repeated until no more new significant reads were found. The ORFs (Open Reading Frames) of the validated sequences were obtained through the tool ORFinder, from the NCBI homepage (http://www.ncbi.nlm.nih.gov), and their protein sequences were generated through the translate tool found in the ExPASY (http://www.expasy.ch) protein database. The protein sequence alignments were performed by the ClustalW program (Thompson et al., 1994), using default parameters.

Phylogenetic analysis

The putative functionally of the deduced amino acid sequences of coffee transcripts, compared to homologs from other species, was assessed by phylogenetic trees performed by the MEGA software, version 4.0 (Tamura et al., 2007), with neighbor-joining comparison model (Saitou and Nei, 1987), p-distance method and pair-wise suppression. Bootstrap values from 1000 replicates were used to assess the robustness of the trees (Sitnikova et al., 1995).

In silico gene expression analysis

In silico qualitative gene expression profiling was performed using virtual Northern blot analyses of the coffee EST database. For each EST-contig and EST-singlet, frequencies of reads that form each EST contig and EST singlet in the libraries in which they were expressed were calculated. This procedure required that the data were previously normalized to give a more accurate idea of the expression degree of the sequences in each treatment and plant organ when all of the libraries were considered in this work.

The normalization consisted in multiplying each read by the quotient between the number of reads from the library where it was expressed and the sum of reads of all libraries where expression was found. The results were plotted in a matrix and gene expression patterns among ESTs and libraries were obtained by hierarchical clustering, performed by the Cluster v.2.11 program (Eisen et al., 1998). Graphic outputs were generated by the TreeView v.1.6 software (Eisen et al., 1998) and presented in a color scale from black to red, where as closer to red color higher the expression level. No expression was represented by gray color.

RESULTS AND DISCUSSION

According to the results obtained in the CAFEST database, a total of 166 reads related to ERF were found and clusterized into 24 contigs and 9 singlets, with all sequences encoding for a complete or partially complete ERF domain, which characterize these transcriptional factors. According to the amino acid identity within the ERF domain, these sequences could be separated into the two subfamilies that form the ERF family (Sakuma et al., 2002): eight contigs and six singlets belonged to CBF/DREB (C-repeat/DRE-Binding Factor / Dehydration-Responsive Element Binding proteins) subfamily, not involved in the ethylene signaling pathway; 16 contigs and three singlets belonged to ERF subfamily, which participate in the ethylene signaling, and were analyzed in this work.

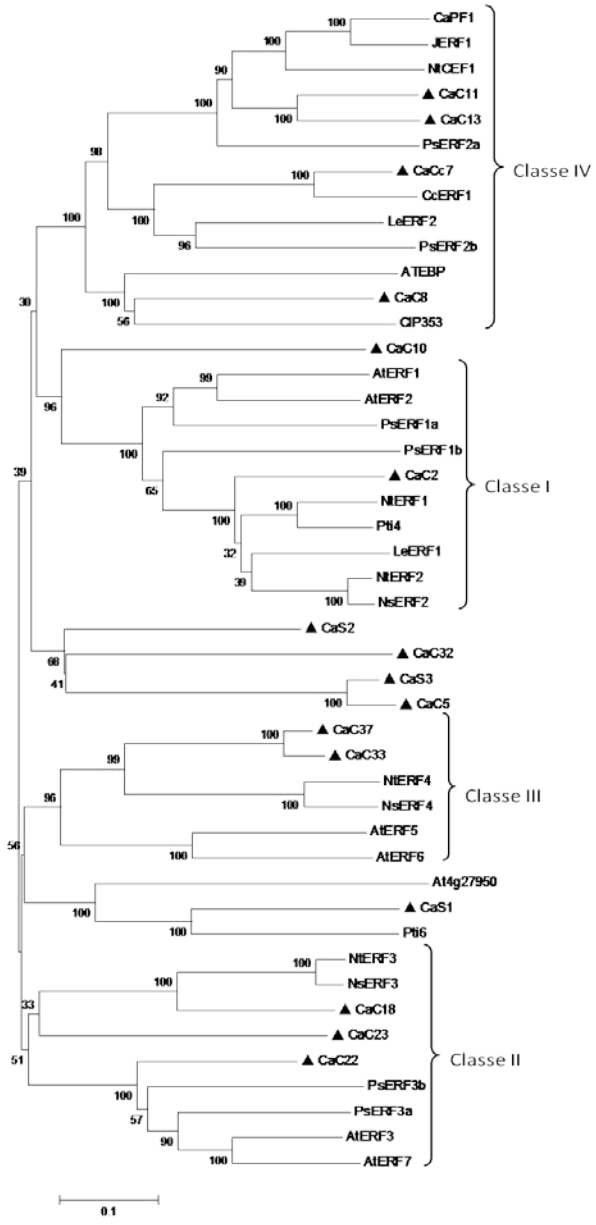

Figure 1. Phylogenetic analysis of putative coffee ERF-like proteins (▲) and homologs sequences obtained from the NCBI database related to ERF transcriptional factors.

Multiple alignments between the putative *Coffea arabica* ERF proteins and previously described ERF sequences from different plant species highlighted a number of conserved motifs and structural similarities that are commonly associated with the AP2/ERF family of transcriptional factors, allowing their classification into the four ERF proteins classes previously described (Figure 1) (Fujimoto et al., 2000; Tournier et al., 2003). The *in silico* Northern showed that these putative transcriptional factors are involved in a wide range of processes, since an expression in 24 different libraries, involving different tissues, development stages and stress conditions, could be observed (Figure 2).

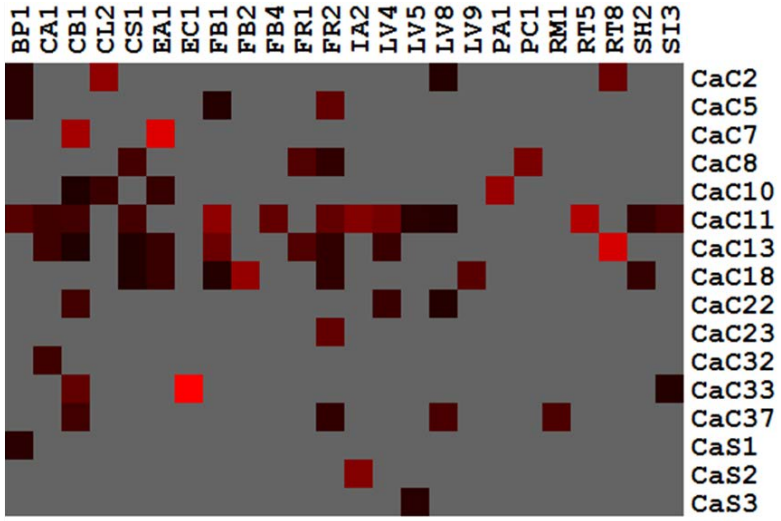

Figure 2. In silico expression profile of putative coffee ERF-like genes. The normalized numbers of reads for the transcripts in each library are represented in a scale from black to red. The EST contigs and singlets are represented as lines and coffee libraries as columns. Coffee libraries are as follows (Vieira et al.., 2006): Suspension cells treated with acibenzolar-S-methyl (BP1); Non embryogenic calli with and without 2,4 D (CA1, PC1); Suspension cells treated with acibenzolar-S-methyl and brassinoesteroids (CB1); Hypocotyls treated with acibenzolar-S-methyl (CL2); Suspension cells treated with NaCl (CS1); Embryogenic calli (EA1,IA2); Embryogenic calli from Coffea canephora (EC1); Flower buds in different developmental stages (FB1, FB2, FB4); Flower buds + pinhead fruits + fruits at different stages (FR1, FR2); Young leaves from orthotropic branch (LV4, LV5); Mature leaves from plagiotropic branches (LV8, LV9); Primary embryogenic calli (PA1); Leaves infected with leaf miner and coffee leaf rust (RM1); Roots with acibenzolar-S-methyl (RT5); Suspension cells with stressed with aluminum (RT8); Water deficit stresses field plants (pool of tissues) (SH2); Germinating seeds (whole seeds and zygotic embryos) (SI3).

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Modifications in the Carbohydrates Metabolism in Seedlings of Coffee Tree Progeny Siriema under Drought Conditions

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SUMMARY

Water deficiency is an important issue that can cause significant losses of production in crops. Climate changes are occurring and longer periods of drought are becoming more common. The study of physiological responses of plants to drought can help to understand the mechanism to further develop tolerant cultivars. This work aimed at studying the effects of drought on carbohydrate concentrations in *Coffea arabica* seedlings cultivar Siriema. Seedlings were growing in a greenhouse under non-irrigated and daily irrigated conditions. Plants were evaluated every three days until they reached a permanent wilting point, or up to a maximum of 30 days of water withholding. In addition, plants under different drought conditions (from three to 30 days) were irrigated and evaluated 24 and 48 hours later. There was a significant increase in the levels of total soluble sugars and reducing sugars, both in the leaves and roots of the non-irrigated plants. The re-irrigated plants behaved like the non-irrigated plants, however, lower levels of sugars were detected in them. The leaves and roots of the non-irrigated plants also showed a significant reduction in the levels of starch.

INTRODUCTION

Plants have developed biochemical responses to adjust their metabolism according to environmental changes. Responses may be complex and may occur at the morphological, physiological and molecular level; however, they will depend on the plant genotype, stress intensity and duration, plant growth stage and the nature of the stress. The solutes intracellular storage and distribution in response to water stress conditions and salinity are important mechanisms to tackle water stress (Turner, 1986).

The osmotical adjustment is considered one of the most efficient mechanisms to maintain the cell turgent (Chaves, 1991). It controls mainly the stomatal opening and photosynthesis under low soil water potential. Several investigations have been conducted to study tolerance to water stress in coffee. Most of them have been conducted in *Coffea canephora* (Pinheiro et al., 2005) and *C. arabica* (Dias et al., 2007). The Siriema cultivar has an ability to maintain its leaf area and vigour under drought conditions (Dias et al., 2007). To date, drought tolerant coffee plants have been catalogued empirically because there is a lack of knowledge about their physiological responses to water stress (Damatta and Ramalho, 2006).

Understanding which mechanisms are involved in drought responses will generate relevant information to assist in coffee breeding and/or genetic engineering to obtain new commercial varieties with drought tolerance. Therefore, the objective of this research was to study the

effect of drought conditions on the carbohydrate concentrations in Siriema coffee seedlings. It was also aimed at evaluating Siriema's capacity to recover from water stress conditions.

MATERIAL AND METHODS

Seedlings were growing in a greenhouse under non-irrigated and daily irrigated conditions to a maximum of 30 days. Also, each 3 days a subgroup was re-irrigated and evaluated 24 and 48 hours later.

Every day during the experiment, between 5 to 6 am the water potential was measured and samples of leaves and shoots were taken for laboratorial analysis. The content of the total soluble sugars and reducing ones were measured as described by Silva et al. (2003).

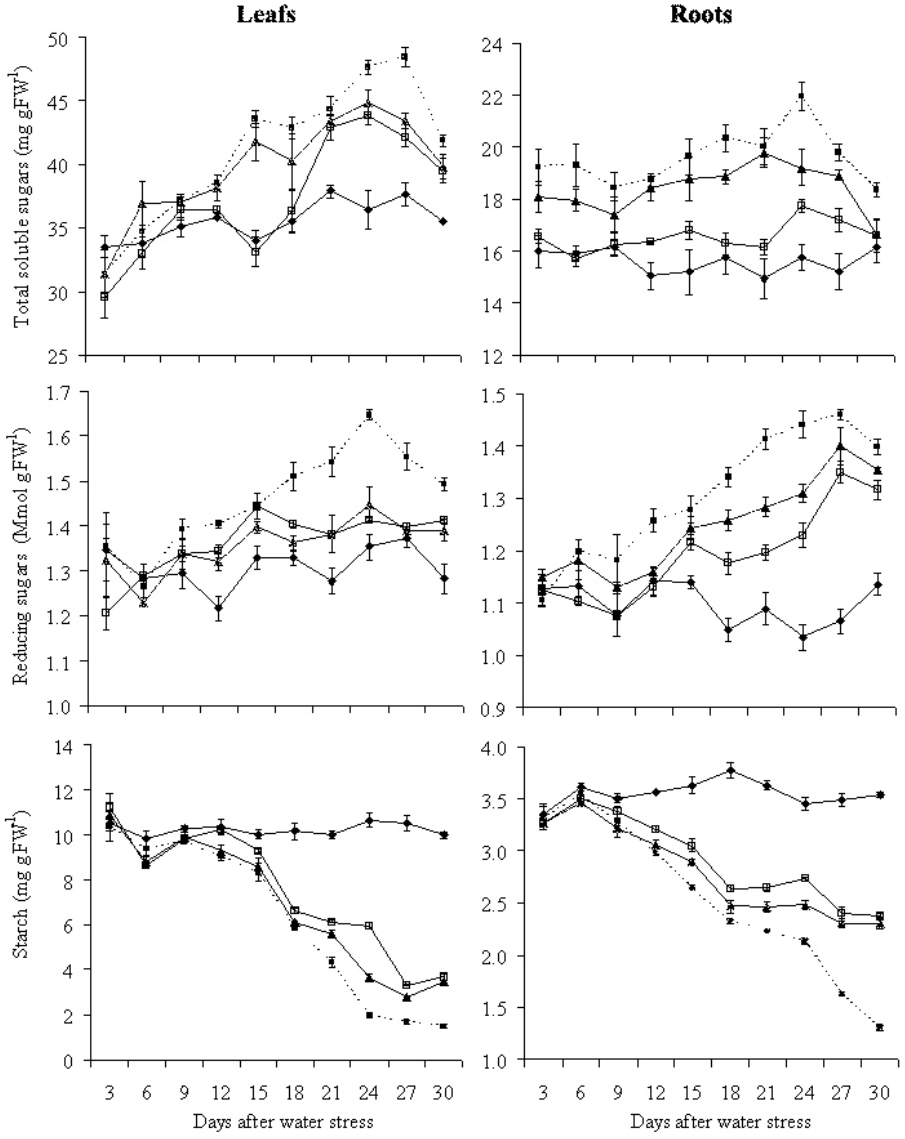

Figure 1. Carbohydrate concentrations in leaves and roots of seedling of the Siriema coffee cultivar under drought conditions. Plants were grown under drought conditions for a 30 day period. Four treatments were tested: Irrigated plants (–♦–), non irrigated plants (--■--), plants that were re-irrigated and evaluated 24 (–▲–) and 48 hours (–□–) after a water stress period. Mean values are presented, dashes shows the standard errors. FW means fresh weight.

RESULTS AND DISCUSSION

Reducing sugars increase under drought conditions in coffee leaves and roots. While the irrigated plants maintained a constant concentration of reducing sugars in the leaves (Figure 1), the plants subjected to the non-irrigated treatment increased their concentration of reducing sugars after day 9. On day 24 the plants reached their highest concentration (1.65 Mmol / g · MF^{-1}), but the levels of reducing sugars decreased after this day. The same trend was observed in roots, which means that reducing sugars increased under water stress conditions to potentialize oxi-reduction reactions. This increase could happen as a result of invertases activity to decrease the water potential if the hexoses released by this enzyme contribute to the osmotical adjustment and prevent additional water-stress-induced cell damage (Valliyodan and Nguyen, 2007). Similar results were observed in the Siriema cultivar, whereas opposite changes in carbohydrate levels were found in the drought-stressed plants that were re-irrigated.

Starch is degradated as a drought response in the leaves and roots of the Siriema cultivar. Under drought conditions, the starch content decreases after 12 days of water stress and reaches values as low as 1.48 mg / FW · g^{-1} (in leaves) and 1.30 mg / FW · g^{-1} (in roots) after 30 days of drought (Figure 1). Re-irrigation increases the starch content (3.69 mg / FW · g^{-1} at day 30). This results shows that a coffee plant's starch reserves are hydrolyzed to overcome drought conditions.

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Evolutionary Changes of the Gene Expression Pattern Mediated by Exonized Transposable Element Sequences in Coffee Genomes

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SUMMARY

A wave of public information has enabled the report of innumerable examples where transposable elements (TEs) fragments are incorporated as exons into mRNAs, however, the proposition that such exonized TEs encode functional proteins is controversial. In order to characterize the gene expression pattern of TE-derived coding sequences identified in the *Coffea arabica* genome, we analyzed the macroarray profiling and digital northern of transcripts with distinct molecular functions of sequences containing TE-cassettes or not. The evolutionary changes of the gene expression pattern mediated by exonized TE sequences using probes of tissues and stress conditions derived from *C. arabica* and *C. canephora* are presented and discussed.

INTRODUCTION

As a major component of many eukaryotic genomes, transposable elements (TEs) has been shown to be a main player on their evolution, because they can induce profound changes in genome dynamics and reorganization (e.g. translocation, deletion, segmental duplication) (Shapiro and von Sternberg, 2005); lead to genome expansion and contraction (Bennetzen, 2002); have an important impact on transduction and amplification of host gene fragments (Jiang et al., 2004). Moreover, of particular interest is the TE contribution to protein coding sequences, because they can directly influence the phenotype by altering protein sequences. This aspect is well documented, however, only at the transcript level, in a broad range of species within the past few years: *Homo sapiens* (Nekrutenko and Li, 2001), *Mus musculus* (DeBarry et al., 2005), *Bos Taurus* (Almeida et al., 2007), *Oryza sativa* (Sakai et al., 2007), *Coffea spp* (Lopes et al., 2008), *Drosophila melanogaster* (Ganko et al., 2006) and *Caenoharbidtis elegans* (Ganko et al., 2003).

Evolutionary consequences of the TE exaptation would be more profound if TE-cassettes were present not only at the transcript level but also at protein level because “protein, rather genes or mRNA, represent the key players in the cell” (Pradet-Balade et al., 2001), by determining the cellular phenotype, and thus directly affecting fitness. Despite of the broad plethora of TE cassettes in transcripts, there are few reports of potentially functional proteins containing TE-encoded fragments (Gerber et al., 1997; Hilgard et al., 2002; Hoenicka et al., 2002), as well as there is one unique evidence that supports the existence of such proteins in

vivo (Gotea and Makalowski, 2006). On the other hand, the presence of TE cassettes in transcripts does not guarantee their translation, because eukaryotic cells contain several quality control mechanisms that can initiate the degradation of the transcript and even of the protein product immediately after translation (Wagner, 2002). For example, alternatively spliced exons that carry premature termination codon in frame are used to down regulate the amount of protein products of certain genes by being targets of the non-sense mediate mRNA decay (NMD) pathway (Wagner, 2002). Many TEs can provide alternatively spliced exons with premature terminate codons, thus a potential role in regulating gene expression post-transcriptionally needs to be taken seriously into consideration. Therefore, the mRNA surveillance onto TE-harboring spliced isoforms needs to be evaluated.

MATERIALS AND METHODS

Plant Material

For the probe synthesis, total mRNA was extracted following tissues and treatment conditions from *C. arabica*: a) Hydric stress: Cultivars tolerant (Iapar59) and sensitive (Rubi) to drought maintained in field conditions with (ψpd= -0,5 MPa) and without (ψpd= -1,7 MPa) water; b) Cell culture: embriogenic callis cv. Catuaí Vermelho maintained in multiplication medium; c) Inhibition tretament: embriogenic callis treated with the protein biosysthesis inhibitor Cycloheximide (CHX: 10 mg/mL in alcohol) was added to cell culture to final concentration of 100 ug/mL. *C. canephora:* a) Hydric stress: clones var. Conillon tolerant (14) and sensitive (22) to drought were selected by the INCAPER (Ferrão et al., 2000) and grown in greenhouse with (unstressed condition) or without (stress, ψpd leaves = -3.0 MPa) water.

Macroarray experiments

Our expression analyses were carried out using transcripts with distinct molecular functions whether containing TE-cassettes (110) or not (93); both sequences highly related by sequence similarity, identified in the *Coffea arabica* genome in our previous study. The 10 total RNA samples were extracted from cells using Concert™ reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer´s protocol. A total RNA sample (10 µg) treated with DNAse was used per array and converted in cDNA and labeling using *SuperScript III First-Strand Synthesis System for RT-PCR* (Invitrogen, Carlsbad, CA) with [α-^{33}P]-dCTP.

The PCR products of each interest transcript was spotted onto *Performa II nylon filters* (Genetix Limited, Hampshire, UK) using the high-throughput robot system *Q-BOT* (Genetix Limited). To increase the signal homogeneity in the amount of PCR product among spots and filters, the set of 203 cDNAs were spotted in duplicate in a 2×2 array into two identical arrays arranged in the same nylon filters (222 × 222 mm). The filters were prehybridized 2 h at 65 ºC in *Modified Church and Gilbert Buffer* (0.5 M Phosphate Buffer pH 7.2, 7 % SDS, 10 mM EDTA) and hybridized overnight with cDNA sample probes. Membranes were washed for 15 min three times with 0.1 % SDS/1 × SSC and three times with 0.1 % SDS/0.1 × SSC at 65 ºC. After washing, the filters were exposed in imaging plates *BAS-MS 2340* (Fujifilm, Tokyo, Japan) for 72 h and scanned using fluorescent image analyzer *FLA3000* (Fujifilm). The radioactive intensity of each spot was quantified by *Array Guage* software (Fujifilm), corrected by level of local background, normalized to the average intensities of the ubiquitin reference gene and for the differences of probe labelling using the average signals of all genes studied. The homogeneity of the spot replicates were evaluated and represented by average

value using *limma* of the Bioconductor package (Gentleman, 2005). The final expression values were analyzed by *heatmap* using R (http://www.r-project.org).

RESULTS AND DISCUSSION

Many of the transcripts modified by TE insertions presented either low or null expression. Preliminary, it can be observed that four different transcripts present higher expression compared to the expression of the reference gene (ubiquitin) in the five tissues analyzed. They are the histone subunity H3, a putative protein of the family of the hidroxilases - CER1 and a protein with unknown function. It is worth to be highlighted also the protein of rust resistance type Rp1-D just expressed in callis and those just expressed in the four tissues submitted to water stress: transmembrane protein of the MLO family; a protein containing associated domain the heavy metals and the ribossomal 50S protein of the family L21.

Interestingly, the five transcripts containing TE fragments, expressed in the five tissues of *C. arabica* (hybrid specie between *C. canephora* and *C. eugenioides*) also had their expression detected in the *C. canephora* tissues suggesting the origin of the CDS mosaic in one of the parental genomes and the maintenance of their transcriptional activity after the polyploidy process. Surprisingly, they also had increased expression in embryogenic callis treated with the protein biosynthesis inhibitor Cycloheximide. The same can be observed with several transcripts silenced in *C. arabica*, but expressed in the clone 22 irrigated of *C. canephora*.

Interestingly, the analysis of expression of 109 CDSs without fragments of TEs, but related to those with TEs, also present very low or null expression in the *C. arabica* and *C. canephora* tissues and the expression restored or even increased in callis treated with Cycloheximide. We cannot discard the possibility of these CDSs do contain TEs in other regions besides in that represented in the unigenes used in our *in silico* analyses because the transcriptome of the *Coffea* species present in the PGCB database were composed by the 5' end sequence of the mRNAs. Taken together, the results of the isoforms profiles containing or non TEs highlight the complexity of the evolutionary and physiologic impact of TE insertions in the eukaryote coding genes.

Our gene expression analyses using probes of cell cultures and of plants under water deficit stresses obtained both from *C. arabica* and from one of its parental genome, *C. canephora*, led us to conclude that most of these TE-derived mRNAs are not expressed. In some cases, the chimerical isoforms present low gene expression levels restrict to stressful or tissue-specific conditions. Surprisingly, several of these transcripts had their expression reestablished in embryogenic cells treated with the protein biosynthesis inhibitor Cycloheximide; a drug that allows the accumulation of previously silenced transcripts by reverting a mechanism named of nonsense-mediated mRNA decay (NMD).

Recent studies have shown that many TE-derived coding sequences (CDSs) are alternatively spliced and that many of these introduce premature terminate codon (PTC), namely PTC^+ mRNA. We propose here that the degradation of TE-containing PTC^+ mRNA occurs as a quality surveillance pathway, before of the translation of potentially negative and truncated proteins. By such process the eukaryote genomes can identify and degrade aberrant mRNAs and assure the fidelity of gene transcription. Therefore, our results show that many of the chimerical mRNA isoforms are degraded by NMD rather than translated to make protein. Most significantly, this study provides the first evidence of TE-derived CDSs are subject to alternative splicing and that TE-harboring spliced isoforms are subject to NMD, thus functioning as major players in the post-transcriptional regulation in eukaryotes.

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FINNANCIAL SUPPORT

This study was supported by grants provided by the Brazilian agencies FAPESP and CNPq.

Effect of Different Levels of Fertilization on Bean Biochemical Composition in *Coffea arabica* cv. Rubi

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SUMMARY

The edaphoclimatic conditions may affect coffee quality (*Coffea arabica* L.). In the case of coffee, adequate fertilization results in increased production and may also affect the final biochemical composition and also cup quality. The sweetness is one of the desirable flavour in coffee gourmets, and the presence of certain compounds in green coffee can serve as standards for quality evaluation. The goal of this study was to evaluate the effects of different levels of NPK fertilization on the biochemical composition of coffee beans. The levels of certain biochemical compounds (caffeine, lipid, sucrose, trigonelline and chlorogenic acids) in coffee beans produced under different levels of fertilization, during three consecutive harvest periods (2007 up to 2009), were evaluated by spectroscopy near infrared (NIRS).

INTRODUCTION

Several environmental factors such as the edaphoclimatic conditions may affect coffee quality (Silva et al., 2005; Vaast et al., 2006). Adequate fertilization during coffee cultivation results in increased production and may also affect the final biochemical composition and also cup quality. The goal of this study was to evaluate the effects of different levels of NPK fertilization on the biochemical composition of coffee beans. In order to initiate such studies, levels of some biochemical compounds of coffee fruits (caffeine, total lipids, sucrose and chlorogenic acids) were evaluated by the near-infra red spectroscopy (NIRS) in beans produced from adult plants of *Coffea arabica* cv. Rubi, cultivated with different levels of NPK fertilization under field conditions. The coffee beans were obtained from an experimental trial set up on a factorial design (NPK x 5 levels x 3 repetitions) located at a Brazilian Cerrado area. In this region, the climate is characterized by a rainy season (from October till April) during summer and a long dry season (from May till September) during winter. In such conditions, coffee cultivation is only possible under irrigation to avoid deleterious effect of water stress on coffee plants. The NIRS analyses were performed using beans produced during three consecutive harvest periods (2007 up to 2009).

MATERIALS AND METHODS

Plant material and field experiments

Field trials were conducted using the 6 years old plants of Rubi cultivar of *Coffea arabica* grown in field condition. at the experimental station of the Embrapa Cerrados center (Planaltina-DF, Brazil, 15°35'43"S - 47°43'52"O) in full-sun condition under five hydric regimes (RH) characterized as follows: RH0, without irrigation (only subjected to natural rainfall); RH1, always irrigated during the year; RH2, without irrigation during 30 days of the winter season; RH3, without irrigation during 60 days of the winter season and RH4, without irrigation during 90 days of the winter season. During the 3 years of this experiment, the date of the return of irrigation was fixed for the 4th of september for RH2 to 4, the flowering that occurred around 10 days after this date. Afterwards, from flowering up to the bean harvest, water soil moisture was controlled and regular irrigations are performed by a circular pivotal system to always maintain water level upper than 0,27cm^3 H_2O.cm^{-1} even during the rainy period. Different fertilization conditions of N, P and K were also tested as follows: N Treatment (N1:0, N2: 100, N3: 250, N4*: 500 and N5: 800 Kg N.ha^{-1}), P treatment (P1:0, P2: 50, P3: 100, P4*: 200 and P5: 400 Kg P_2O_5.ha^{-1}) and K treatment (K1:0, K2: 100, K3: 250, K4*: 500 and K5: 800 Kg de K_2O.ha^{-1}). For each condition, 3 biological repetitions were used leading to 225 samples studied (3 NPK treatments x 5 doses NPK x 3 biological repetitions x 5 RH conditions). (*): fixed values of fertilizer used in combination of tests. A scheme of the field trial is presented on Figure 1.

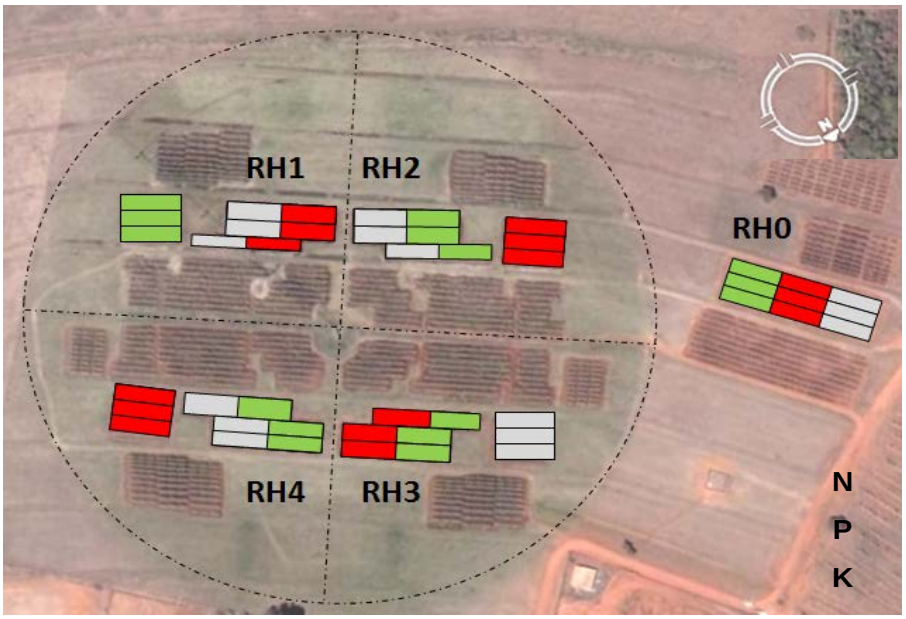

Figure 1. Map of field trials Embrapa Cerrados center (Planaltina-DF, Brazil). Blocks of *C. arabica* cv. Rubi are indicated as well as RH (irrigated: RH1 to RH4 and non-irrigated: RH0). Blocks of plant with variated doses of N, P and K fertilizers are respectively in red, green and grey. Map obtained from Google Earth (15°35'43"S - 47°43'52"O).

Fruit sampling and processing

Under the present conditions, and depending of year rainfalls, water stress accelerated fruit ripening by approximately one month, with a full ripening of fruits estimated at 210 DAF and

240-260 days after flowering (DAF) for RH0 and RH1 to 4 conditions, respectively. Fruits were harvested at maturity that corresponded to cherry fruits with red pericarp turning to purple and containing hard white endosperm (over-ripe cherries with a dried pericarp turning brown to black, were not considered). Fruits were sun-dried for 15-20 days until beans reached 10-12% humidity levels and then processed mechanically to remove dried pericarp and endocarp (parchment).

Chemical analysis

Chemical analyses were performed on green coffee beans by near infrared spectrometry (NIRS) by reflectance (Decazy et al., 2003) of green coffee (50 g) using a NIR spectrometer system (NIRS model 6500, FOSS, Port Matilda, Pa.). Sucrose, total lipids, caffeine and chlorogenic acids (CGA) contents were measured using dry beans (30-50 g) that had been equilibrated (for 6 days at 60% humidity and 28 °C) prior to being frozen in liquid nitrogen and ground (<0.5 mm). A NIR spectrum was acquired in reflectance (R) mode in the 1,104- to 2,456-nm range (Downey and Boussion, 1996) and compared with previously established calibration curves (Downey et al., 1994) Data were treated by Winisi 1.5 (NIRS2 4.0) software (Intrasoft Int., Port Matilda, PA).

Statistical analysis

Within each RH condition, three randomized blocks of five plots were set up for each fertilizer N, P, and K, and each fertilizer was applied at five levels in the three blocks with a constant level of the two other fertilizers. Three distinct experiments were therefore considered and analysed separately. Data of each year were analysed separately because of very different climatic conditions between the three years. Data were analysed using a mixed model analysis of variance. Factors RH and fertilizer were considered fixed, and factor block within RH was considered random. For each fertilizer, the observed value Y_{ijk} corresponding to RH i, bloc j within RH i, and fertilizer level k, was modelled as follows:

$$Y_{ijk} = m + a_i + B_{ij} + c_k + (ac)_{ik} + E_{ijk}$$

where m (overall mean), a_i (fixed effect of RH i), B_{ij} (random effect of bloc j within RH i), c_k (fixed effect of fertilizer k), $(ac)_{ik}$ (interaction RH x fertilizer) and E_{ijk} (random residual error).
All the computations were performed using the statistical software package *R* (Pinheiro et al., 2007), version 2.6.0. The analyses of variance were performed using the R package Nlme (R Development Core Team, 2007).

RESULTS AND DISCUSSION

The results presented on figure 2 were only focused on the effects of the NPK fertilization, excluding interactions with RH conditions (see also Vinecky et al., in the same issue).

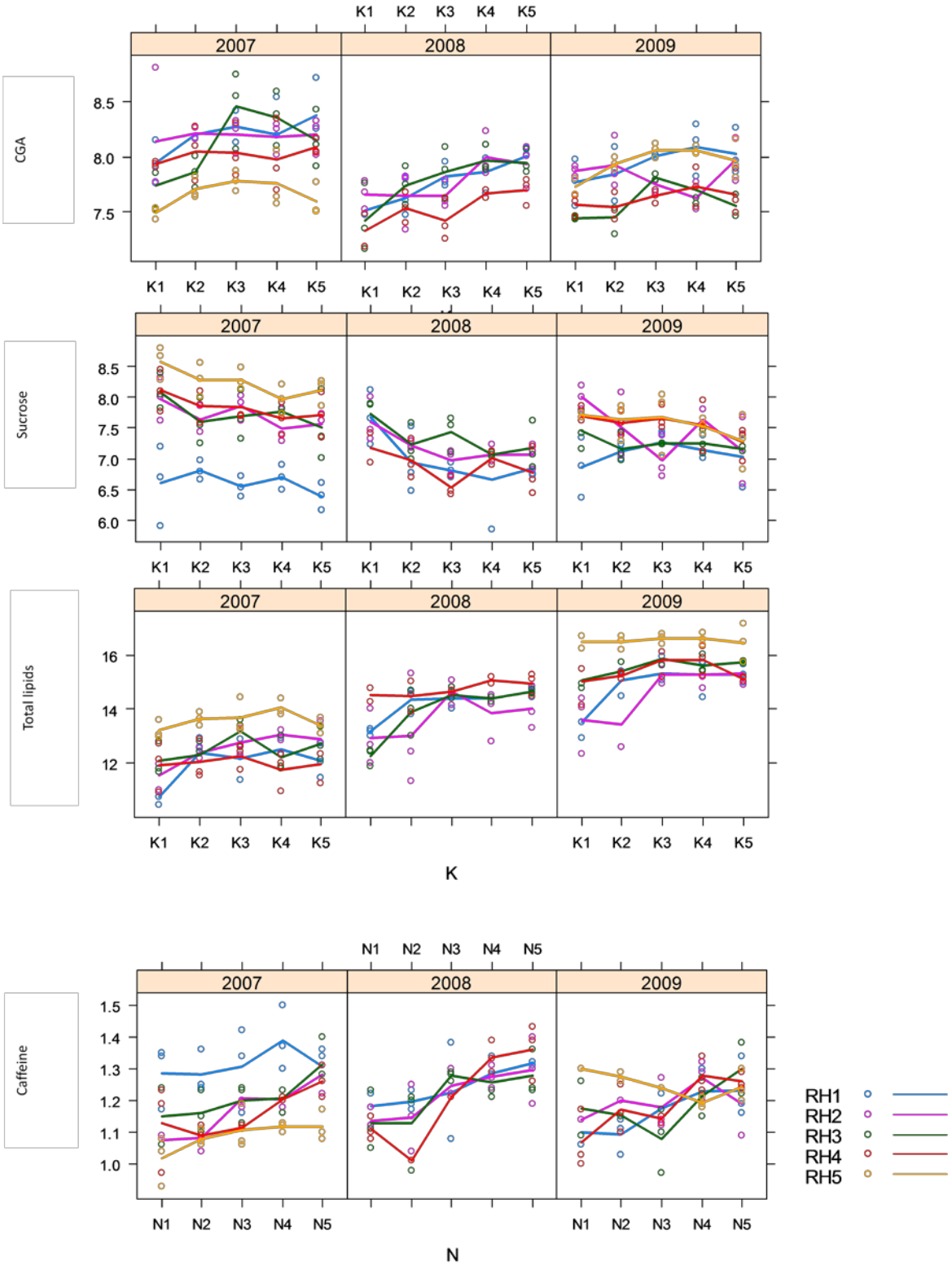

Figure 2: Evolution of biochemical contents of caffeine, total lipids, chlorogenic acids (CGA) and sucrose expressed in % of dry matter of mature beans with with different levels of N and K and the year of harvest. Due to a severe drought period during in the winter 2007, RH0 fruits were not produced in 2008.

The main conclusions of this study are:

- contents of caffeine, chlorogenic acids [CGA] and sucrose were relatively similar during the 3 years of analysis,
- contents of total lipids were subjected to major fluctuations, ranging for 12-14% in 2007 to 15-16% in 2009,
- There is a significant effect of K on total lipids, chlorogenic acids [CGA] and sucrose with increasing contents of lipids and CGA with increasing levels of K . In the case of sucrose we observed decreasing levels.
- There is a significant effect of N on caffeine content with increasing contents of these biochemical compound with increasing levels of N.

ACKNOWLEDGMENTS

This work was supported by FINEP (Qualicafé), CNPq, CBP&D/Café, Cirad and FAPEMIG.

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Heterogeneous Characteristics during the Development of *Coffea arabica* Somatic Embryos

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SUMMARY

Somatic embryogenesis technique makes it possible to produce clonal coffee planlets in a homogenous mass production. In vitro culture of *Coffea arabica* L. in liquid medium consisted of somatic embryos of different sizes, colors, and developmental stages. Thirty miligram of embryogenic callus were cultured on a liquid medium during six weeks culture. The aims of the study were to observed their morphological variations with respect to embryo's size, color, and developmental stage over one passage of embryo maturation phase. Embryo maturation medium was a modified-MS medium with half-strength of macronutrients containing 1mg/L Kinetin and 0.1 mg/L NAA. At the end of culture passage, fresh weight of embryo increased by 3740 miligram or increased 124 fold compared early culture. The average of embryo length was 6.97 mm. At the initial of cultured, 100 % of the embryos were yellowish. At the end of the cultured the composition of yellowish embryos were 6.25 %, whitish embryo were 28.6%, greenish embryo were 21.1%, reddish embryo were 10.83%, creamy embryo were 33.22%. At the initial culture, 100% of the embryos were at the globular. At the end culture, there are 3.18% globular, 0.66% at heart, 45.80% at torpedo, 16.16% cotyledon and 7.36% germination stage. Beside that, there were embriogenic callus which is consisted of 26.57% friable callus. This study indicates that was possible to generated coffee trees commercially with normal performance from embryogenic suspensions, although there were morphological variations during the development of *Coffea arabica* L. somatic embryos.

INTRODUCTION

Arabica coffee (*Coffea arabica L.*) plantation has important economical meaning as export comodity. Coffee plantation have brighter future because we can predicted that world requirement of coffee always increase. Propagation using cutting, oculation and top grafting have many limitation in amount of materials. Tissue culture methode expect to overcome those problems, so that can produce amount of clonal seedling in relatively short time. Most of applied methode to regenerate planlet from tissue culture is by somatic embryogenesis (Williams and Maheswaran, 1986). Somatic embryogenesis get many attention because can produce unlimited propagule in relatively short time. In long term and short term storage, somatic embryo consider to be an ideal materials to store because can regenerate to somatic seedlings. Somatic embryogenesis is a process, where somatic cell (haploid or diploid) develope become new plant by specific embryo development stage without gamet fusion. The heterogeneous of somatic embryos of *Coffea arabica* was influenced by the concentration of auxin and cytokinin in the medium. Somatic embryogenesis culture is usually present in all stages of development (globuler, heart, torpedo and cotyledonary) at any one time because of repetitive (secondary) embryogenesis (Akula and Dodd, 1998; Akula et al., 2000).

In *C. sinensis*, Jha et al. (1992) identified three different types of somatic embryos: white 'seed-like' embryos, green 'cup-shape' embryos and globular embryos. However, the formation of these different types of embryos was not related to plant growth regulator in the media (Jha et al., 1992). The morphological variations of somatic embryos limited the scaling up of *in vitro* mass propagation (Tautorus and Dunstan, 1995) and the development of synthetic seed technology (Attree and Fowke, 1993; Onishi et al., 1994). This experiment was conducted to determine the extent of heterogeneous characteristics during coffee somatic embryo development on liquid medium with respected to developmental stage, embryo size and color. An understanding of this phenomenon may help in improving the cultural procedure and conditions of coffee somatic embryogenesis and in developing synthetic seed.

MATERIALS AND METHODS

Somatic embryos initiation of *Coffea arabica* clone BP 416 A were induced from flush leaves and used solid MS modification medium refered to Jos van Boxtel dan *Berthouly* (1996). Mostly callus embryogenic weigh about thirty milligram in total were culture on 50 ml of maturation medium in a 250 ml erlenmeyer. The maturation medium was a Murasige and Skoog (MS) medium (Murashige T and Skoog F, 1962) with a half macro and micro salt, 20g/L sucrose, 1mg/L Kinetin, 0,1mg/L NAA. Culture medium were adjusted to pH 5,5 and autoclaved at 121 °C and 1.0 kg/cm^2 for 30 min. Culture were placed in shaker with 100 rpm light condition. An observation was used to determine somatic embryo development during 6 weeks of culture by harvesting embryo in 30 erlenmeyer selected randomly every week. Growth and development were measured by embryo fresh weight and number. The length of each embryo was measured using a digital caliper and its color and developmental stage were determined. Embryo heterogenity was determined by embryo size and coefficient of variance (CV). In addition, morphological variations of somatic embryo were also represented by different embryo colors and developmental stages at a specified time of culture.

RESULTS AND DISCUSSION

The fresh weight of *Coffea arabica* somatic embryos increased slowly during the first 6 weeks in culture and then sharply thereafter (Figure 1). The fresh weight of embryos was almost 120.6 times of the initial weight by the end of experiment (6 weeks). Until the end of the culture, the number of embryo somatic increased around 18 times started earlier at the week (Figure 1). Addition of somatic embryos number shows that there were formation new somatic embryo (secondary embryo) and mature embryo from embriogenic callus. This addition did not affect the weight significantly because of their very small size. The average size of *Coffea arabica* somatic embryos at the initial culture was 0.792 mm (Figure 2). The embryo size had increased slowly up to sixth week (9.031 mm). The incerased, due to there were formation secondary embryogenesis from primary embryo. Although, the total fresh weight of somatic embryos at sixth week was almost 120.6 times of the initial weight (Figure 1), the average size of somatic embryos over one culture passage did not change significantly (Figure 2). All stages of developing embryos with different size were present at any one time over one passage of coffee somatic embryo culture (Figure 3).

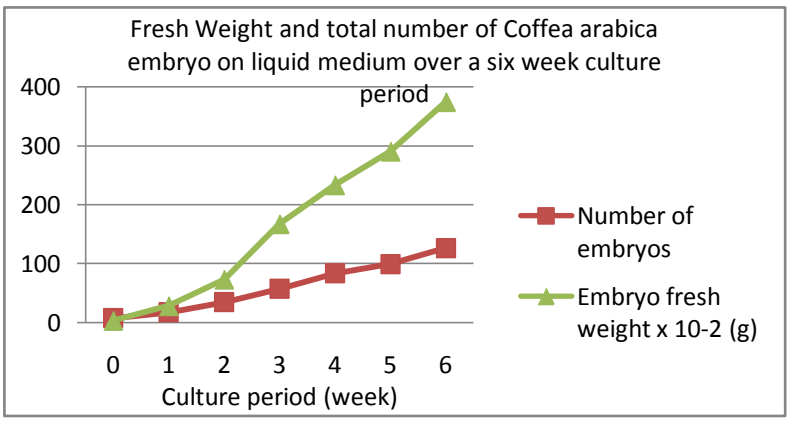


Figure 1. Fresh weight and total number of *Coffea arabica* embryos on liquid medium over a six week culture period.

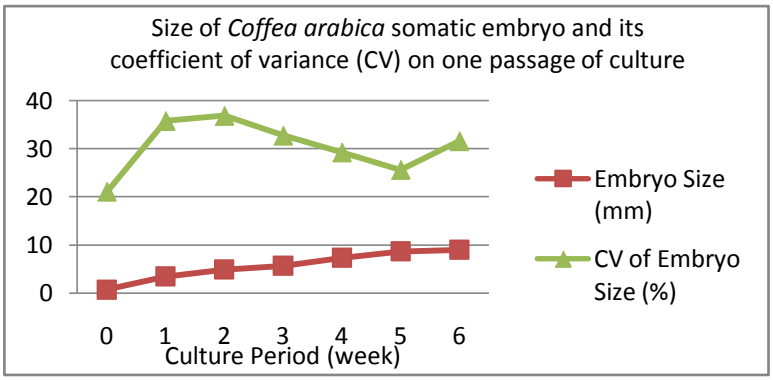
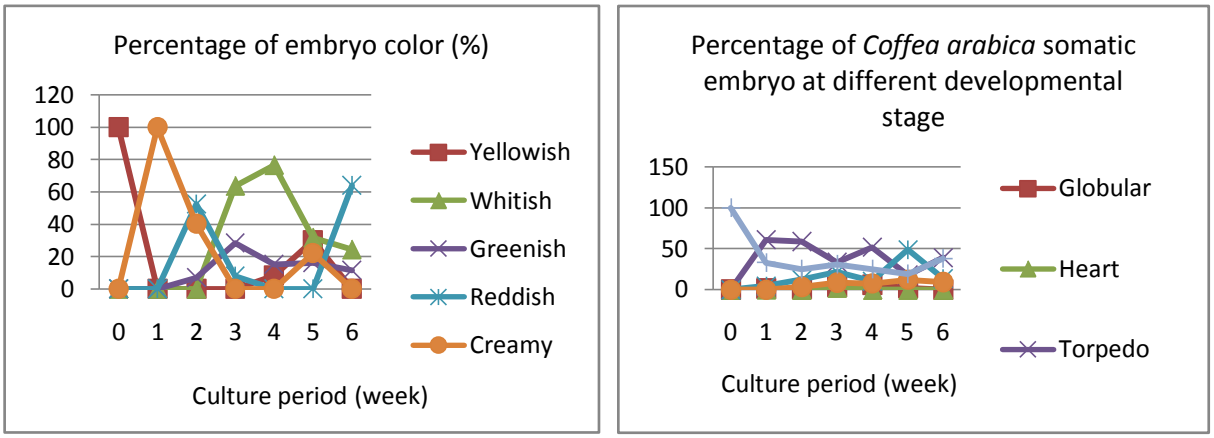


Figure 2. Size of *Coffea arabica* somatic embryo and its coefficient of variance (CV) on one passage of culture.

Figure 3. Somatic embryo culture of *Coffea arabica* on liquid medium where different sizes, colors and developmental stages of embryos were found at the same time during the culture.

A group of coffee somatic embryos on a liquid medium were consisted of different colors. At the initial culture, there were 100% yellowish embryos. The percentage of the yellowish embryos decreased gradually up to third week, while the whitish, greenish, reddish and creamy increased. By the end of the experiment, there were not yellowish and creamy embryos. Most of the embryos were whitish, greenish and reddish (Figure 4a). There were 60.86% torpedo and 33.03% were at embryogenic callus at the initial culture. During the second and third weeks, some of the embryogenic callus had grown the globular, heart, torpedo, cotyledon and germination. This can be observed by the decrease and increase in

each embryo development stages. Embryogenic callus still produce on the last weeks because of the multiplication rate was high (Figure 4b).



(a) (b)

Figure 4. a) Color changes of *Coffea arabica* somatic composition over one passage of cultur; b) Percentage of *Coffea arabica* somatic embryo at different developmental stages over one passage of culture.

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In Vitro Inoculation of Arabica Coffee Derived from Somatic Embryogenesis with *Beauveria bassiana*

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SUMMARY

Entomopathogenic fungi, *Beauveria bassiana* have been widely studied, and methods for their production and application in coffee agroecosystem have been developed. Various fungal entomopathogens have already been reported as endophytes and the various methods used to inoculate coffee plants with *B. bassiana* were partially effective. Objective of this research is to investaigate the possibility of in vitro inoculation of *B. bassiana* in coffee plantlet derived from somatic embryogenesis. The experiment was arranged by Randomized Complete Design with 10 replication. Concentration treatment of *B. bassiana* spores were 0,2; 0,4; 0,6;0, 8; 1; 2, and 3 g/L. Modification of ½ MS medium used as Culture Medium. Inoculation of *B. bassiana* suspension by injection culture medium using Syringe Filter 0,2µm, 25 mm diameter in the laminar air flow. Embryo was planted in the bottle, 5 plantlets each bottle. The result showed that colony of *B. bassiana* as an endophyte was recovered from roots, stem and leaves of Arabica coffee plantlets in 2, 3 and 4 months post-inoculation of *B. bassiana.* Inoculation of *B. bassiana* up to 1 g spore /l concentration gave no negative effect on coffee plantlet growth. Spore concentration of 2 and 3 g/l inoculated in culture medium, influenced on number of leaves and height of coffee plantlets.

BACKGROUND

Entomopathogenic fungi have been widely studied and their production methods and application in coffee agroecosystem have been developed. In Pest management of coffee berry borer (CBB) *Hypothenemus hampei* Ferr. it was known that entomopathogen fungi, *Beauveria bassiana* is an effective biological agent for controlling CBB. In addition,it is important to develop cost-effective ways to spray it. One particularly interesting concept involving *B. bassiana* involves its possible use as a fungal endophyte in biological control programs. Various fungal entomopathogens have already been reported as endophytes (Vega et al., 2008b *cit.* Vega et al., 2009) and the various methods used to inoculate coffee plants with *B. bassiana* were partially effective (Posada et al., 2007). Objective of this research is to investigate the possibility using of *B. bassiana* as an endophyte through in vitro inoculation on coffee plantlets derived from somatic embryogenesis

MATERIAL AND METHODS

The experiments were arranged in Randomized Complete Design with 10 replications. Each replication consists of 5 explants. Concentration treatments of *B. bassiana* spores are without *B. bassiana* (Control), 0.2, 0.4, 0.6, 0.8, 1, 2 g dry spores/l. Modification of ½ MS medium was use as culture medium. Inoculation of *B. bassiana* suspension by injection culture medium using Syringe Filter 0,2µm, 25 mm diameter was conducted in the laminar air flow. Embryos of Arabica coffee BP 416 clone were planted in the bottle, 5 plantlets in each bottle.

Observation of *B. bassiana* colony recovered as an endophyte were conducting in 2, 3 and 4 month post-inoculation by plated the root, stem, and leaf tissues on potato dextro agar (PDA) culture medium. Plantlet growth parameter were observed on number and length of roots, number of leaves and plantlets height.

RESULT AND DISCUSSION

The recovery of *B. bassiana* from coffee tissues indicates that this entomopathogen fungi can become established as an endophyte in coffee plantlets through in vitro inoculation. Based on recovered of colony *B. bassiana* from root, stem and leaf of Arabica coffee plantlets on 2, 3 and 4 month post-inoculation, it was known that entomopathogen *B. bassiana* can move throughout internal plant tissues. Observation on percentage of recovery *B. bassiana* colony two month after inoculation, it was known that highest presence of *B. bassiana* as an endophyte from root was found in concentration *B. bassiana* of 0.8 g spores/l, but three and four months post- inoculation of *B. bassiana*, the highest presence was obtained on high concentration treatments of 2 and 3 g spores/l. Previous research conducted by Posada and Vega (2006) showed that *B. bassiana* became established as an endophyte in coffee seedling grown in vitro and inoculated with *B. bassiana* suspensions in the redicle.

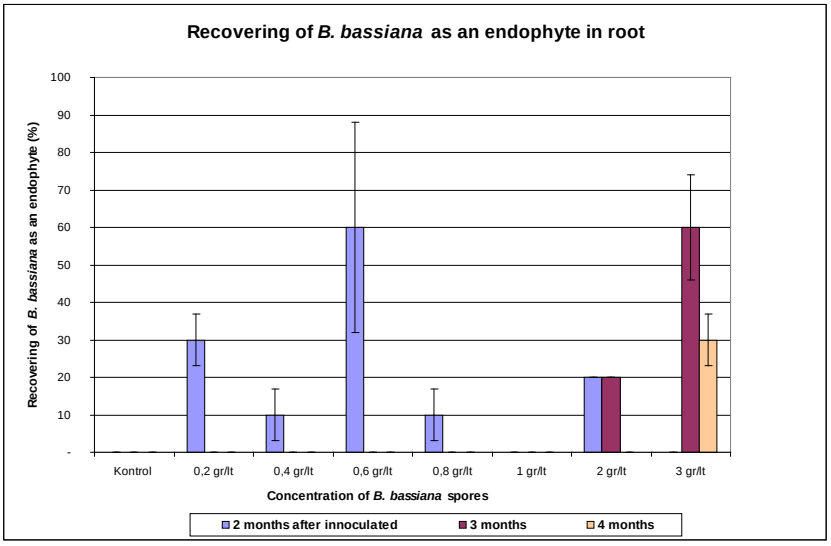


Figure 1. Percentage of *B. bassiana* recovered as an endophyte from root tissues.

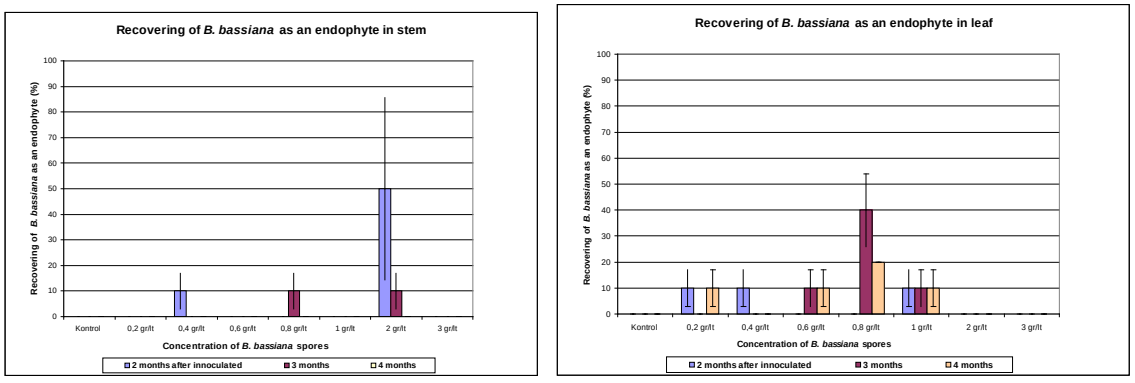


Figure 2. Percentage of *B. bassiana* recovered as an endophyte from stem and leaves of coffee plantlets.

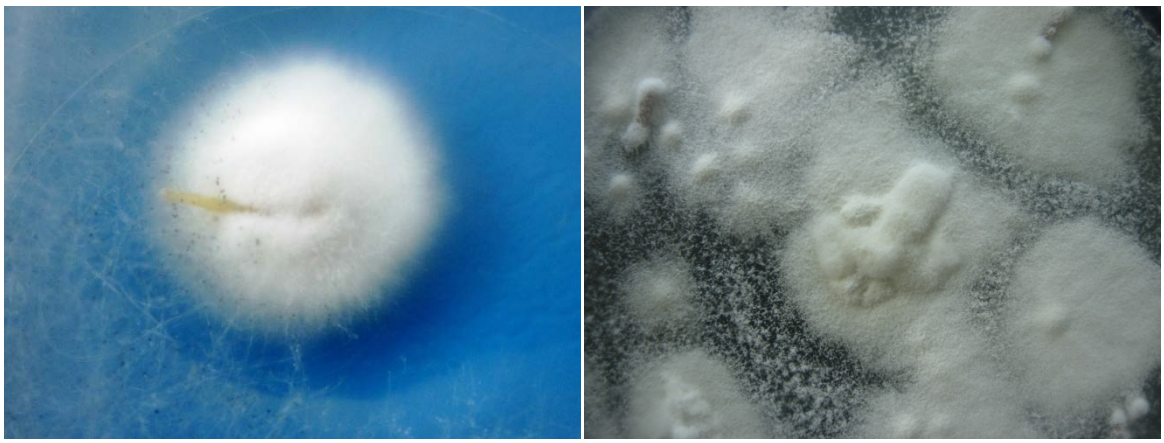
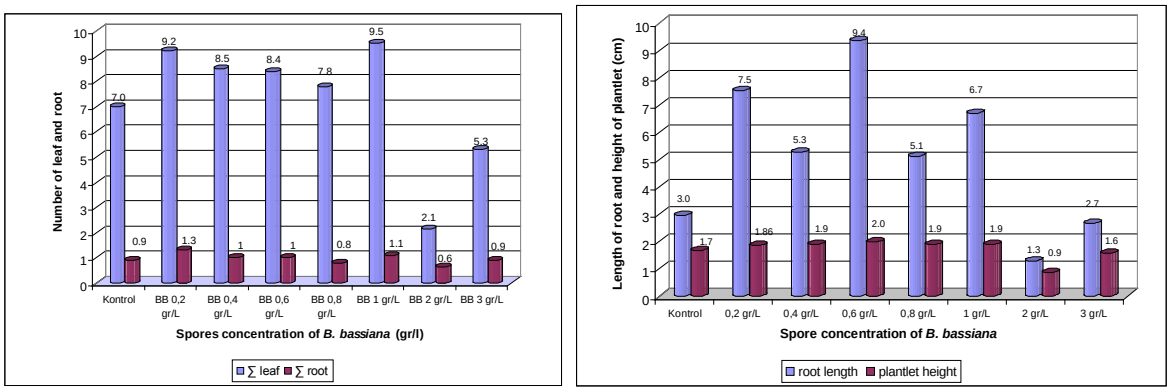


Figure 3. Colony of *B. bassiana* recovered as an endophyte from root and stem tissues of coffee plantlets.

Observation on plantlet growth, it was known that inoculation of *B. bassiana* up to 1 g/l spore concentration were not negative effect on coffee plantlet growth, but concentration of 2 and 3 g spore/l were influence on the number of leaves and height of coffee plantlets. Number of leaves on treatment of 2 and 3 g spore/l were 2.1 and 5.3 respectively lower than control which reached 7 leaves. Height of coffee plantlets on treatment of 2 and 3 g spore/l were also lower compared to control, and there were significantly different with the treatment of 0.2-1 g spore/l which heigth of plantlet reached 5.1-9.4 cm.



a. b.

Figure 4. (a).Number of root and leaf of coffee plantlet six month post-inoculation of *B. bassiana* and (b). Length of root and height of coffee plantlet six months post-inoculation of *B. bassiana.*

CONCLUSION

- Colony of *B. bassiana* as an endophyte was recovered from roots, stems and leaves of Arabica coffee plantlet 2, 3 and 4 month post-inoculation of entomopatogen fungi *B. bassiana* .
- Inoculation of *B. bassiana* suspension up to 1 g/l spore concentration gave no negative effect on coffee plantlets growth
- Spore concentration of 2 and 3 g/l inoculated in culture medium, were influence on number of leaves and height of coffee plantlets.

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Predicting the Performance of Introduced *Coffea canephora* Germplasm under Recurrent Selection

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SUMMARY

Benefits derived from recurrent selection of allogamous and heterogeneous species justify the adoption of population breeding for *Coffea canephora* improvement in Ghana. Germplasm introduced from Côte d'Ivoire in 1977, comprising seed-lots of five half-sib families were evaluated together with two locally selected families in a completely randomised design for 10 vegetative and six bean- and berry-quality traits and yield. Genetic variation and heritabilities were estimated from variance components. Estimation of selection differential was based on 21% selection intensity with an index involving bean yield and weight, which are of major importance in *Coffea canephora*. Selection response was predicted for bean yield and weight, and the effect of selection for these traits on other important traits in the population assessed. The families were also assessed for similarity based on 21 agro-morphological traits including yield and leaf characters. The families varied significantly ($0.05 > P < 0.001$) for all traits except dry-/wet berry ratio, span and length of primary branches. Cluster analysis grouped the families into six distinct groups, further indicating high variation among the genotypes. The means of the selected individuals and the population varied significantly for bean yield ($P < 0.001$), bean weight ($P< 0.01$), pea-berries and number of primary branches ($P< 0.05$). Heritability estimates were high (35-100%) for bean yield and bean and berry quality traits but low to moderate (5-51 %) for the vegetative traits. Predicted selection responses as percentage of the population mean were positive for 11 of the 17 traits and high for bean yield (117%), % pea-berry (6.8%), bean weight (5.9%) and number of primary branches (2.1%). Negative selection response was predicted desirably for four traits and no response for two. Two cycles of selection were predicted to change the mean of the population for yield from 0.58 to 1.93 kg/tree and bean weight from 13.36 to 14.94 g/100 beans.

INTRODUCTION

In Ghana low yields of local *Coffea canephora* genotypes, ranging between 100 and 200 kg/ha clean coffee annually on smallholder farms, have been identified as the main disincentive to coffee cultivation, and hence the low production of the crop (Anon, 1996). Germplasm was, therefore, introduced from Côte d'Ivoire in 1977, as a basic material for breeding and improvement of the crop.

Coffea canephora is an allogamous and heterogeneous species. A long-term breeding strategy for the crop should aim at a continual increase in genetic gains of agronomic traits most of which are quantitative with low heritabilities. Recurrent selection for population improvement is an effective method for improving such cross-pollinated species (Acquaah, 2008). It involves the systematic testing and selection of desirable parents through truncation selection from a population and crossing them randomly to form a new population (Falconer and Mackay, 1996). Increase in the frequency of favourable alleles from each cycle results in the

accumulation of gains over time, leading to significant improvement, while maintaining genetic variation of the population. Predicted gains from recurrent selection in a population may be determined by using estimates of means, variance components, and heritabilities (Falconer and Mackay, 1996). Using these population parameters, predictions of genetic gains under recurrent selection have been applied successfully to coffee (Leroy et al., 1994, 1997; Montagnon et al., 2003; Yapo et al., 2003; Mistro et al., 2004). It is therefore necessary to estimate genetic parameters for the main traits of agronomic importance to enable prediction of selection response under recurrent selection. The objective of this study was, therefore, to estimate genetic parameters and determine whether progress could be made under recurrent selection for yield, green bean quality and other traits of agronomic importance in introduced Robusta coffee populations.

MATERIALS AND METHOD

Plant material and Experimental design

The materials used for the study comprise seven half-sib families – five seed-lots (A, B, C, D, E) introduced from Côte d'Ivoire and two (F and G) collected locally. The families were evaluated in a completely randomised design, with individual plant randomisation of 18 to 49 plants per family, in a compact plot of 0.25 ha at a spacing of 3m x 3m. Planting was done in 1978. The plants were raised on three stems.

Traits observed

Ten vegetative traits and six bean/berry quality traits were recorded during fourth cycle growth on 48-months old regenerated stems in 2006. Vegetative traits assessed included girth (taken at 10cm above the ground in cm), span (cm) taken as the width of the canopy measured at the widest portion of the tree canopy, height measured from the base to the apex of the plant, number of primary and secondary branches counted as an average per stem per plant. Length of primary branches (measured from the point of attachment to the main stem to the apex in cm), diameter of primary branches (10 cm from the main stem in mm) and number of nodes per primary branch were estimated as an average value of the six longest branches at the middle of the stem per plant. Inter-node length of primary branches (IL of prim.) was estimated as average length of primary branches divided by average number of nodes per primary branch, and inter-node length of main stem (IL of stem) estimated as height divided by number of primary branches per stem.

Yield was recorded over two production cycles, 1980/81-1984/85 and 1986/87-1991/92. Yield (average annual) was recorded as weight of fresh berries and converted to economic yield using a conversion factor of outturn of individual stands. Assessment of bean/berry quality traits was done using samples of 300 berries harvested from each tree, at the yellow-/red-ripe stage. Three 100 g samples were used. These were weighed, dried and reweighed and average weights used in estimating the ratio dry-: wet berries. For each sample, the berries were cracked individually in a mortar and the beans separated from the pericarp. Dry beans/-berry was recorded as the weight of the dry beans expressed as a percentage of the dry berries. Empty locules and pea-berries were recorded and expressed as percentage of total berries in sample. 100-bean weight was recorded as the weight of 100 dry beans counted for each sample. Outturn was estimated as an average of weight of dry beans divided by weight of wet berries.

Data for cluster analysis was based on the 17 vegetative and berry/bean quality traits observed and yield, in addition to leaf length, breath, length/breath ratio, and leaf petiole length, all estimated as average for fifteen leaves sampled from the middle of the sixth to eighth primary branches counted from the apex of the plant.

Statistical analyses

Analysis of variance (ANOVA, General Linear Model) was performed using the MINITAB release 12 statistical software (MINITAB, 1997). Estimates of the observational components of variance between the families (σ^2_b) and within families (σ^2_w) were made and used in estimating the causal components of variance as: Variance additive (V_A) = σ^2_b x 4; Variance Phenotypic (V_P) = $\sigma^2_b + \sigma^2_w$. Heritabilities were calculated using estimates of variance components as V_A/V_P. Twenty-one percent of the population was selected through truncation selection by an index (Amaravenmathy and Srinivasan, 2004) involving yield and bean weight. Selection differential (S) was estimated as the difference between the mean value of the population before selection and the mean of the selected individuals for all the traits. T-test testing the difference was by standard procedure (Steel et al., 1997). Genetic gains from selection (GA) was estimated as a product of the heritability (h^2) and the selection differential and expressed as a percentage of the mean as: (GA/X) x 100, where X = half-sib mean for a given trait. Genotypic (GCV) and Phenotypic (PCV) coefficient of variation were estimated as: GCV = 100 x ($\sqrt{V_A}$/ X); PCV = 100 x ($\sqrt{V_P}$/ X), where V_A = Additive genetic variance and V_P = Total phenotypic variance. Clustering of the families was done using mean values of the traits after standardisation with the complete linkage method measured in Euclidean distance, using MINITAB release 12 statistical software.

RESULTS AND DISCUSSION

Variance components

Analyses of variation among the seven half-sib families for the traits are presented in Tables 1 and 2.

The six bean/berry quality traits studied and yield all showed highly significant ($P < 0.001$) variation except dry-/wet berries. Phenotypic (PCV) and genotypic (GCV) variation coefficients were highest (>15%) for yield, empty locules, 100-bean weight and pea berries. For the vegetative characters, significant variation ($P < 0.05$) was observed among the families for eight of the 10 traits studied. PCV and GCV were highest for number of primary and secondary branches. The significant variation observed among the families show that between family-selection is possible for these traits. High PCV and GCV are indications that, for these traits, there is a lot of variation at both the phenotypic and genotypic levels available for selection and improvement of these traits.

Table 1. Variation for bean/berry characters and bean yield in seven half-sib families of Robusta coffee.

Family	Pea-berry (%)	Empty locules (%)	100-bean wt.(g)	Dry bean/-berry (%)	Outturn (%)	Dry-/wet berry (%)	Yield (wet berries kg/tree/yr.)	Yield (clean coffee (kg/tree/yr.)
A	35.2	3.4	13.0	54.4	22.5	41.4	2.896	0.662
B	38.7	3.5	12.3	54.1	22.1	40.8	3.715	0.828
C	35.5	2.3	15.4	57.5	23.1	40.2	2.392	0.561
D	33.4	2.1	13.7	53.6	21.5	39.6	2.934	0.629
E	35.4	2.1	13.4	56.9	23.0	40.7	4.010	0.912
F	21.4	3.0	13.0	59.0	24.6	40.8	0.893	0.222
G	22.2	3.6	12.4	54.8	23.1	40.9	1.064	0.247
Mean	32.4	2.9	13.4	55.8	22.9	40.7	2.630	0.580
P-value	***	***	***	***	***	NS	***	***
PCV (%)	52.1	64.8	16.8	9.1	10.6	6.4	80.44	83.06
GCV (%)	35.9	38.6	15.3	6.3	6.8	1.0	86.30	85.78

*** *Significant at 0.1%; NS = Not Significant*

Table 2. Variation for vegetative characters in seven half-sib families of Robusta coffee.

Family	Girth (cm)	No. Prim branches/ stem	No.sec. branches/ stem	Height (cm)	Span (cm)	Nodes /prim branch	Dia. Prim. (mm)	Lgth prim (cm)	IL on prim (cm)	IL on stem (cm)
A	5.8	88.2	5.0	303.3	169.8	17.6	5.9	95.4	5.5	3.63
B	5.3	86.3	6.9	286.1	168.8	18.8	5.6	92.9	5.1	3.57
C	5.7	80.0	3.3	288.1	172.6	17.8	6.0	94.5	5.4	3.99
D	5.8	95.9	7.2	297.7	183.7	15.6	6.1	96.9	6.3	3.39
E	5.5	90.8	6.1	283.2	186.3	18.6	6.4	101.1	5.6	3.21
F	5.2	70.1	3.1	266.8	162.6	15.8	6.1	91.5	5.9	4.01
G	5.1	79.6	6.2	269.9	180.1	18.6	6.4	98.3	5.4	3.57
Mean	5.5	84.2	5.3	285.5	174.3	17.8	6.0	95.8	5.5	3.63
P-value	**	*	*	***	NS	*	**	NS	***	*
PCV (%)	15.6	31.9	98.9	12.6	18.2	22.2	14.5	15.8	15.5	29.6
GCV (%)	8.6	13.7	22.4	7.9	6.9	10.2	7.4	3.5	11.0	11.8

****Significant at 0.1%; **Significant at 1%; *Significant at 5%; NS = Not Significant*

Cluster analysis

Cluster analysis grouped the families into six groups based on similarities (Figure 1). The first comprises two families and the rest singletons. The first made up of families A and B is characterised by fairly high yield, tall plants, high number of pea-berries and %dry-/wet berries, high proportion of empty locules, narrow leaves, short inter-node length and small diameter of primary branches. The C-family is characterised by large beans and long inter-nodes on the main stem. Plants in the D-family have high values for height, span, number of primary branches, girth, and inter-node length of primary branches. They also have the lowest values for nodes on primary branches, %dry bean/-berry, outturn, empty locules and %dry-/wet berry. The highest yield was recorded by the E-family. The E-family is also characterised by highest values for span, diameter and length of primary branches, shortest inter-node length on the main stem, and few empty locules. The F and G families have lower values for most traits including yield. However, the F-family recorded very high values for outturn, dry bean/berry, inter-node length on main stem, and leaf width and petiole length, whilst the highest values for empty locules and leaf petiole length were recorded by the G-family. The results of the cluster analysis further emphasise the presence of considerable variation among the families.

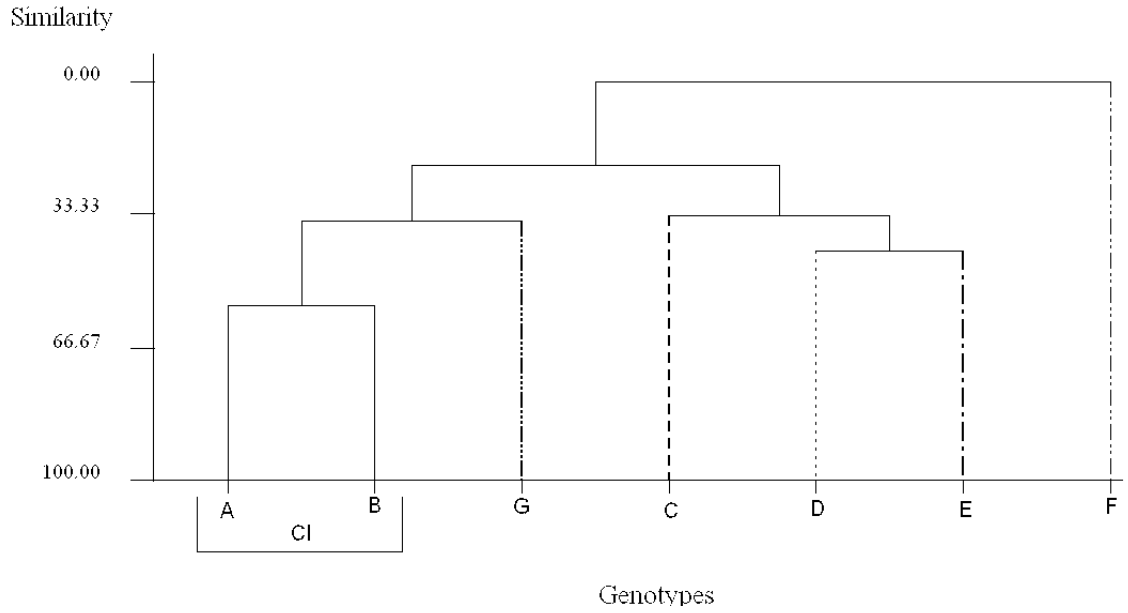

Figure 1. Dendrogram of cluster analysis of seven half-sib families of Robusta coffee classified according to 22 agro-morphological traits.

Heritability, Selection differential and Genetic advance

Heritability, selection differential and genetic advance for the traits are presented in Table 3. Heritability estimates were high (35-100%) for yield and the berry/bean quality traits, except for dry/wet berry which had very low heritability. Low to moderately high heritability estimates (4.8-50.7%) were recorded for the vegetative traits. The model used for the analysis of variance assumed the families were half-sib from a random mating population. Any planting arrangement among the parental population which encouraged assortative- or sib-mating, could have resulted in the unusually high heritability estimated for yield in this study. The compact nature of the trial plot could also have resulted in low environmental variance, hence increasing heritability estimates.

Table 3. Heritability (h^2), selection differential (S), and Genetic advance (GA) of vegetative and bean/berry quality traits, and yield of seven half-sib families of Robusta coffee

Trait	Mean of population	h^2	Mean of selected (21%)	t-population vs. Selected	S	GA	GA/m x 100
Pea-berry (%)	32.42	0.474	37.06	*	4.64	2.20	6.78
Empty locules (%)	2.87	0.353	2.68	NS	-0.19	-0.07	2.34
100-bean weight(g)	13.36	0.833	14.31	**	0.95	0.79	5.9
Dry bean/-berry (%)	55.84	0.471	57.19	NS	1.35	0.64	1.14
Outturn (%)	22.90	0.444	23.40	NS	0.504	0.22	1.0
Dry-/wet berry (%)	40.71	0.026	40.90	NS	0.19	0.005	0.01
Yield, clean coffee (kg/tree/yr.)	0.58	1.000	1.257	***	0.6	0.677	117
Girth(cm)	5.49	0.303	5.58	NS	0.09	0.03	0.05
No.prim branches	84.15	0.185	93.74	*	9.59	1.77	2.11
No.Sec. branches	5.29	0.224	5.29	NS	-0.02	0.00	0.00
Height(cm)	288.5	0.390	290.40	NS	4.90	1.91	0.67
Span (cm)	174.3	0.142	180.80	NS	6.50	0.92	0.53
Nodes/prim. branch	17.79	0.212	18.14	NS	0.35	0.074	0.42
Diameter of Prim.branch (mm)	6.00	0.260	6.00	NS	0.00	0.00	0.00
Length of prim. branch (cm)	95.81	0.048	95.49	NS	-0.32	-0.02	-0.02
Inter-node length on prim.(cm)	5.52	0.507	5.43	NS	-0.09	-0.05	-0.83
Inter-node length on stem (cm)	3.63	0.159	3.37	NS	-0.26	-0.04	-0.01

****Significant at 0.1%; **Significant at 1%; *Significant at 5%; NS = Not Significant*

Significant variation was observed among the mean of the population before selection and the mean of the selected individuals for yield ($t<0.001$), 100-bean weight ($t<0.01$), pea berries and number of primary branches ($t<0.05$). These observations tend to suggest that, plants with high yield and bean weight also have relatively higher number of primary branches than the rest. This is expected since many studies showed positive genetic associations between coffee bean yield and number of primary branches (Walyaro and Van der Vossen, 1979; Cilas et al., 1998; Anim-Kwapong and Adomako, 2010).

Expected genetic gain from selection was positive for 11 of the 17 traits and high for the bean/berry quality traits, but low to moderate for the vegetative traits. Expressed as a percentage of the mean, the highest positive genetic gains were predicted for bean yield

(117%), pea berries (6.8%), 100-bean weight (5.9%) and number of primary branches (2.1%). High negative value was predicted for empty locules (-2.3).

The proportion of selected individuals from the different family groups, together with their yields and bean weights are presented in Table 4. The highest number of selected individuals were from the E-family, followed by the B, A, and C families. These families, therefore, show combined superiority for yield and bean weight.

Table 4. Mean annual yield and bean weight of selected individuals from seven half-sib families of Robusta coffee.

Family	No. Planted	No. Surviving	No. Selected	% of selected individuals	Yield (clean coffee(kg/tree/yr.)	Bean weight
A	37	34	9	19.2	1.240	14.6
B	36	29	9	19.2	1.440	13.8
C	35	31	7	14.9	0.969	16.8
D	18	15	4	8.5	1.179	14.6
E	36	32	17	36.2	1.622	14.2
F	49	43	1	2.1	0.927	14.7
G	49	40	0	0.0	-	-

CONCLUSION

There is considerable genetic variation among the population. Heritability estimates were also high for most of the traits observed. The population can therefore be improved through selection. Two generations of recurrent selection is expected to shift the mean of the population for clean coffee yield from 0.58 to 1.93 kg/tree and bean weight from 13.36 to 14.94 g/100beans. Negative responses for length of primary branches, inter-node length of primary branches and of main stem, although low, are desirable for the development of plants with compact growth habit suitable for high density planting.

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Genotype X Environment Interaction on Yield of Selected *Coffea canephora* Clones in Ibadan, Oyo State, South Western Nigeria

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SUMMARY

Genotype x environment interaction for yield of Robusta Coffee (*Coffea canephora*) was studied on the selected clonal materials of Robusta coffee in the Coffee Seed Garden established within Cocoa Research Institute of Nigeria Headquarters, Ibadan, Nigeria. The clonal materials under evaluation were C111, C36 and T1049.The yields data were collected over three years (2006, 2007 and 2008). Climatic data recorded over the years varied. Combined analysis of variance showed that the effect of Genotype and Year are highly significant ($p<0.01$). The average mean yield for the berries was highest in the third year (2008); while the average mean yield was the highest in clone C111, however the interaction between the Genotype and year did not have much effect on the yield .The mean yield for the clonal genotypes varied over the years due to variation in climatic conditions during the period under consideration.

INTRODUCTION

Coffee is one of the most important commodities in the international agricultural trade, representing a significant source of income to several Latin American, African and Asian countries. Currently, *Coffea arabica* L. (Arabica coffee) accounts for about 65% of the coffee produced, and *C. canephora* Pierre (Robusta coffee) accounts for the rest. In Brazil, total coffee yield was about 48,500 million bags (60 kg/ bag) of green beans in 2002/2003 (which amounts to 41% of the world production) which was predicted to decrease to around 28,000 million bags in 2003/2004 largely due to unfavorable climatic conditions (Damatta, 2004). Not only in Brazil but also in several coffee growing countries unfavourable condition is considered to be the major environmental stress affecting coffee production. Gene expression is subject to modification by the environment; therefore, genotypic expression of the phenotype is environmentally dependent (Kang, 1998). The development of new cultivars involves breeding of cultivars with desired characteristics such as high economic yield, tolerance or resistance to biotic and abiotic stresses, traits that add value to the product, and the stability of these traits in target environments. Identification of yield-contributing traits, knowledge of GE interactions and yield stability are important for breeding new cultivars with improved adaptation to the environmental constraints prevailing in the target environments. In Nigeria, the climatic condition over the year varies from one year to other due to different factors thus militating against ideal coffee production environment. There is a need to evaluate the genetic stability of some of the available clonal materials. The objective of this study therefore is to determine the genetic stability of three clones of *C. canephora* in the field in Ibadan, Oyo State, Nigeria.

MATERIALS AND METHOD

Three selected clones of *C. canephora* were established, using rooted stem cuttings, in Coffee Seed Garden plot located at Zone 1 at Cocoa Research Institute of Nigeria headquarters in 2003. The clones were C36, C111 (Quillou) and T1049 (Java). Each of these clones was planted in rows using augmented design in 2 replicates. Berries were harvested between September - December in 2006, 2007 and 2008. The climatic condition for the three years were evaluated. Berries yields were evaluated clonally each year. Data were subjected to statistical analysis of ANOVA (Mackay, 2004).

RESULTS AND DISCUSSION

Average berry yield for C111, C36 and T1049 were 1.27-1.61, 1.05-148 and 1.08-1.56 t/ha respectively (Table 1). The Analysis of Variance (ANOVA) for the clones (Genotype) across the years is shown in Table 2. The effect of genotype on the yield is highly significant at ($p<0.01$) so also the effect of years on the yield. However the Genotype X Year which is not significant at ($p<0.01$) is as a result of genetic stability of the clonal materials. The results show that the interaction between the clonal materials is not significant on the yield. The evaluation of genotypic performance in a number of environments provides useful information to identify their adaptation and stability (Crossa, 1990). However the genotype and the year influence the yield of coffee berries independently.

Table 1. Mean yield of three clones of Robusta coffee over three years (2006-2008).

Clone	Berry yield t/ha					
	Replication 1			Replication 2		
	2006	2007	2008	2006	2007	2008
C111	1.27	1.46	1.48	1.35	1.61	1.56
C36	1.05	1.09	1.38	1.12	1.14	1.48
T1049	1.08	1.30	1.46	1.33	1.46	1.56

Table 2. Analysis of Variance for berry yield in the selected clones of Robusta coffee.

Source	df	SS	MS	Fcal
Genotype	2	0.17	0.08	9.33**
Year	2	0.23	0.11	12.56 **
Genotype X Year	4	0.12	0.03	3.33 ns
Error	8	0.06	0.09	

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Identification and Partial Characterization of *Sepallata* Genes in *Coffea arabica L.*

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SUMMARY

The *SEPALATA* genes play an important role in the flowering process being responsible for the determination of the four floral whorls. Among the factors that affect coffee quality, the sequential flowering found in this species plays a central role, leads to an asynchronous ripening process and consequently a lower cup quality. In order to get a better understanding coffee (*Coffea Arabica*) flowering process, this study aimed to identify and characterize the putative coffee *SEPALLATA* (SEP) genes present in the coffee expressed sequence tag (CAFEST) database. The phylogeny of the sequences found was assessed by phylogenetic trees and their expression profile was assessed by *in silico* Northern. The putative coffee SEP genes were cloned, sequenced and then compared to the sequences obtained in the CAFEST. Three putative SEP genes (C1, C8, C14) were identified, and the *in silico* expression profile showed that they're expressed in different flower development stages and also in during fruit development, which is in accordance with the function displayed by SEP genes in reproductive tissues. The comparison of the sequenced sequences with those found in CAFEST, allowed the observation of a great similarity between these sequences and confirmed the importance of previous bioinformatic studies in molecular analysis.

INTRODUCTION

The MADS-box gene family of transcription factors, which is characterized by the MADS domain, plays an important role in many plant development process such as meristem identity, flower determination, pollen fertility, and ovule and fruit development (Shitsukawa et al., 2007). Some of these transcriptional factors, such as the SEP genes, are part of the ABCE model which has been proposed to explain the formation of floral organs (Causier et al., 2002).

According to the ABCE model, floral formation is achieved by the interaction of the four homeotic gene classes that compose this model. The E class is formed by the SEP genes which was shown to be required for the formation of the four floral whorls: sepals, petals, stamens and carpel. Multiple mutants for this gene class may have all floral whorls converted to sepals or even leaf whorls, indicating the central role in flower development displayed by the SEP genes (Pelaz et al., 2000; Honma and Goto, 2001; Ditta et al., 2004).

Among the factors that affect coffee quality, the sequential flowering found in this species plays a central role, contributing to an asynchronous ripening process and consequently leading to a lower cup quality. Thus, in order to get a better understanding of the coffee flowering process, this study aimed to identify and characterize the putative coffee (*Coffea arabica*) SEP genes found in the coffee expressed sequence tag (CAFEST) database.

MATERIAL AND METHODS

Database search and alignments

In order to identify homologs of functionally characterized SEP genes, data mining in the CAFEST database (Vieira et al., 2006) (http://bioinfo04.ibi.unicamp.br) were carried out using plant gene (BLASTn) and protein (tBLASTn) sequences as bait, as well as keyword searches. The sequences showing a reliable similarity were clusterized, annotated and analyzed for the presence of conserved domains.

Phylogenetic analysis

The putative functionally of the deduced amino acid sequences of coffee transcripts, compared to homologs from other species, was assessed by phylogenetic trees performed by the MEGA software, version 4.0 (Tamura et al., 2007), with neighbor-joining comparison model (Saitou and Nei, 1987), p-distance method and pair-wise suppression. Bootstrap values from 100 replicates were used to assess the robustness of the trees (Sitnikova et al., 1995).

Identification of common motifs

The identification of common motifs was performed by the MEME (*Multiple Expectation Minimization for Motif Elicitation,* http://meme.sdsc.edu/meme/meme.html/) program (version 3.5.4) (Bailey and Elkan, 1994).

In silico gene expression analysis

In silico qualitative gene expression profiling was performed using virtual Northern blot analyses of the coffee EST database. For each EST-contig and EST-singlet, frequencies of reads that form each EST-contig and EST-singlet in the libraries in which they were expressed were calculated. The results were plotted in a matrix and gene expression patterns among ESTs and libraries were obtained by hierarchical clustering, performed by the Cluster v.2.11 program (Eisen et al., 1998). Graphic outputs were generated by the TreeView v.1.6 software (Eisen et al., 1998) and presented in a gray scale.

Molecular analysis

DNA was extracted from coffee (*Coffea arabica*), Rubi cultivar, using a modified CTAB (Doyle and Doyle, 1991) protocol. Total RNA extraction were performed by the Hot borate method (Birtic and Kranner, 2006). After DNase treatment, cDNA synthesis was performed using the Super Script III First Kit - Strand Synthesis Super Mix from Invitrogen. Specific primers, designed from the sequences obtained from the *in silico* analysis, were used to clone the putative coffee SEP genes. The cloning process was performed using the pCR ® II TOPO TA vector (Invitrogen) and bacteria transformation was done by the heat shock method. Plasmid purification was performed by the alkaline-lysis method and the sequencing process by the Sanger method (Sanger et al., 1977).

RESULTS AND DISCUSSION

The search for SEP genes in the CAFEST database generated 790 reads related to these genes and the motif analysis showed that only three contigs displayed the conserved domains that characterize the SEP genes, being selected for further analysis.

Multiple alignments between the proteins encoded by the putative coffee SEP genes found in this work and SEP proteins from other species allowed the identification of six conserved motifs, which were present in almost every coffee sequences (Figure1). MADS proteins of type II shows a conserved structural organization called MICK, which were designated according to the domains present in these proteins from the N to the C-terminal direction (Alvarez-Buylla et al., 2000; Riechmann et al., 1996): MADS-box (M), intermediate (I), Keratin-like (K) and carboxy terminal (C). The motif 1 and 2 represent the MADS and K-box domains, respectively, which are the most conserved domains of the MADS-box transcription factors (Figure 1). According to Malcomber and Kellogg (2005) the motif 3 and the motifs 4, 5, and 6 belongs to the I and C Domains, respectively, which are less conserved domains (Figure 1).

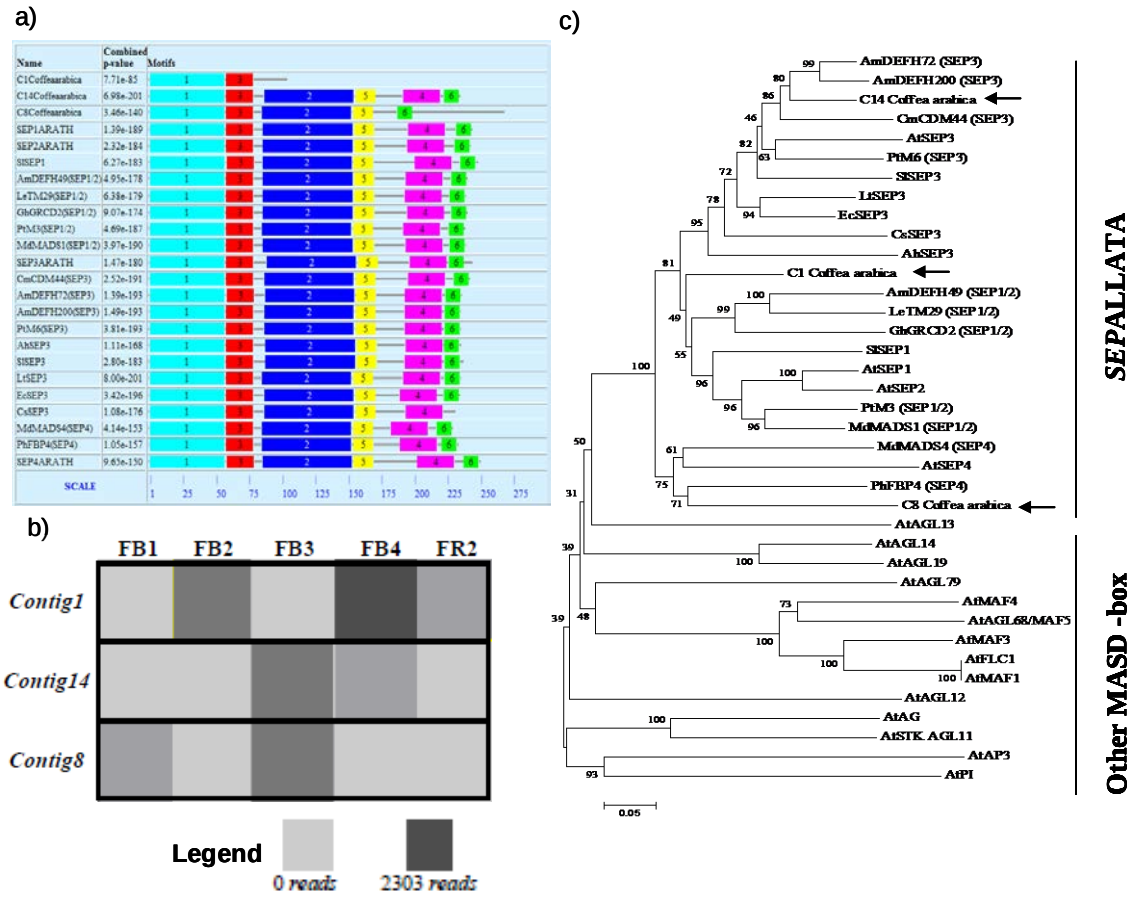

Figure 1. motif analysis of putative coffee SEP protein sequences (C1, C8, C14) and SEP proteins from other species obtained from the NCBI databse (a); *in silico* expression profile exhibited by the putative coffee SEP genes found in the CAFEST database, coffee libraries are as follows (Vieira et al., 2006): Flower buds in different developmental stages (FB1, FB2, FB3, FB4); Flower buds + pinhead fruits + fruits at different stages (FR2) (b); phylogenetic analysis involving the putative coffee SEP protein sequences and SEP and MADS-box proteins sequences from other species obtained from the NCBI database (c).

The phylogenetic analysis between the putative coffee SEP protein sequences, SEP proteins from other species and other than SEP MADS-box proteins sequences, both obtained from the NCBI database, showed a separation of these sequences into two major groups, with the SEP proteins clustered in a distinct group from the other MADS-box proteins (Figure 1). All of the

three sequences found in this study were clusterized into the SEPALLATA group, indicating that they may encode for putative coffee SEP proteins. The *in silico* expression profile exhibited by C1, C8 and C14 is in accordance with the role of SEP genes in reproductive tissues, and also corroborates to the fact they're putative coffee SEP proteins. C1, C8 and C14 were found to be expressed in libraries comprising different flower development stages and also in a library generated from fruits at an early development stage (Figure 1). According to the similarity of C1, C8 and C14 to previously described SEP proteins, these contigs were identified as SEP1/2 (C1SEP1/2), SEP4 (C8SEP4) and SEP3 (C14SEP3), respectively.

The sequence comparison between the sequences found in the *in silico* analysis with those obtained from the sequencing process, allowed the observation of a great similarity between these sequences, except for the contig C8SEP4 that failed to be completely sequenced. The contig C14SEP3 showed a nucleotide identity of 85% and 80% with SEP3 of *Lycopersicon esculentm* and *Antirrhinum majus*, respectively. The contig C1SEP1/2 were shown to display a nucleotide identity of 77% with SEP1 from *Cucumis sativus*, *Citrus sativus* and *Antirrihinum majus*. These results corroborate with those found in the phylogenetic analysis and confirm the importance of previous bioinformatic studies in molecular analysis.

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Coffee Seeds Isotopic Composition as a Potential Proxy to Evaluate Minas Gerais (Brazil) Seasonal Variations during Seed Maturation

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SUMMARY

Plant seeds reflect the prevailing climate conditions and the physiological response to those conditions. The aim of this work was to evaluate the potential use of green coffee seeds as a proxy for seasonal climatic conditions during coffee bean maturation, through an array of isotopic composition determinations. Determinations of carbon, nitrogen, oxygen and sulfur isotopic composition by IRMS (Isotope Ratio Mass Spectrometry) were carried out, as well as strontium isotope abundance by MC-ICP-MS (Multicollector Inductively Coupled Plasma Mass Spectrometry), on green coffee beans harvested at different times at Minas Gerais, Brazil. The isotopic composition data were combined with air temperature and relative humidity data observed during coffee bean developmental period, and with the parent rock strontium isotopic composition. Results indicate that coffee seeds indeed integrate the interactions between plant physiology and local climate variations, as well as the particular soil geology.

INTRODUCTION

Previous work has demonstrated that isotope analysis of bulk coffee bean leads to geographical origin discrimination (Rodrigues et al., 2007) and this has been correlated with local climatic and geological data (Rodrigues et al., 2010). Carbon and oxygen isotopes are known to fractionate in plant leaves but less is known concerning fractionation in seeds (*e.g.*, the coffee bean). However, most of the seed organic matter should derive from leaf photosynthesis, probably with minor contribution of seed photosynthesis. Also, oxygen isotope composition of plant organic material is known to reflect that of source water and leaf evaporative conditions at the time the material is synthesized. Based on this, we postulated that coffee beans will reflect local climate as well as the climatic variations observed along successive harvest periods. Green coffees from Minas Gerais state, Brazil were collected in 2004 and 2008. Oxygen, carbon, nitrogen and sulfur isotopic composition of bulk coffee beans were determined by EA-IRMS. The observed isotopic trends were compared to variations in air relative humidity, daily temperatures and vapor pressure deficit (VPD). The same approach was applied to caffeine extracted from the coffee beans, whenever possible.

Additionally, strontium isotope ratio (MC-ICP-MS) of coffees corresponding to the four regions of Minas Gerais state was also addressed. The study is a part of the ISOGEOCOFFEE project – Pursuing Green Coffee Geographic Origin Discrimination through Relations between Isotopes and Environmental Factors (PTDC/AGR-AAM/104357/2008 – Portuguese Science Foundation) (https://sites.google.com/site/isogeocoffee).

MATERIALS AND METHODS

Green coffee bean samples from the 2008 harvest period were obtained in Minas Gerais State at the Cerrado region. Previous harvest year samples were supplied by Novadelta, S.A. (Campo Maior, Portugal). Carbon, nitrogen, oxygen and sulfur isotopic composition Sulfur Isotopic Ratio were determined by EA-IRMS as described in Rodrigues et al. (2007; 2010)**.** Strontium isotope abundance was determined by MC-ICP-MS as in Swoboda (2008). Green coffee bean total caffeine was extracted according to Weckerle et al. (2002)**.**

Local climate data were acquired from metereological station Araxá, a part of CPTEC/INPE (Centro de Previsão de Tempo e Estudos Climáticos do Instituto Nacional de Pesquisas Espaciais), Brazil.

Local geological information was obtained at the Advanced Data Management in Solid Earth Geochemistry (2003). Mean annual $\delta^{18}O$ of precipitation was acquired on the Online Isotopes in Precipitation Calculator (OIPC) (2010).

RESULTS AND DISCUSSION

Our results show that oxygen and strontium isotopes constitute the best discriminatory system for Minas Gerais coffee producing regions (Figure 1). Coffees from the Cerrado region discriminated from all other coffees based on strontium isotope abundance ratio. This is an important result as this coffee is certified and has a high market price in comparison to coffees from the other three regions. Two outliers (from South of Minas and Chapada region) were found. The coffee from South of Minas showed higher $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ values, which do not match with $^{87}Sr/^{86}Sr$ values reported for parent rock in this region (0.7026 to 0.7177). In what refers to Chapada region outlier, this coffee was cultivated at low altitude and higher latitude which can be related to a lower $\delta^{18}O$ values in precipitation and organic matter, and consequently in the coffee bean.

The results of carbon isotope ratio analysis of caffeine extracted from the different coffee bean samples suggest a relation between carbon isotope ratio of the bulk green coffee bean and climate variations (Table 1). The endosperm development period correspondent to harvest year 2008 shows lower average air relative humidity, higher average maximum daily temperature and maximum daily vapor pressure deficit (VPD), which are in accordance to more positive values of $\delta^{13}C$ of the coffee bean (Table 1).

Differences in $\delta^{13}C$ have been observed in total coffee bean but not in total caffeine extracted from the green coffees of 2004 and 2008 (ANOVA). The observed variation in seeds may be related to higher stomatal conductance and a consequence of a higher expression of RuBisCo ^{13}C discrimination, due to favorable climatic conditions along the year. The lower maximum VPD and higher RH conditions during 2004 may be some of the environmental conditions that rule stomatal opening and consequently the observed $\delta^{13}C$ value in organic material. The absence of a similar pattern in caffeine, suggests that the $\delta^{13}C$ of caffeine does not reflect the

average observed differences in humidity, temperature and VPD (Table 1). One of the main reasons could be due to the fact that caffeine is biosynthesized during coffee plant leaflet emergence, a short period during the first 5 weeks of fruit development (as a chemical defense). However, less is known about the *de novo* biosynthesis of caffeine in the seed. Additional physiological studies may clarify the observed differences in $\delta^{13}C$ of the coffee bean and of the extracted caffeine.

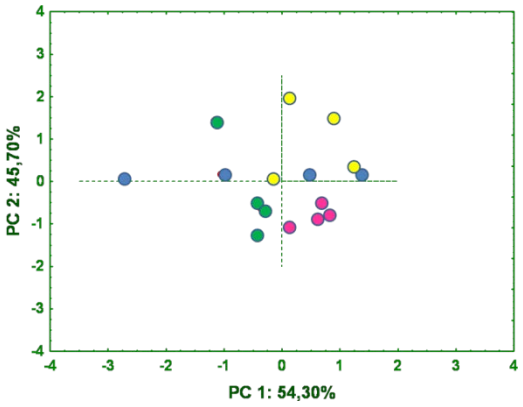

Figure 1. Minas Gerais coffee producing regions discrimination based on oxygen isotopic composition and strontium isotope abundance ratio of 2008 harvest green coffee beans. Legend: blue – Matas region; green – South of Minas Gerais; yellow – Chapada region; pink – Cerrado region.

Table 1. $\delta^{13}C$ of green coffee beans and extracted caffeine from harvest years 2004 and 2008, and precipitation, air relative humidity, maximum daily temperature and daily vapor pressure deficit (VPD) correspondent to the endosperm development period (average of values observed between mid-October and mid-February).

Parameter	2004	2008
$\delta^{13}C_{coffee\ bean}$	-28.1 (± 0.2)	-26.9 (± 0.5)
$\delta^{13}C_{caffeine}$	-27.6 (± 0.1)	-27.8 (± 0.4)
Precipitation (mm)	115 (± 100)	135 (± 107)
Air Relative Humidity (%)	86 (± 19)	75 (± 25)
Maximum Daily Temperature (ºC)	28 (± 3)	29 (± 3)
Maximum Daily VPD (KPa)	11 (± 10)	21 (± 8)
Average Daily VPD (KPa)	27 (± 7)	27 (± 7)

In short, isotope fingerprinting potential to proof authenticity of Minas Gerais coffee has been demonstrated. Additionally, impacts and climatic alterations that result in changes to coffee plants' environment appear to be reflected on changes of the isotope composition of their seeds, the green coffee bean. The green coffee bean isotopic signature is, therefore, the result of close interactions between climate and geology of the location where the plant and respective seeds are developed.

ACKNOWLEDGEMENTS

This work was financially supported by the Austrian Science Foundation (FWFSTART grant 267N11), Fundação para a Ciência e Tecnologia, through the project PTDC/AGR-AAM/104357/2008, co-financed by the european fund FEDER, and by the grant SFRH/BD/28354/2006 (C. Rodrigues), co-financed by the portuguese PIDDAC program and European Social Fund, under the 3rd framework program.

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Impact of Different Coffee Shade Trees on Soil Quality Parameters in Relation to Organic Coffee Farming Potential in Yayu District, Southwestern Ethiopia

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SUMMARY

Agro-forestry as a sustainable agriculture can maintain soil fertility through organic matter accumulation, nitrogen fixation, erosion control and employing conservation mechanisms. The impact of various shade trees types and density on soil quality and fertility was studied in Yayu District employing stratified random sampling method. The soil within 0-30 and 30-60 cm depth is dominated by clay loam and clay textural classes respectively; which are fine textured to accommodate nutrients for coffee and reduce soil erodibility. Generally, the bulk density of the soil is low with high soil porosity. Soil chemical quality evaluation under forest, SFL and SFNL shade trees showed non- significant difference ($P>0.05$) for organic matter, exchangeable bases (Ca, Mg, K, Na), CEC, exchangeable acidity, pH, total nitrogen, C:N ratio, PBS apart from phosphorus. The fertility of the soil under semi-forest shade trees was high in terms of total organic matter and nitrogen. Soil fertility and quality of the semi-forest were thus, found statistically similar to the undisturbed natural forest. Therefore, the district has got high potential for organic coffee farming.

INTRODUCTION

Agro-forestry thus, as a sustainable agriculture can maintain soil fertility through maintenance of organic matter, nitrogen fixation, erosion control using conservation mechanisms. Most farmers in the coffee growing areas prefer nitrogen-fixing trees to non-fixing shade trees to get a benefit of biological fertilizer for their coffee tree (Grossman et al., 2006). Decline in soil fertility potential is a major problem facing small-scale coffee farming in Ethiopia. Hence, to optimize the fertility potential of the soil, evaluating the impact of shade trees on the quality of the soil is very crucial. Therefore, the impact of different shade trees on selected soil properties of Yayu District in relation to organic coffee farming was studied.

MATERIALS AND METHODS

The study was conducted in Yayu District of Illubabor Zone, in Oromia Regional State of Southwestern Ethiopia. The altitude of the district ranges 1160-2581 masl and study covers 1336 to 2070 masl. Soil physical and chemical parameters were collected under forest, semi-forest leguminous shade trees, semi-forest non-leguminous shade trees using stratified random sampling design at uniform farm sites of 30 m X 30 m quadrate size. The physical parameters, bulk density, particle density porosity, texture, soil color were studied at a depth of 0-100 cm. Soil chemical parameters, pH, exchangeable acidity, organic carbon, CEC, Na, K, Ca, Mg, PBS, ESP, N, P were collected at depths of 0-30 cm and 30-60 cm. All the parameters were collected along the ranges of lowland (<1500 masl), midland (1500-1800

masl), and highlands (>1800 masl) altitude. All soil parameters were analyzed using standard laboratory procedures. The data obtained from laboratory results were subjected to analysis of variance to compare the effect of shade tree on soil quality parameters using SAS Software.

RESULTS AND DISCUSSION

Soil Physical Parameters

Soil bulk and particle density showed increasing trend with the increase of profile depth across forest and semi-forest coffee vegetation (Table 1). Low bulk and particle density for the surface layers indicates the accumulation of soil organic matter in the form of litter fall and root residues of vegetation. Forest soils were more porous than semi-forest soils due to high organic matter content. Porosity of the soil at the study area is more than 44% even at a depth of 100 cm profile, which indicates the potential of the soil for organic coffee production (Table 1). More than 50% of the sampled soils in the area constituted fine particles (clay+ silt) at both surface (0-30 cm) and subsurface 30-60 cm). This indicates high nutrient and water holding capacity of the soils in the district.

Soil Chemical Parameters

Variations in soil pH were not significant for coffee shade types and density at ranges of altitude and depths of soil. The mean soil pH (H2O) range in the region was 5.44 to 6.63 for 0-30 cm soil depth, which is within suitable range for organic coffee production according to Snoeck and Lambot (2004). The accumulation of OM was not significantly different among forest, SFL, and SFNL vegetation categories. Nevertheless, there was statistically significant difference ($P<0.01$) in OM percentage among soils of low, mid and high altitudes. Similarly, OC was significantly different for 0-30 cm and 30-60 cm depths of the soils as the deposit decreased along the layers of soils (Table 2). According to Snoeck and Lambot (2004), optimum OM in the coffee soil ranges from 2 to 5%. Soils at different locations of the study area has got high organic matter content which is greater than 5.67% for soil depths of 0-30 cm. The difference in total nitrogen content was significant ($P<0.01$) among altitudinal variation and depths but was not significant among the soil for forest, SFL and SFNL. The content of total nitrogen was generally high in the soils of the district according to the rating of Baruah and Barthakur (1997).

Total nitrogen content of the soil of SFL was greater than forest and SFNL at 0-30 cm due to biological nitrogen fixation. The mean pH for the district ranges from 5.44 to 6.63 at 0-30 cm soil depth for SFL and forest respectively, which is within suitable range of biological nitrogen fixation. Nitrification and nitrogen fixation by leguminous trees takes place vigorously at soil pH of >5.5 (Rai, 2002). Variations of P was significant ($P<0.01$) along altitude and shade trees but was not significant with soil depths. Available P was very high under forest vegetation and lowest under SFL shade trees, which could be attributed to relatively higher and lower soil pH values respectively. The pH of forest coffee soils was higher within suitable range of availability of P to the coffee plant and the deposition of organic matter contributed for greater quantity of available P in the soils. According to the Jaiswal (2003) ratings, the amount P (ppm) in the soil was high in forest soil (60.71 ppm) at 0-30 cm soil depth. At 0-30 cm, available P was medium in soils under SFNL (14.22 ppm) and SFL (9.20 ppm) vegetation as result of P fixation.

Table 1. Effects of shade tree types and density along altitudinal range on soil physical parameters in Yayu District, Southwestern Ethiopia.

Locations	Farm site layers	Depth (cm)	Bulk density (g/cm^3)	Particle density (g/cm^3)	Porosity (%)	Color types
Lowland	Forest I	0-29	1.29	2.57	49.77	Very dark reddish brown
Lowland	Forest II	29-47	1.48	2.62	43.60	Dark red
Lowland	Forest III	47-100	1.53	2.80	45.56	red
Lowland	SFL I	0-14.5	1.21	2.67	54.70	Very dark reddish brown
Lowland	SFL II	14.5-33	1.21	2.76	56.14	Dark reddish brown
Lowland	SFL III	33-100	1.31	2.79	53.12	Dark red
Lowland	SFNL I	0-15	1.27	2.46	48.29	Very dark reddish brown
Lowland	SFNL II	15-43	1.46	2.68	45.54	Dark reddish brown
Lowland	SFNL III	43-59.5	1.39	2.81	50.67	Very dark reddish brown
Lowland	SFNL IV	59.5-100	1.37	2.61	47.40	Dull reddish brown
Midland	Forest I	0-25	0.97	2.59	62.73	Reddish black
Midland	Forest II	25-46	1.04	2.74	62.20	Very dark reddish brown
Midland	Forest III	46-100	1.26	2.75	54.09	Very dark reddish brown
Midland	SFL I	0-16	1.16	2.72	57.49	Dark reddish brown
Midland	SFL II	16-28	1.11	2.90	61.78	Very dark reddish brown
Midland	SFL III	28-48	1.23	2.83	56.54	Dark red
Midland	SFL IV	48-100	1.24	2.86	56.51	Dark red
Midland	SFNL I	0-20	1.14	2.48	53.99	Very dark reddish brown
Midland	SFNL II	20-45	1.18	2.65	55.41	Dark reddish brown
Midland	SFNL III	45-100	1.23	2.79	55.79	Dark red
Highland	Forest I	0-48	0.79	2.46	67.76	Reddish black
Highland	Forest II	48-68	1.20	2.78	56.66	Very dark reddish brown
Highland	Forest III	68-100	0.98	2.67	63.31	Grayish red
Highland	SFL I	0-40	1.03	2.67	61.27	Very dark reddish brown
Highland	SFL II	40-100	1.25	2.75	54.63	Dark reddish brown
Highland	SFNL I	0-30	1.07	2.53	57.47	Very dark reddish brown
Highland	SFNL II	30-100	1.18	2.72	56.41	Dark reddish brown

SFL=semi-forest legume; SFNL= semi-forest not legume; I, II, III, IV stands for soil layers with depth according to their color difference

Table 2. Mean soil quality parameters affected by shade tree types and density, altitude and depths of the soil.

Shade trees	Depth (cm)	Na	K	Ca	Mg	PBS	ESP	CEC	OM	N	C:N	P	pH	Exchangeable acidity
Low altitude														
Forest	0-30	0.07	0.15	15.49	3.36	60.93	0.22	31.33	5.15	0.32	9.14	23.67	5.46	0.52
	30-60	0.05	0.08	14.54	2.33	56.80	0.16	35.55	2.91	0.25	6.78	7.60	5.14	0.35
SFL	0-30	0.05	0.28	16.78	3.64	47.46	0.13	41.13	5.20	0.44	7.00	8.33	5.44	0.32
	30-60	0.06	0.21	13.98	3.69	46.82	0.18	36.63	3.10	0.29	6.30	3.33	5.47	0.40
SFNL	0-30	0.07	0.42	25.44	4.35	69.69	0.18	43.35	6.67	0.43	9.00	15.87	5.89	0.38
	30-60	0.07	0.28	12.38	3.78	43.19	0.21	38.17	2.96	0.27	6.33	5.00	5.50	0.35
Middle altitude														
Forest	0-30	0.05	0.63	29.80	5.25	66.95	0.09	57.46	6.81	0.40	9.94	83.27	5.52	0.34
	30-60	0.08	0.67	22.39	6.46	61.43	0.16	47.79	4.05	0.29	8.57	55.73	5.70	0.28
SFL	0-30	0.04	0.18	10.94	3.41	41.95	0.12	35.21	4.66	0.36	7.67	13.47	5.01	0.34
	30-60	0.06	0.15	23.12	4.17	74.44	0.17	36.69	3.79	0.26	8.61	5.67	4.97	0.50
SFNL	0-30	0.07	0.33	22.16	6.33	62.28	0.15	48.60	5.76	0.36	9.13	17.00	5.59	0.28
	30-60	0.07	0.23	14.28	4.04	51.74	0.25	38.03	2.98	0.25	7.22	8.40	5.45	0.27
High altitude														
Forest	0-30	0.08	0.48	22.10	4.80	71.72	0.22	37.75	7.75	0.53	8.50	75.2	6.63	0.32
	30-60	0.06	0.35	26.23	3.96	59.69	0.12	49.78	5.48	0.46	6.89	92.2	6.23	0.43
SFL	0-30	0.06	0.47	27.50	4.81	67.47	0.12	52.92	6.76	0.50	8.05	13.67	5.77	0.35
	30-60	0.06	0.19	11.32	2.80	36.57	0.16	39.20	3.69	0.27	8.00	10.80	5.26	0.57
SFNL	0-30	0.09	0.27	18.11	3.89	55.10	0.20	42.07	6.23	0.48	7.78	9.80	5.85	0.58
	30-60	0.07	0.41	12.22	3.06	55.53	0.23	33.11	3.80	0.32	7.32	26.87	5.17	0.50

SFL = semi-forest legume; SFNL = semi-forest not legume; (Na, K, Ca, Mg, CEC, Exchangeable acidity in Cmol+/ Kg soil);(PBS, ESP, OM, Total N in %); (P in ppm); (Depth 0-60 in cm).

Analysis of variance showed not significant variation of exchangeable Ca, K, and Na for coffee shade trees (forest, SFL and SFNL), for altitude difference and depth of soils. However, exchangeable Mg was significantly different ($p<0.01$) with altitudinal gradients but was not significant among coffee shade type, density and depths. Generally, exchangeable K, Mg, Ca and Na were abundant at shallower depths (0-30 cm) of the soil which are readily accessible to coffee root than subsurface (30-60 cm).

Percent base saturation (PBS) and exchangeable sodium percentage (ESP) were not significantly ($P>0.05\%$) different for shade tree type and density and altitude gradient as well for the upper and lower depths of the soil. Cation exchange capacity did not significantly differ among shade types and density, altitude and soil depth. Generally, CEC of semi-forest coffee (SFL and SFNL) soils in the area are greater than that of deep natural forest at surface (0-30 cm) soil layer and it was highest under SFNL shade trees followed by SFL and forest. The value of CEC was highest under SFNL at surface soil layer, which can be due to the impact of OM and clay content under the shade tree. The average CEC for surface layer (0-30 cm) under different vegetation types of Yayu District was very high (> 40 Cmol+/ kg of soil). According to Landon (1991), top soils having CEC > (40 Cmol+/ Kg of soil) are rated as very high and 25-40 Cmol+/kg of soil considered as high value. Exchangeable acidity of showed significant variation only for the altitudes of coffee growing locations due to differences in soil conservation practices under the coffee.

CONCLUSION

Fertility and quality of the soil under SFL and SFNL shade trees were found statistically similar to undisturbed natural forest, which showed the potential of coffee farm sites for organic coffee production. The pH was convenient for biological nitrogen fixation and coffee cultivation and exchangeable acidity found low. The soil was also rich in basic cations, organic matter, nitrogen; percent base saturation and with high cation exchange capacity, relatively low bulk density, high porosity and with fine textures. Therefore, the quality of the soil is very good under various shade trees and it is a great opportunity to keep on organic coffee cultivation in the region with maintenance and conservation of the soil fertility.

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Impact of Different Shade Trees on Coffee Associated Parameters under Different Production Systems of Yayu District, Southwestern Ethiopia

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SUMMARY

Arabica coffee still grows wild in natural forest covered by heterogeneous species of overhead shade trees. The coffee associated parameters are collected using stratified random sampling method. Significant difference ($p<0.01$) were observed in coffee leaf area among forest, SFL and SFNL shade trees. Coffee leaf area under the shade trees was different due to the difference in the percentage of light transmitted through their canopies. The percentage of light transmitted through different shade trees canopy was significantly differed ($p<0.01$). Though coffee fruit size was not significantly affected by the amount of light transmitted through shade tree canopy, it showed significant difference ($p<0.01$) for (low, mid and high) altitude. The forest coffee ecosystem had highly diversified weed flora than under semi-forest coffee farm where the weed ecology interference by the farmers is high. Thus, coffee leaf area, fruit bean size, weeds categories, light transmitted through shade trees showed variation under different coffee production systems in the study area.

INTRODUCTION

The world-wide domesticated Arabica coffee originated from the Ethiopian highlands. The natural forest is covered by heterogeneous species with some recommended shade trees. Under the forest coffee management, coffee shrubs grow up to 4-6 m high with only few fruiting branches at the top of the tree (Workafes and Kassu, 2000). Semi-forest coffee production systems are thought to be descended from natural forest coffee management system (Paulos and Demil, 2000). When deep shade trees heavily screen the percentage of light passing to coffee tree, the coffee leaf responds to the situation by expanding the leaf area to trap maximum amount of light (Franck *et al.,* 2006). High shade intensity restricts types of weeds infestation that are mainly of soft broad-leaved weed categories that can be controlled by slashing (Lambot and Bouharmont, 2004a). Growth and productivity of coffee plants is more improved under moderate light regimes (25-75%), but significantly lower in full sun or under very low light intensity (Yacob *et al.,* 1996). The study was thus, carried out with the objective of evaluating impact of various shade trees on coffee related parameters under different production systems of Yayu District, Southwestern Ethiopia.

MATERIALS AND METHODS

The field study was conducted at lowland (<1500 masl), middle altitude (1500 masl-1800 masl) and highland (>1800 masl) of Yayu District. Twenty-seven coffee farm sites were selected based on shade tree type and density. These are forest, semi-forest legume (SFL) and semi-forest not legume (SFNL). From nine coffee farm sites three forest, three SFL and three SFNL farm sites were selected. From each plot (30 m X 30 m), 20 and 30 coffee trees were

randomly selected for leaf area and fruit bean size measurement respectively. Stratified random sampling method was employed. Three leaf samples and five fruits were collected from the middle branches of sample coffee. A quadrate of 1 m X 1 m was taken under shade trees in the plot and weeds found in the quadrate were categorized. The light percentage of the shade trees in the plot was measured using digital light meter. The data collected for coffee leaf area, fruit bean size and light percentage parameters were subjected to analysis of variance to compare effects of coffee shade tree type and density on the parameters.

RESULTS AND DISCUSSION

Significant difference ($p<0.01$) were observed in coffee leaf area among forest, SFL and SFNL shade trees. Coffee leaf area under the shade trees was different due to the difference in the percentage of light transmitted through their canopies. Thus, forest vegetation, which transmitted very low amount of light, resulted in the largest coffee leaf area. Relatively small coffee leaf area was measured under small leaved leguminous shade trees (*Albizia* spp.) due to better transmission of incoming sunlight to coffee trees (Table 1). The negative correlation result of leaf area to light percent received by coffee tree was significant ($p<0.001$). The percentage of light transmitted through different shade trees canopy was significantly differed ($p<0.01$). However, the variation of sun light percentage was not significant for shade trees of different altitudes within study area. The percentage of light taken by digital light meter under dense ranged from 0.58% to 6.26%. Light percent passing through *Albizia* spp. of the best quality shade tree runs 19% to 44%, which lies within the suitable light ranges for coffee.

The area of coffee leaf could be determined using square paper by counting square area under drawn leaf. More precisely leaf area can be determined by scanning method using digital leaf area meter. Coffee researchers can alternatively use model developed by randomly selecting 527 from 727 scanned leaves collected from highland, middle altitude and lowland areas. Model of determining leaf area for shaded coffee:

Estimated area = leaf length X width;

Actual area (measured area through scanning);

$$\frac{Actual}{Estimated} = K \text{ (constant factor);}$$

Calculated leaf area= Estimated X K;

K=0.624;

closely similar K value was obtained (0.640) for 13 varieties under shade at Gera Research Center (1940 masl), Southwestern Ethiopia using square paper drawings.

Coffee fruit size was not significantly affected by the amount of light transmitted through shade tree canopy. However, analysis of variance for coffee fruit bean size showed significant difference ($p<0.01$) for locations (low, mid and high) altitude. Hence, fruit size showed decreasing pattern as altitude increased from lower level to the upper position across the district however, the reverse is true for fruit weight. Fruit sizes were larger at lowland areas (<1500 masl) than that of middle altitude (1500-1800 masl) and highland (>1800 masl). The different scenario might have observed due to the difference in temperature. Nevertheless, analysis of variance was not significant ($p>0.05$) for bean sizes among shade types and

density. However, fruit sizes of coffee grown under SFL (*Albizia* spp.) shade tree were greater than that of grown under forest and SFNL shade trees at lowland and middle altitude.

Table 1. Effect of shade tree types and density on coffee leaf area, fruit size and transmitted light percentage across coffee farm sites.

Coffee farm sites	Leaf area (cm^2)	Fruit bean size (mm^3)	Transmitted light (%)
Semi-forest legume (Albizia *spp*.)	50.26	1942.80	30.74
Semi-forest not legume (Broad leaf species)	57.07	1874.61	8.99
Forest	65.21	1932.79	2.74
CV %	6.89	7.4	29.80
S.E.	0.76	27.33	0.81
LSD (0.05)	3.9	NS	4.15

In the district multiple purpose shade trees serves as a sources of food, feed, medicine, firewood, and timber, habitat for fauna and flora, and supply essential ecological maintenance, soil and water protection. The forest coffee ecosystem had highly diversified weed flora than under semi-forest. Very few types grass species were naturally grown under broad-leaved shade trees and under deep natural forest of the area. Under deep shading effect, broad leaf weed species similar to coffee can adapt to small amount of sun light having high capacity of utilizing limited quantities of radiation. On the contrary, relatively greater amount of grassy weed flora found under small leaved coffee shade trees receiving better quantities of sun light percentage.

CONCLUSION

Farmers in the study area uses different multiple purpose trees as shade for coffee.Shade grown on coffee farms of Yayu District, Southwestern Ethiopia resulted in variations with respect to coffee leaf area, fruit bean size, transmitted light percentage, weed categories in coffee production system. Coffee leaf area and weed categories are affected due the amount of light passed through the canopy of the shade trees. Coffee cultivars and weed species found under deep natural forest shade showed broad leaf system to trap maximum amount of limited light. Thus, coffee leaf area, fruit bean size, weeds categories, light transmitted through shade trees showed variation under different coffee production systems in the study area.

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Traditional Coffee Husbandry Practices in West Hararghe, Ethiopia

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SUMMARY

A survey was conducted in January 2009 in Darolebu wereda representing the major coffee producing region of West Hararghe, Ethiopia. The objectives are to characterize and document farmers' indigenous knowledge of coffee seed preparation, nursery and field management practices and identify production and marketing constraints and opportunities. A team of researchers consisting of an agronomist, plant breeder, soil scientist and extension agent participated in the survey. The team made discussion with experts and development agents (DAs) of the Wereda Agricultural and Rural Development Office and selected two peasant associations (PAs), namely Serero and Gudise. From each PA 15, farmers were selected to represent highly innovative, late adopters and those who are too skeptical of agricultural technologies. The selected farmers were interviewed using structured questionnaires for their knowledge of coffee nursery and field management practices, coffee harvesting and processing, markets and marketing systems and some relevant information about the farming systems of the study sites. Besides, assessment of coffee production constraints in the selected PAs was made by questioning 15 selected farmers, and 15 wereda experts and DAs. Besides, observation was made on the farming system of the study area through transect walk. Information regarding farmers indigenous knowledge on coffee seed preparation, nursery and field management practices, diseases and insect pests, method of harvesting and processing, and marketing conditions, major pressing problems in the system and suggested solutions is discussed in this paper. The information will help researchers and policy makers to conduct demand driven and client oriented research and develop policies and strategies that could improve production, productivity and quality of the crop and enhance the livelihood of farmers in the area.

INTRODUCTION

Coffee production and management practices in Ethiopia may vary with region and its environmental conditions, which, in turn, govern the farming systems of a given area. Identification of such practices and indigenous knowledge of the coffee culture has a paramount importance for farmers, researchers and policy makers. In view of this, diagnostic survey was conducted in coffee based farming systems of Darolebu wereda, West Hararghe Zone of the Oromiya Region, with the objectives to characterize and document farmers' indigenous knowledge of coffee seed preparation, nursery and field management practices, method of harvesting and processing, and marketing conditions. It also attempted to identify major constraints in the process. The information gathered will help to identify and prioritize major coffee production constraints, and propose appropriate technologies that could improve coffee productivity of the area.

METHODOLOGY

A survey was conducted in January 2009 in Darolebu wereda, representing the major coffee producing wereda of West Hararghe Zone of the Oromiya Region (Figure 1). The area is known for its high cup quality Harar coffee brand, which fetches premium prices in the world market.

A multidisciplinary team of researchers consisting of an agronomist, plant breeder, soil scientist and extension agent participated in the survey. The team made discussion with experts and development agents (DAs) of the Wereda's Agricultural and Rural Development Office on the objectives of the study, and selected two peasant association (PAs), namely Serero and Gudise, based on their production potential, their accessibility and representativeness of the farming system. From each PA, 15 farmers were purposively selected to represent innovative, late adopters and those who are skeptical of agricultural technologies. The selected farmers were interviewed using structured questionnaires for their knowledge of coffee seed preparation, nursery and field management practices, coffee harvesting and processing, markets and marketing systems and some relevant information about the farming systems of the study sites. Besides, assessment of coffee production constraints in the selected PAs was made by questioning 15 selected farmers, and 15 wereda experts and DAs. The questionnaire data were supplemented by direct observation of the farming system of the study area through transect walk. It also visited the selected farmers' field in each PA and had an overview of the existing cropping system, vegetation cover and associated farm elements. Other quantitative secondary data were also collected from the Agricultural and Rural Development Office of the study wereda, the respective DAs in each PA, and Jima Research Center (JRC). The collected data were analyzed using Statistical Product and Service Solutions (SPSS) software.

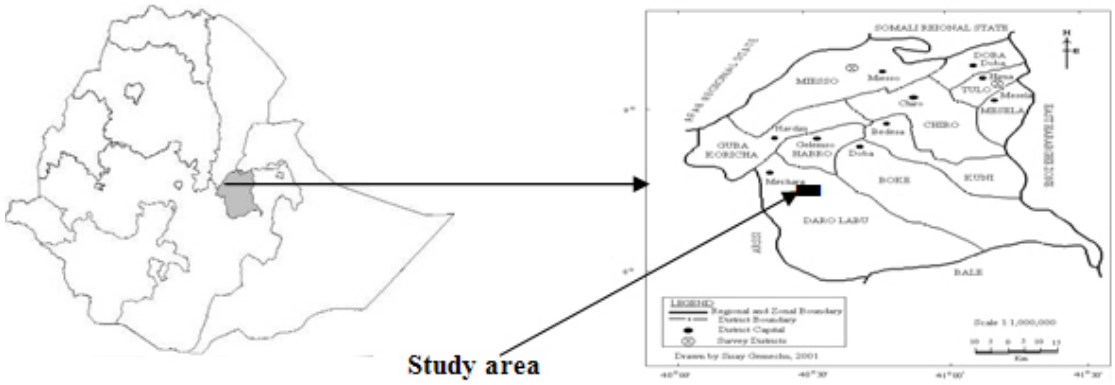

Figure 1. Map showing Darolebu wereda and study area in West Hararghe Zone. Source: Mashilla (2004).

RESULT

Seed preparation and nursery management

The majority of the respondent farmers raise their own coffee seedlings. They get coffee seeds either from the Agricultural and Rural Development Office or from their own seed orchard. Farmers practice two methods of seed preparation: **(1)** collect ripe red cherries from local

coffee types and dry their two seeds under shade or **(2)** remove the pulp from the collected cherries by hand and dry the parchment seed under shade usually in their backyards. Farmers keep the dried cherry and the parchment seed under room temperature on clean ground in well ventilated container until sowing.

Farmers do not produce polythene grown seedlings because unavailability and associated costs. The major reasons that limit farmers not to grow coffee seedlings using polythene are: **(1)** polythene is not available in the local market around the study area, **(2)** preparing the media and filling the pots with the media demands large amount of labor, and **(3)** polythene grown seedlings require frequent watering, which is not practical in the study area mainly due to unreliable amount and erratic distribution of the rainfall, and the inaccessibility of underground water, rivers and springs to many farmers.

Farmers raise seedlings in fine seed beds. First they prepare seedbed of about 1 m width and 5-10 m length by digging a trench of about 20 - 30 cm depth, and latter they incorporate the trench with mixture of plant debris (chopped plant leaves and young stems of *Erythrina*, and maize and sorghum stock), and topsoil. The seedbed is elevated on the trench with a mixture of fine soil and manure and/or compost with varying amount (10-12 kg per bed). In addition to this, when they prepare seedbed they also burry plant debris around or between the beds. The practice helps to supply moisture for the seedlings by releasing water slowly from the buried plant parts and it also increases the level of soil organic matter after decomposition.

Farmers practice two methods of seed sowing. They use either dried cherry with its seeds *jenfel* or parchment seed. The *jenfel* is sown in the nursery bed by drilling with no space between *jenfel* within rows and 15-20 cm between rows. Similarly, the parchment seeds are sown at a distance of 10 to 15 cm within and between rows to a depth of 2-3 cm with the grooved side of the seed down ward. In both methods sowing is accomplished in April.

After sowing, the nursery beds are heavily mulched with grasses or tree leaves or chopped maize or sorghum stover of 3-5 cm thick. At the same time, over head shade of 50-60 cm height above the ground are constructed and all sides of the bed are covered with heavy grass pads or plant stems and leaves to minimize evapotranspiration. Seeds are left under this condition with no supplementary watering till germination.

After germination, the mulch is removed from the germinated seeds. Seedlings are cultivated by breaking the soils to fine tilth to cover soil cracks, a practice known as soil mulch, which is also useful to conserve soil moisture. Seedlings are left without supplementary watering until field planting, unless sever plant wilting, which often occurs at a time of extreme dry weather. It takes 12 to 14 months until field transplanting takes place after sowing up. The planting operation is completed between April and July.

Farmers believe that seedlings obtained from such a dry nursery are tolerant to adverse situations and their subsequent field performance is superior to seedlings produced by the improved technologies.

Field management

Holing and on field planting

Before marking the land for holing and refilling, bench terraces are constructed on all kind of slopes following the contour of the land and a convenient square or rectangular individual ridge, locally known as *katara*, is built for coffee seedling (Figure 2).

A 1 m wide × 1 m deep or 1 m wide × 50 to 60 cm deep holes are dug regardless of the growth nature of coffee varieties. Holes are refilled layer by layer with young E*rythrina* stem or plants having fibrous stems, top soil and manure and/or compost with varying amount, mostly ranging between one and three shovels. The holes are refilled up to the surface of the soil but below ridges. Usually holing is done from February to April and refilling is done in April.

Unlike most coffee producing regions of the country that use recommended spacings, farmers in the area plant coffee at spacings of 2 to 3 m between plants and 3.5 to 4 m between rows (Figure 2). Planting is usually done 3 to 5 cm deeper than the normal nursery soil level of the seedling (collar level).

Planting coffee trees at wider spacing has the following advantages: **(1)** allow farmers to intercrop their coffee with perennial and/or annual crops and utilize resources in their limited holdings more intensively and efficiently, **(2)** avoids competition for moisture and nutrients, and **(3)** make weeding practice easy, and **(4)** allows aeration and hence reduce the incidence of diseases and insect pests.

After planting, coffee trees are left to grow free with one central vertical stem. Suckers are removed to strength the tree and to use their leaves for making a drink boiled with milk, locally called *Hoja* or served alone, *Kuti*.

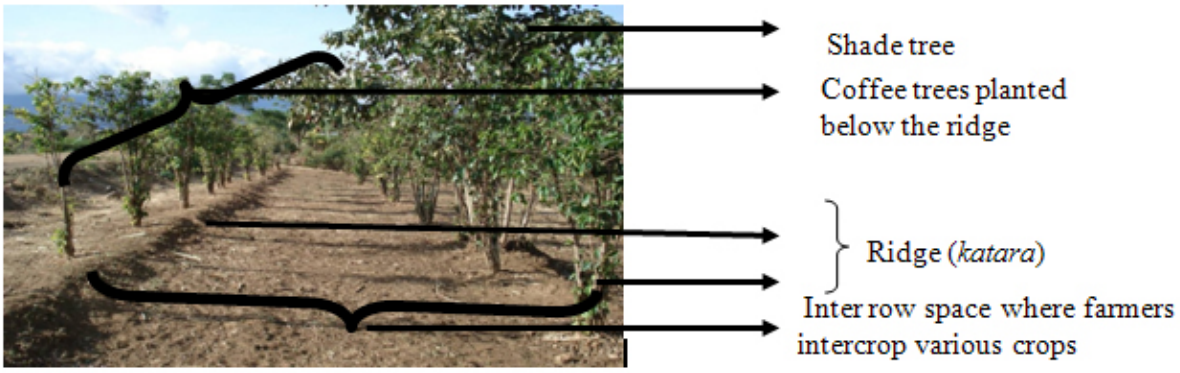

Figure 2. Coffee trees planted in 2.5 m × 4.0 m spacing at Serero, Darolebu wereda, West Hararghe Zone.

Shading

Farmers in Serero PA grow coffee without shade in an open field. Consequently, biennial bearing and overbearing die-back are common problems. Besides, the wide spacing between coffee trees and the absence of shade increases the risk of soil erosion. Although the interviewed farmers are aware of their contribution to coffee husbandery, they don't grow

shade trees with coffee. This is mainly because they believe that shade trees compete with coffee and other intercropped crops for the limited available soil moisture. Besides, shade trees occupy spaces which could have been used for growing other crops. On the other hand, farmers in Gudise PA plant coffee trees under naturally established shade trees. The most commonly used shade trees, in decreasing order of farmers preference are *Korch/Gorgo/ Wolensu* (*Erythrina burana*), *Gerbi-Adi and Gerbi-Guracha* (*Acacia* spp), and *Wanza* (*Cordia africana*). Farmers responded that they have chosen these shade trees on ability to boost coffee yield, ameliorate soil fertility and provide feed to livestock.

Stumping and pruning

Sixty-four respondent from Gudise and all respondents in Serero PAs practice stumping to change the cycle of unproductive old coffee trees. It is done in January after crop harvest. Old coffee trees are stumped at 0.40 to 1 m height above the ground. The number of bearing heads left on stumped coffee trees varies from 3 to 4. Farmers leave these verticals to grow free. Most farmers practice handling and desuckering and remove dead branches when the need arises. However, a few farmers remove dead branches while they harvest coffee cherry, usually from September to January.

Soil fertility management

The high cost, scarcity and timely unavailability of inorganic fertilizers and the absence of credit system force farmers not to apply mineral fertilizers to their coffee trees. Instead they depend more on organic fertilizer sources (farmyard manure and/or compost) to fertilize their coffee trees. Depending on its availability, farmers apply 1-10 kg of farm yard manure and/or compost per tree in April to June.

Some farmers believe that if a coffee field sufficiently manured once, it can nourish the plant for two to three consecutive years compared to commercial fertilizers, which need yearly application. In the absence of enough amounts of organic sources and abundance of competition from other crops intercropped with coffee, farmers manure their fields every year.

Cultivation and mulching

Farmers commonly practice digging and hoeing or harrowing using spade and hoe 2 to 3 times per annum in September, April and July depending on the growth of weeds, moisture conditions, labor availability and other practices like intercropping. This destroys weeds, facilitates water infiltration and aeration, reduce the adverse effect of soil cracking, enhance the capillary movement of soil moisture, and create catchments around each coffee tree for water harvesting during the rainy seasons.

Farmers use soil mulch by plowing the land to the tilth and cover it with soil dust at the end of the rainy season (usually in October) and beginning of the rainy month, mostly in May. Soil mulch is applied more frequently than organic mulch materials to prevent evaporation from the soil. This is mainly caused by shortage of organic mulch materials in the area. These techniques of long standing moisture conservation techniques indicate the traditional innovative and creative capability of the Hararghe farmers.

Soil and moisture conservation

The area is known for its short annual rainfall, which leads to moisture stress at some time during the growing periods. The problem is more pronounced when the rainfall comes late and stops early or at times of uneven distribution. Rugged topography of the area, which exposes the land to soil erosion, aggravates the problem. Farmers have adopted cultural practices aimed at reducing moisture loss through run off. Farmers employ a technique called *Katara* which is similar to ridging. They construct the ridge in April or June, if it is for the first time, and renew it in March or April. They also apply soil mulch in October and May whereby soil cracks are regularly covered with soil dust to reduce evaporation and conserve soil moisture. These traditional land preparation methods effectively conserve soil and moisture in the farm and around each individual coffee tree.

Weed control methods

Grassy and annual broad leaf weeds are the major weeds in the coffee fields (Table 1). *Cypres* spp, *Cynodon* spp and *Digitaria* spp are some of the grassy weeds which require much labor for control. In general, coffee fields are kept free of weeds throughout the year using the following cultural practices: **(1)** digging, hoeing or plowing between rows of coffee trees (if the field is to be intercropped) in March orApril, June and September, **(2)** dig out the weeds manually using a locally made tool known as *Dengora*, and **(3)** pulling out weeds by hand in inter and intera rows of coffee trees

Farmers weed their coffee farm by the above methods using family labor and/or *Guza*, community labor. However, slashing, which is the most common weed control method in other coffee growing areas, is not implemented in the study area. Similarly, farmers is not use herbicide because it is not affordable for them. Thus improved and affordable technology should be generated for effective control of weeds.

Table 1. Major weed species in coffee fields of the study area.

Scientific name	**Common name**	**Type of weed**
Parthenium hysterophorus	Congress weed	Broad leaf
Tagetus minute	Mexican marigold	" "
Amarantus spp.	Pigweed	" "
Striga spp.	Witch weed	" "
Commelina spp.	Water grass	" "
Guizotia scabra	Tufo (Afan Oromina)	" "
Bidens pilosa	Black jack	" "
Galinsoga parviflora	Gallant soldier	" "
Lanthana spp*	-	" "
Cynodon spp.	Bermuda grass	Grass
Eleusine indica	Wild finger millet	"
Cyprus spp	Nut sedges	"
Digitaria spp.	Couch grass	"

**Infest grassy land and around coffee farm*

Diseases and insects

Diseases

Coffee in the study area has been increasingly threatened by coffee beery disease (CBD) followed by coffee leaf rust (CLR) and branch die-back. CBD is sever in medium to high altitude, while CLR is problem in lower altitudes. During prolonged dry season, the coffee trees become more susceptible to rust. Farmers believe that the problem have been aggravated by the absence of locally screened coffee types resistant to the disease, and lack of improved cultural control method to CLR.

However, farmers have some indigenous knowledge and experience to control the diseases. This includes planting coffee seedlings in deeper and wider hole to attain well established trees capable of absorbing ground water so as to make the tree less susceptible to the diseases, planting coffee trees under shade to reduce transpiration and make them less stressed (not to be easily attacked by the diseases), application of farmyard manure and/or compost, frequent cleaning and burning of fallen leaves, fruits and other plant debris and screening of local coffee lines relatively resistant to the diseases are some of disease control measure practiced by farmers. Although the use of resistant land race cultivars is effectively control the diseases, it should be supported by research findings. The current land race development program run at Mechara by JRC and Mechara Research Center is inline with the farmers' effort.

Insects

Ants, termites, antestia, thrips, leaf miner and stem borer are the major pests attacking the crop. The coffee trees become more susceptible to the pest when there is a prolonged dry season during production years.

Coffee growers apply different traditional management practices, such as adding ash around coffee trees, flooding or burning or digging mound, and killing the larva manually by inserting stick into a hole burrow by insect, for the control of ant, termite and stem borer, respectively. But such traditional control methods may not be adequate for effective control. Local pest resisting materials control methods should be investigated by research so as to provide the farmers with efficient and economical pest control methods.

Harvesting and processing

Coffee trees flower from January to April. However, the main flowering occurs in April. Harvesting is mainly done in the months of October to December and rarely, early maturing local cultivars are harvested in September. Farmers collect red cherries from trees and those cherries fallen down by wind, animals and rain. They do not allow the cherries to dry while they are on the trees. The cherries collected from the trees and from the ground are sun dried separately on clean ground or a canvas lay on the ground. Farmers store the dried cherry, known as *jenfel or buni*, in jute sack (sisal bag) in their house until taken to the market. All respondents sell their produce only in *jenfel*.

Table 2. Principal farmers' problems and suggested potential solutions.

Major problems	Suggested potential solutions
1. Extended drought and moisture stress	Developing drought tolerant varieties, developing improved soil and moisture conservation technologies that effectively conserve moisture, screening early maturing crop varieties, developing crop varieties compatible to the rainfall pattern of the area, strengthen the existing soil and water conservation activities, launching afforestation program and promote farmers to adopt water harvesting technologies
2. Diseases (CBD, rust) and insects pests	Developing integrated pest management options that has long standing solutions for the control of the disease(s), screening and evaluating local land races for diseases and insect pests, and drought resistant and good quality, developing improved cultural and chemical control methods and screening natural enemies that effectively control the diseases
3. Shortage of arable land	Generate information on the right rate of fertilizers to be applied at sowing (planting) and subsequent periods, promote the inclusion of agro forestry practice into the farming system and assessing appropriate combinations of crop for double, multiple and relay cropping and intercropping
4. Overbearing die-back	Promote planting of recommended level of appropriate shade trees in coffee farm
5. Soil fertility degradation	Introduction of adaptable cover crops that improve soil fertility and serve as livestock feed, promote the use of organic mulch, selecting or adopting appropriate approaches of soil conservation techniques , identify multipurpose trees and shrubs suitable for the area to meet problems of soil fertility, erosion, feed and wood, and selecting or adopting appropriate approaches of soil conservation techniques
6. Absence of training	Education campaign through public media, use of local and public media to create awareness, training on management and post harvesting activities should be organized by the staff of Mechara Research Center in collaboration with concerned other research centers to farmers, DAs and expertise , dissemination of information using different mechanism, such as leaflets and posters, on coffee production, processing and marketing to farmers, DAs and experts, building research capacity at Mechara Research Center, establishing strong linkage with farmers' training centers (FTC) in different villages so as to raise the farmers' knowledge, attitude and practices of using improved technologies, establishing on farm and station demonstration fields and strengthening Research extension Advisory Council role
7. Labor shortage	Developing improved technologies profitable for more than one crop, using modern agricultural technologies like herbicide etc, screening efficient herbicides and screening and planting crop variety that mature at different time

Marketing

The coffee produce is sometimes sold by women in pieces on market days and is mainly meant for minor expense, but large quantities are sold by household head to coffee collectors or whole seller. If the coffee farmers are not in need of money, they store the dried cherry up to one year to attain better price.

Premium price is not paid for good quality harvest and drying procedure. This discourages farmers not to harvest and processed their coffee with great care. This signals that, if the trend continue unabated, it is very likely that the genetic basis of the typical Moka flavor of Harar coffee will be face irreversibly quality deterioration. Furthermore, farmers claimed that they could not get fair share that would cover the cost of production. As a result, most farmers tend to shift their labor and land from coffee to chat (*Catha edulis*). If this condition is not reverted, both the culture and coffee germplasm of the area will be lost in a short life span.

Production constraints

Farmers in the survey area have experienced several major problems caused by natural and social factors and/or policy related issues. However, they have their own strategies to overcome the problems. The major principal problems and suggested solutions are listed in Table 2.

CONCLUSION

Farmers in the study area relay on their indigenous traditional husbandry practices in crop production. Dry nursery seedling production practice should be studied in depth to investigate the scientific reasons of the success. Attempts should be made to investigate if dry nursery practice can be improved to produce large amount of healthy and strong seedlings with the introduction of some improved technologies.

Coffee pricing should give due emphasis to quality so that farmers would be encouraged to produce quality coffee. This will improve and maintain the quality, productivity and sustainability of coffee production in the area. To achieve this, interactive, more rigorous and timely intervention is needed by government as well as non governmental organizations.

Drought, unreliable and uneven rainfall distribution, diseases and insect pests, soil fertility degradation, shortage of arable land, shortage of labor, food and feed shortage, low price for coffee, lack of credit and saving, absence of training on coffee production and processing are some the principal production constraints in the study areas. Farmers' alleviate these problems and ensure sustainable food supply for their own family by using local varieties and husbandry practices. Research and development interventions focusing on these aspects should be done to complement the local varieties and farmers traditional knowledge.

ACKNOWLEDGMENTS

The authors are deeply indebted JARC for the financial support from European Union through Coffee Improvement Project (CIP) IV. Special thanks also go to the CIP IV Project Coordinator, Dr. Taye Kufa, who facilitates the necessary logistics and expenses for the survey work. The continuous support and advice of Dr. Wondyifraw Tefera throughout the study is highly acknowledged.

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Pre-Sowing Arabica Coffee Seed Management in Ethiopia: a Review

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SUMMARY

Pre-sowing seed management includes all operation involving seed collection, preparation and handling, and pre-germination seed treatment. With regard to this, research results show that seeds of red ripe cherries dried with intact parchment under shaded and ventilated condition showed enhanced germination. Coffee seeds with moisture content greater than 40% when stored in moisture vapor barrier containers, *viz.* glass jar and polythene bag had retained their viability and vigor for a longer period. However, sowing coffee seeds immediately after harvesting and processing was found to be the best option for higher germination rate and better seedling growth. Pre-germination of coffee seeds is the primary cause of multiple and crooked tap roots and eventual tree death in the field. Sowing clean coffee seeds after soaking in cold water for 24 hours hastened germination and seedling growth.

INTRODUCTION

Arabica coffee is the major commodity crop of Ethiopia contributing 38% of the total export earnings of the country. A quarter of the population of the country also depends directly or indirectly for its livelihood in the production, processing, transport and marketing of the crop (?). Despite the existence of enormous genetic diversity and its importance in the country's economy, productivity of the crop is very low (0.66 ton ha^{-1} green coffee) (Central Statistical Agency, CSA, 2006). Such low level of productivity of the crop stems from erroneous management of the plant during the initial stage of establishment in the field and the use of weak and whippy seedlings with undesirable shoot and root growth for field planting. This emanates mainly from poor seed preparation and handling, use of deteriorated seeds and growing media not suitable for germination and seedling growth, improper depth of seed sowing, and pre-germination practices (Wondyifraw, 1994; Tesfaye et al., 1998; Anteneh et al., 2008).

In view of this, several pre-sowing seed management practices have been tested aiming at improving the viability and germination of seeds and production of quality coffee seedlings in the country. These include seed preparation and handling, pre-sowing seed treatment and pre-germination practices. Thus, available research findings and techniques generated so far in the aforementioned areas in Ethiopia are summarized and discussed in this paper.

EXPERIMENTAL RESULTS

Seed preparation and handling

Stage of fruit maturity and seed drying

Stage of harvest of the cherries, the condition of processing and drying affect germination of coffee seeds. In line with this, results revealed that red ripe cherries are the best stage of maturity for seed purpose (Figure 1a). After pulping the cherries and removing the floaters, drying parchment intact seeds in a well aerated, cool and shaded condition till they attained the desired moisture level before sowing/planting or further storage ensured higher germination percentage (Figure 1b).

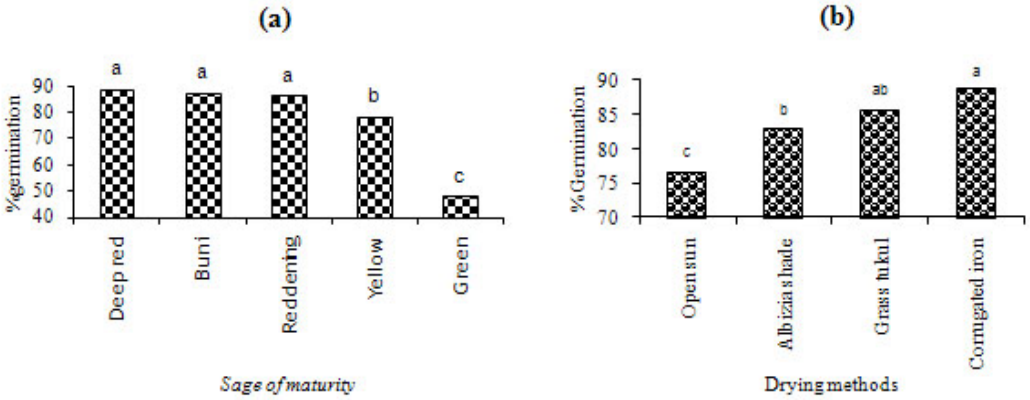

Figure 1. Germination of coffee seeds as effect by stage of fruit maturity (a) and drying condition (b). Bars capped with same letter(s) are not significantly different at 0.05 probability level. Source: Tesfaye (1998) and Anteneh et al. (2008).

Seed storage time

Studies revealed that coffee seed germination percentage (Figure 2), percentage of seedling emergence (%E) and seedlings attained first true leaves (%FTL) (Table 1) decreased gradually since the second month and rapidly after the third month of storage. Besides, mean days to germination (MDG) and FTL (MDFTL) consistently delayed with prolonged storage time (Table 1). Thus immediate sowing after harvesting and processing is always the best option for higher germination and subsequent growth (Wondyifraw, 1994; Tesfaye et al., 1998).

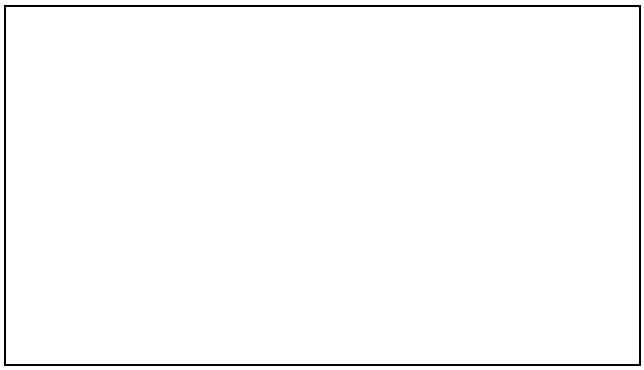

Figure 2. Effect of time of storage on germination of coffee seed. Source: Tesfaye et al. (1998).

Table 1. Effect of time of storage on coffee seed germination and subsequent growth performance of seedlings.

Storage time in month	%E	%FTL	MDG	MDFTL
0*	93.9a	88.4[a]	32.2[f]	94.2[f]
1	84.4b	76.2[b]	39.6[e]	99.4[e]
2	81.0c	76.9[b]	41.6[d]	105.4[d]
3	78.0d	69.2[c]	44.[7c]	109.5[c]
4	55.5e	51.7[d]	52.1[b]	114.2[b]
5	51.0f	43.6[e]	59.9[a]	116.3[a]

*Figures followed by same superscript letters within a column are not significantly different at 0.01 probability level. *The time just at the date of storage. Source: Wondyifraw (1994)*

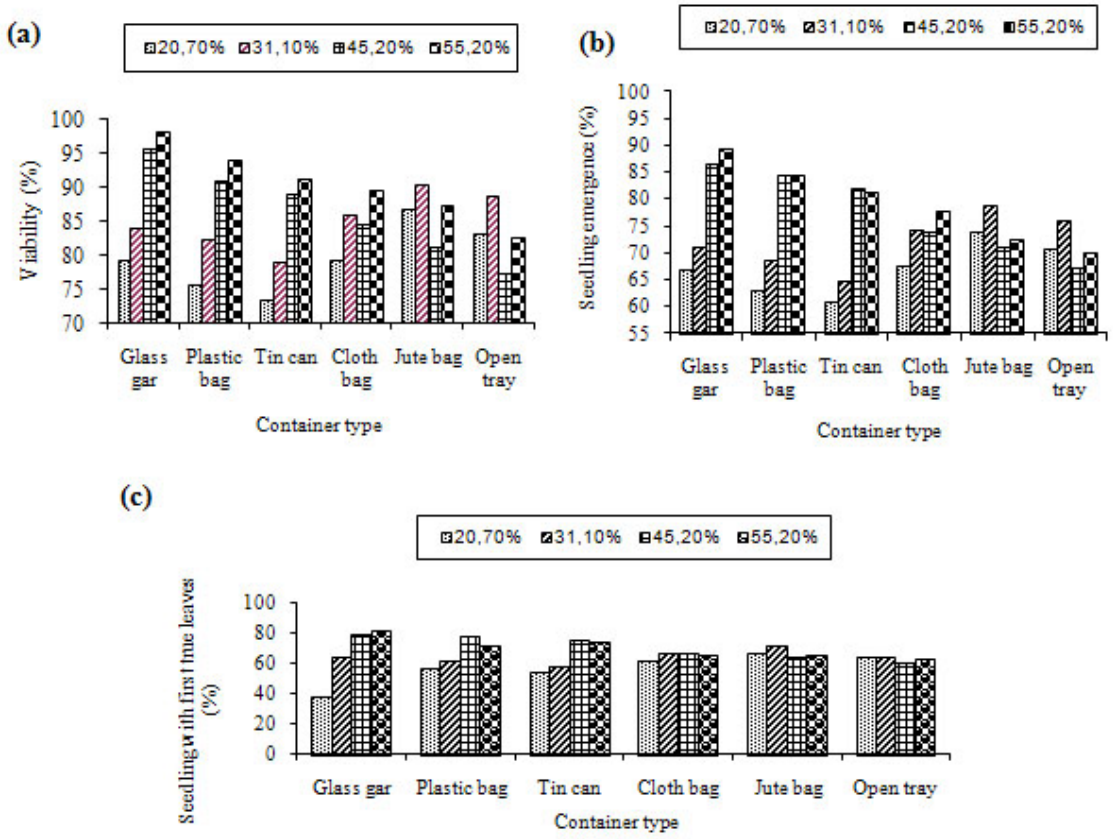

Figure 3. Effect of initial seed moisture level and type of container on percentage of seed viability (a), seedling emergence (b) and seedling attain first true leaves growth stage (c). Source: Wondyifraw (1994).

Seed moisture content and types of container for seed storage

A combination of high initial seed moisture level (not less than 40%) and moisture vapor barrier containers relatively better preserved coffee seed viability longer and improve growth of coffee seedlings. Accordingly, Wondyifraw (1994) reported a combination seeds with moisture content of 45.2% and glass jar resulted 97.5, 84.5 and 89.1% values for seed germination, seedling emergence and seedlings attain first true leaf stage, respectively, after

five months of storage. While seeds stored at 55.2% moisture content in plastic bag resulted 89.0, 82.0 and 86.3% values for the respective parameters (Figure 3 a, b and c).

Pre-sowing seed treatment

Pre-germination practices

Available reports showed that pre-germinated and planted seeds had resulted large percentage of seedlings with deformed roots, viz. multiple and crooked tap roots (MTR and CTR, respectively) than sowing in situ in permanent bed (direct sowing) (Table 2). The practice can also delay the growth of seedlings and thus large percentage of cotyledon and first pair of true leaves was initiated much earlier from direct sowing than pre-germination practice (Figure 4a and b). Hence, coffee seeds should be seeded directly in seedbeds or polythene tube for the production of seedlings with normal root system than following the pre-germination techniques.

Table 2. Effect of planting normal (not pre-germinated) and pre-germinated coffee seeds in conventional seedbed, and fine (sieved) and course (unsived) soils filled in polythene tube on percentage of MTR and CTR.

Treatments	Fine soil		Coarse soil		Conventional seedbed	
	MTR	CTR	MTR	CTR	MTR	CTR
PGS	31	66	32	70	20	66
DS	1	24	6	20	0	30
LSD $_{(0.05)}$	7.4	21.5	7.4	21.5	-	-
$_{(0.01)}$	10.1	29.5	10.11	29.5	-	-

PGS = Pre-germinated seed, DS = direct sowing (not pre-germinated seeds), MTR = Multiple tap root and CTR= Crooked tap root. Source: Bayetta and Mesfin (2005).

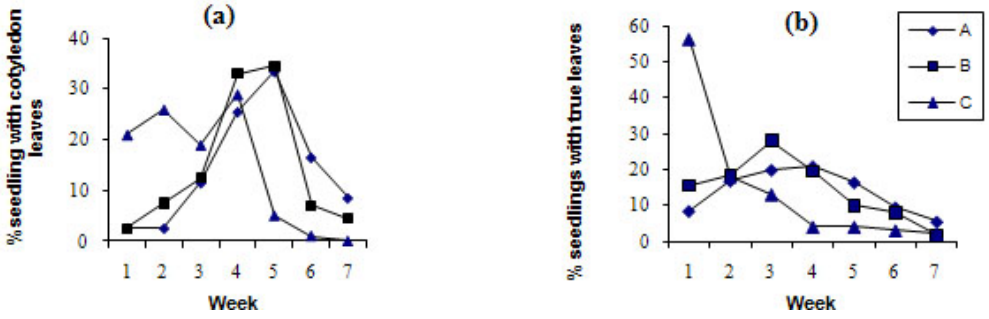

Figure 4. Weekly differences in the proportion of seedlings with cotyledon (a) and first pair of true leaves (b) for treatments A= Pre-germinated and planted in polythene tube, B = Pre-germinated and planted on conventional seedbed, and C= Direct sowing on seedbed. Source: Bayetta and Mesfin (2005).

Parchment removal and seed soaking

Sowing parchment removed coffee seeds had significantly promoted mean days to emergence as compared to parchment seeds (Figure 5). The practice could also enhance seedling growth (Table 3) and shortens the nursery period by about four weeks (data not presented) (Taye and Alemseged, 2007). Though the difference is not considerable, soaking coffee seeds in cold

pure water for 24 hours immediately before sowing had improved rate of emergence, particularly during the early stage after sowing (Figure 5), and produced vigorous seedlings than unsoked seeds (Table 3).

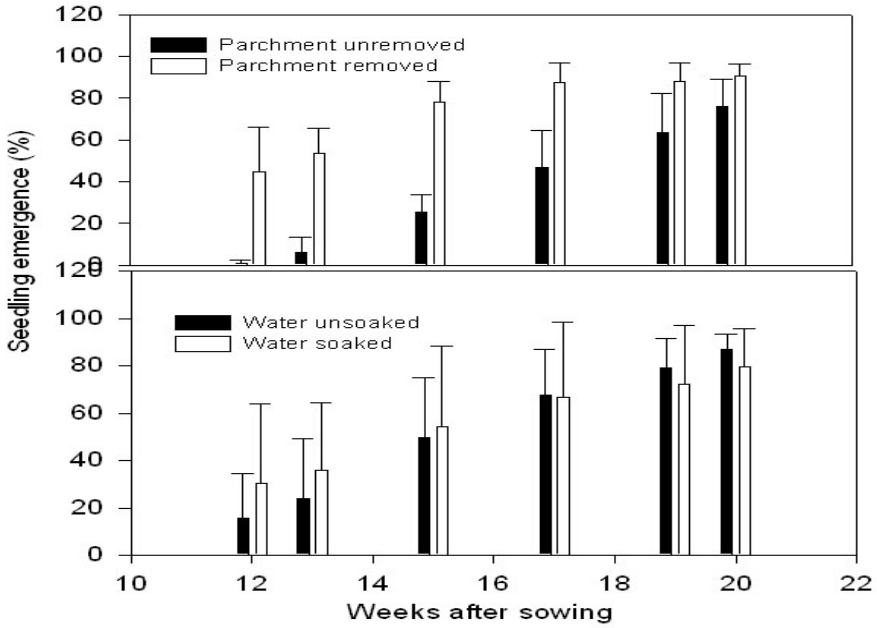

Figure 5. Effect of pre-sowing seed treatment on rate of seedling emergence of Arabica coffee seedlings. Source: Taye and Alemseged (2007).

Table 3. Growth parameters (means ± SD) of coffee seedlings as influenced by pre-sowing seed treatments.

Growth character	Parchment removal		Water soaking	
	Unremoved	Removed	Unsoaked	Soaked
Height (cm)	28.02 ± 6.16	28.77 ± 2.89	27.33 ± 3.24	29.46 ± 5.78
Stem diameter (cm)	0.46 ± 0.08	0.49 ± 0.05	0.46 ± 0.04	0.48 ± 0.08
No. of true leaf pair	7.00 ± 0.32	7.50 ± 0.89	7.00 ± 0.71	7.50 ± 0.63
Shoot dry matter (g)	2.88 ± 1.03	3.60 ± 0.86	3.15 ± 0.63	3.32 ± 1.30
Root dry matter (g)	0.70 ± 0.22	0.77 ± 0.16	0.72 ± 0.19	0.74 ± 0.20
Total dry matter (g)	3.58 ± 1.22	4.36 ± 1.01	3.88 ± 0.78	4.06 ± 1.50
RGR* (g month^{-1})	0.58 ± 0.26	0.74 ± 0.20	0.62 ± 0.21	0.70 ± 0.31

**RGR = Relative growth rate. Source: Taye and Alemseged (2007).*

CONCLUSION AND RECOMMENDATION

Coffee seeds to be used as a seed material should be prepared from cherries picked at red ripe stage. Then, after pulping the cherries and removing the floaters, seeds with their parchment intact should be dried under shade and ventilated conditions as these reduces the drying temperature, which otherwise can injure its germinability. Farmers who want to store coffee seed for sowing should store seeds having initial moisture content of >40% in well sealed moisture proof containers, depending on their availability, cost incurred, durability and

easiness for handling, under cool and dry condition. However, if condition forces to use pores or moisture-vapor permeable container, viz. cloth bags, fiber sacks, open tray, etc., the moisture content of the seed can be reduced to <32%.

Planting pre-germinated seeds should not be practiced by farmers as it result in large percentage of seedlings with malformed root system and eventual early (forth to fifth bearing) tree death in the field. Hence, coffee seeds should be seeded directly in seedbeds or polythene tube. However, if seed viability is doubtful, two seeds per hole should be seeded and then thinned to one plant. Furthermore, coffee seeds should be sown after removing the hard seed cove, parchment, and soaking the seeds in water for 24 hours as the practices enhance germination and seedling growth. These can also be shorten the nursery period and reduce the associated costs.

GAPS AND CHALLENGES

- Farmers' indigenous knowledge of coffee seed preparation and handling and nursery management has not been assessed;
- Research information on coffee seed pre-treatment to improve rate of germination, emergence and seedling growth is limited;
- Coffee seed research unit is lacking; AND
- Lack of awareness and knowledge regarding coffee seed preparation and sebesquient seedling handling.

FUTURE PROSPECTS

- Survey should be designed to collect information regarding farmers' indigenous knowledge about seed preparation and handling.
- Establishment of farmer's research group that prepared coffee seeds for sell to other farmers.
- Training should be given to farmers, development agents, subject matter specialists, private investors, state farms and cooperatives on techniques of coffee seed preparation and handling.
- Focus attention should be given to generate information on seed viability, purity percent, germination rate and other handling practices for each seed lot for farmers who will buy coffee seeds from Jima Agricultural Research Center.

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Early Agronomic Performance of Some New and Existing Arabica Coffee Varieties in Kenya

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SUMMARY

The primary goal of plant breeding is to improve yield, quality and disease resistance. However, majority of reported work on coffee breeding primarily concerns agronomical improvement that directly impinges on either coffee quality or yields. The main objective of this study was to compare the agronomic traits of new Arabica coffee varieties with existing commercial cultivars in Kenya. Field recording of cherry yield and disease infection was done during the cropping seasons of 2007 and 2008. Artificial inoculation for both Coffee Berry Disease (CBD) and Coffee Leaf Rust (CLR) were done in respective screening laboratories using Completely Randomized Design (CRD). Significant variations in yield and disease resistance were observed among the genotypes. There were significant negative correlations between disease scores (both CBD and CLR) and cherry yield.

INTRODUCTION

Coffee is an important export crop and a major foreign currency earner for Kenya (Gichimu and Omondi, 2010). In Kenya, the coffee industry has been the leading foreign exchange earner since independence. However, its performance has been on the decline as evident from the drop in coffee exports, coffee quality and yields (Condliffe et al., 2008). It has since been overtaken by other sub-sectors and now ranks fourth after tourism, tea, and horticulture. Currently, it is estimated that 170,000 ha of Kenyan land is under coffee and that the sector supports approximately five million Kenyans. The industry contributes about 8% of the country's foreign exchange earnings, a drop from a 40% contribution in the good years of 1980's (Coffee Board of Kenya, 2010).

Coffee production in Kenya is seriously constrained by diseases across the coffee growing areas especially the two fungal dieseases; Coffee Berry Disease (CBD) caused by *Colletotrichum kahawae* and Coffee Leaf Rust (CLR) caused by *Hemileia vastatrix* (Omondi et al., 2001). CBD mainly affects the berries. This is also the infection of highest economical importance, especially on green immature fruits, a stage in which it can cause up to 80% crop loss if not controlled and conditions are favourable (Masaba and Waller, 1992). On the other hand, CLR is a disease of foliage that causes premature leaf fall, yield loss and even death of the tree in severe cases. Disease control in susceptible coffee varieties is by intensive spray programmes that accounts for up to 30% of the total cost of production and is a major constraint to economic coffee production especially to the small-holders who find the use of pesticides beyond their financial and technical capabilities (McDowel and Wolffenden, 2003).

In view of the economics and to minimise the chemical input for disease management, the development and cultivation of tolerant cultivars is the most effective and viable option. A Kenyan Arabica coffee hybrid cultivar, Ruiru 11, developed by Coffee Research Foundation, Ruiru, Kenya, and released to growers in 1985, combines resistance to CBD and leaf rust with

high yield, fine quality and compact growth amenable to high density planting (Omondi et al., 2001). In addition, the Foundation has recently developed and released three true-breeding Arabica coffee varieties namely Batian 1, Batian 2 and Batian 3 that are also resistant to CBD and CLR. The main objective of this study was to compare the early agronomic traits of the new Arabica coffee varieties with existing commercial cultivars.

MATERIALS AND METHODS

Study site

The field trial was carried out at Tatu Estate of Socfinaf Co. Ltd. The site is located within the upper Midland 2 agro-ecological zone at latitude 1°05'S and longitude 36°54'E and is approximately 1623 m above the sea level. The area receives a bimodal mean annual rainfall of 1063 mm and mean annual temperature is 19 °C. The soils are classified as a complex of humic nitisols and plinthic ferrasols. The soil pH ranges between 5 and 6.

Experimental Materials and Layout

The test materials included three new true-breeding Arabica coffee varieties namely Batian 1, Batian 2 and Batian 3, which were evaluated alongside two commercial Arabica cultivars, SL28 and Ruiru 11. The site was laid out in a Randomized Complete Block Design (RCBD) with twenty trees per plot planted on a spacing of 2M x 1.5M and replicated three times. Field establishment was done in March/April 2005. Cherry yield for 2007 and 2008 was recorded in kilograms per tree from all the twenty trees per plot. CBD infection was recorded by counting the percentage number of infected berries from five tagged branches per tree and then calculating the mean infection per replicate. A similar method was applied for CLR where percentage leaf fall from five tagged branches per tree were used to calculate the mean percentage CLR infection per replicate.

Laboratory inoculation tests

Laboratory experiments to evaluate resistance to CBD and CLR through artificial inoculations were set up in Completely Randomized Design (CRD) with three replications. Evaluation for CBD was conducted through hypocotyl inoculation tests using the method of Van der Vossen et al. (1976). After three weeks, the seedlings were scored individually on a scale of 1 (no visible symptoms), to 12 (whole seedling dead). Evaluation for leaf rust resistance was carried out using leaf disk inoculation tests (Eskes, 1982). A scale of 1-100 was used to score the CLR infection based on percentage sporulation as follows: 0-20% = highly resistant; 21-40% = resistant; 41-60% = moderately resistant/susceptible; 61-80% = susceptible; and 81-100% = highly susceptible.

Data Analysis

The data was subjected to analysis of variance (ANOVA) using COSTAT software and effects declared significant at 5% level. A combined analysis of variance was performed on yield and disease data for both years. Least Significance Difference ($LSD_{5\%}$) was used to separate the means. Linear correlation and regression analysis was performed using SAS Version 9.1 and Ms Excel respectively to compare the relationship between disease resistance and cherry yield.

RESULTS AND DISCUSSION

The genotypes were evaluated for cherry yields over a two year period. The three new varieties recorded similar or higher mean cherry yields than the existing commercial cultivars. Batian 1 performed significantly ($p<0.05$) better than Batian 2 and Batian 3 whose yields were not significantly ($p>0.05$) different from SL28 which was the least yielding (Table 1). These results were in agreement with findings of similar work that was carried out in Western (Kitale) and Eastern (Meru) Kenya on the adaptation trials of the new varieties (Gichimu and Omondi, 2010).

Disease pressure was low under field conditions for both CBD and CLR. However, artificial inoculation with both *C. kahawae* and *H. vastatrix* pathogens demonstrated significant ($p<0.05$) differences among the genotypes. SL28 was therefore an escape in the field experiment and would have been mistaken as resistant if laboratory experiment was not done. Batian 1 was highly resistant (1-3) to CBD while SL28 was highly susceptible (10-12). The rest including Ruiru 11 were resistant (4-6). Resistance to CBD in Ruiru 11 and the new varieties is mainly controlled by the R gene from Rume Sudan and the T gene from Catimor either directly or introgressed through Hibrido de Timor (Gichimu and Omondi, 2010).

On the other hand, Ruiru 11 and the three new varieties were resistant to CLR while SL28 was susceptible (Table 1). The three new varieties (Batians) are selections from male parents of Ruiru 11 which are useful in imparting resistance to CLR into Ruiru 11. It is therefore expected that the Batians, are also resistant to CLR.

Table 1. Variation in yield and disease resistance.

Genotypes	Cherry Yield (Kg/tree)	FIELD DISEASE SCORES		LAB DISEASE SCORES	
		% CBD Score	% CLR Score	CBD Score	% CLR Score
Batian 1	2.96a	0.53b	1.02b	3.08c	3.33b
Batian 2	2.03b	0.72b	0.86bc	3.96b	8.67b
Batian 3	2.14b	0.70b	0.71cd	4.28b	6.00b
Ruiru 11	1.90b	0.54b	0.50d	4.15b	0.33b
SL28	1.71b	1.06a	2.36a	10.36a	92.33a
LSD (5%)	0.60	0.33	0.24	0.60	13.07
CV%	23.13	35.11	18.35	6.84	36.44

Combined mean cherry yields were more than three times higher in 2008 (3.38 kg/tree) than in 2007 (0.91 kg/tree). Gichimu and Omondi (2010) reported that for newly established coffee plants, when all the other variables are held constant, growth and yield characters are expected to increase almost exponentially up to a certain threshold above which the limits are determined by the management practices applied. Wamatu et al. (2003) also reported that for established coffee trees, biennial bearing phenomenon is common and coffee yields fluctuates from year to year. The level of CBD infection was not significantly ($p>0.05$) different over the two years but there was significantly ($p<0.05$) more CLR infection in 2008 than in 2007 (Table 2).

Table 2. Seasonal effects on berry yields and disease infection.

Year	Mean CherryYield (kg/tree)	Mean CBD Infection (%)	Mean CLR Infection (%)
2007	0.91[b]	0.73	0.02[b]
2008	3.38[a]	0.69	0.16[a]
LSD (5%)	0.38	NS	0.22
CV%	23.13	38.24	18.35

There was significant negative correlation between cherry yields and both CBD (-0.62) and CLR (-0.56) indicating that yields increased with increase in resistance to both diseases. Regression analysis also revealed negative relationship between cherry yields and both CBD and CLR infection (Figure 1). This was an indication of how major coffee diseases can lower coffee yields even in potentially high yielding but susceptible varieties. Gichimu and Omondi (2010) reported that production of resistant coffee cultivars is the most economical and sustainable control strategy.

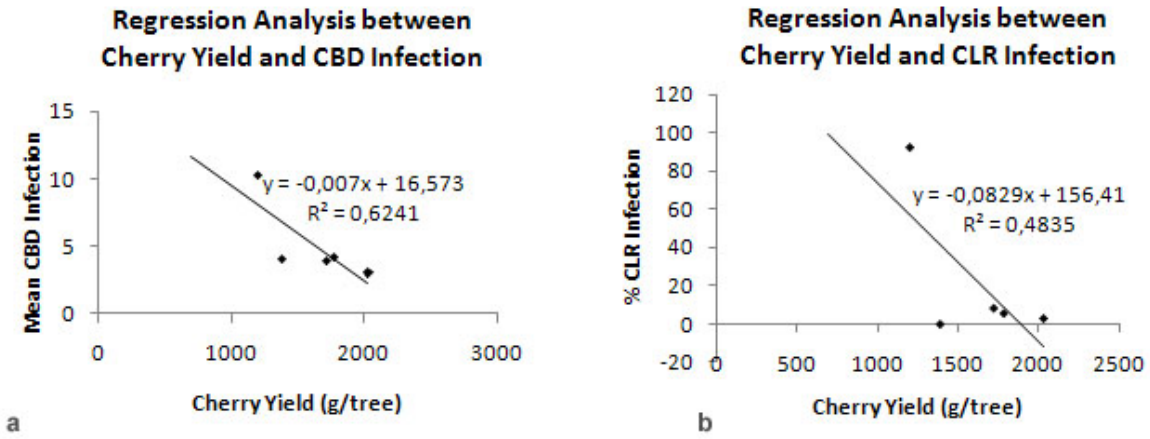

Figure 1. Regression analysis between cherry yields and (a) CBD infection; (b) CLR infection.

CONCLUSIONS

The study demonstrated that the three new varieties have a higher yield potential than the two existing commercial cultivars, Ruiru 11 and SL28. The lines also possess good resistance to CBD and leaf rust comparing well to the improved commercial cultivar, Ruiru 11. The study also revealed that growing resistant coffee varieties can contribute greatly in yield improvement especially where no disease control is applied

ACKNOWLEDGEMENT

This work was co-financed by Coffee Research Foundation (CRF) and the Coffee Leaf Rust Project funded by Common Fund for Commodities and supervised by International Coffee Organization. Thanks are due to the technical staff of both Breeding and Pathology sections who participated in the study. This work is published with the permission of the Director of Research, CRF, Kenya.

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Diversity of Shade Trees on Coffee Based Agroforestry System

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SUMMARY

Shade-grown coffee is an agricultural system that cointans some forest-like characteristics. However structure and diversity are poorly known in shade coffee systems. In 8 coffee-grown plots of Ngantang-Malang structural variables of shade vegetation and coffee yields were measured recording spesies and their use.

Coffee stands had four strata with mean density 3381 trees per hectare and at least there were three spesies on each plot. Diametric distribution resembles of a secondary forest. There is a significant role of shade-grown coffee as diversity refuge for woody plants and maybe associated fauna.

INTRODUCTION

Biodiversity is one aspect that lately become much attention for his role in maintaining the continuity of the ecosystem. Shade coffee plantations have very diverse biodiversity and capable of maintaining soil conditions, climate, and biology the same as natural conditions (Perfecto et al., 1996). However, many farmers manage coffee plantation with only one type of shade trees for protection. As a result, biodiversity is reduced and consequently erosion, and pest attacks increased.

Shade coffee plantations were important as habitat for flora and fauna (Perfecto et al., 1996) and has a complex structure (2) and represents an important biological reservoir for both flora and fauna (Bandeira et al., 2005; Perfecto et al., 1996). However, only a little information about the structural characteristics and plant diversity in coffee plantations that can describe the ideal compositionof vegetation and structure from the ecology and economics perception.

This study aimed to determine (1) whether the pattern of management of coffee plantations will affect the species composition, structure and diversity of the coffee plantation (2) the effect of coffee plantation management patterns of population structure of vegetation in coffee plantations, and (3) provide recommendations pattern coffee farm management best able to maintain environmental health.

MATERIALS AND METHODS

Research was done on coffee-based agroforestry at Ngadirejo and Tulungrejo- Ngantang Malang Regency. Geographically this site was located at 7°40 ˙ 8°00 ˙ LS and 5°30 - 5°40 BT. In this study, it was taken four shade coffee plots and four plots multistrata coffee. 1. Data Collection.

Vegetation sampling transects were taken at 10 x 20 m^2. In any plots were recorded type of vegetation, tree height, diameter (dbh) and canopy cover. Understorey was identified on 2 x 2 m^2 plot. All types of crops included in the plot were recorded either their types nor abundance.

Vegetation structure was measured by calculating the basal area, canopy percentage, and the abundance of trees and non-coffee coffee. Diversity and richness of species between shade coffee and coffee multistrata calculated and compared by t-test.

Shannon Diversity Index (H') was used to know diversity, and is calculated according to the formula:

$$H' = -\sum_{i=1}^{s} pi\ pi\ 2\ lg$$

- H' = Shannon-Wiever Diversity Index
- pi = proportion of i-th species abundance or the proportion between the number of individuals species to-i(ni) of the total number of individual species (N) so that pi= ni?N

RESULTS

Vegetation structure

Vegetation has a complex vertical profile both in shade coffee systems or coffee multistrata. Both of them was found three vertical strata (C, D, E). The lowest strata are dominated by shrubs with a height less than 1 m, while the three other strata are grouped into: 1-4 m, 4-20 m, 20-30 m; and taller than 30m. Each strata has a different species.

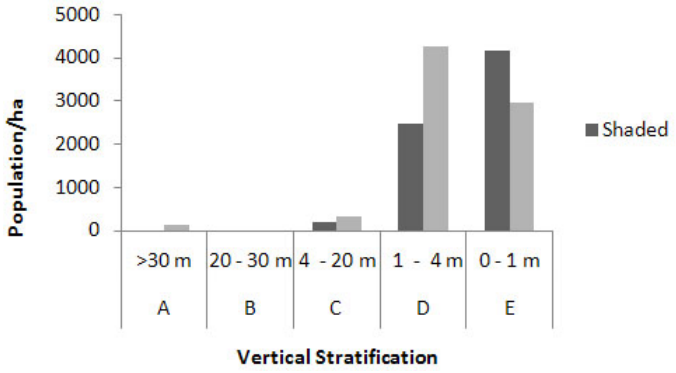

Figure 1. Average population per ha in each of vertical strata in the Coffee Garden Coffee Garden Refuge and multistrata.

Both systems, coffee and shade coffee multistrata vegetation is dominated by small size (2 <dbh <10 cm). Almost 90-95% sample of individuals in shade coffee systems and coffee multistrata has size 2 <dbh <10 cm, medium-sized 10 <dbh <20 cm in only 4-6% and on shade coffee may not find an individual who sized 20-30 cm.

Average abundance of trees was 2525 trees ha^{-1} (shade coffee) and 3937.5 ha^{-1} tree (coffee multistrata) with an abundance varies from 2250 to 5700 trees ha^{-1} Shade tree used (81-85%)

is a woody tree. No difference in the two systems both on the total basal area and canopy closure (t = 1.34 p <0.05). Average basal area is 7.77 m^2 ha^{-1} (shade coffee) and 17, 49 m^2 ha^{-1} (coffee multistrata) was 61.76% canopy cover (shade coffee) and 68.63% (coffee multistrata).

Table 1. Average structural variables and diversity and H 'on the Refuge and Coffee Coffee System multistrata.

Structure	
Density (population/ha)	2,13 ± 805,55
BA (m^2/ha)	1,76 ± 5,52
Canopy closure	1,34 ± 5,14
Diversity	
Species richness	4,74 ± 0,13
H'	4,75 ± 1.31

Vegetation Abundance

Coffee multistrata has 11 species and only three species was found in shade coffee. *Coffea* sp. as main crops and *Gliricidi*a sp. as shade trees has the highest Important Index Value; and bananas at the second order. High value of IIP illustrates their spread and dominance. Average species richness is 3 to 9 species per 200 m^2. Shannon diversity index in both systems were significantly different (t = 4.74, p <0.05) and also shown by different signicificantly numbers of species (t = 4.75, p <0.05).

Table 2. Characteristic vegetation on coffee based agroforestry.

Landuse	Tree Species	Important Value Index (%)	Population/ha	H'
Multistrata	*Gliricidia sepium*	68,88	2212,5	1,88
	Coffea canephora var.robusta	54,13	1587,5	2,06
	Persea americana	8,92	25	0,15
	Musa paradisiaca	21,68	212,5	0,80
	Erythrina subumbrans	9,22	37,5	0,19
	Durio zibethinus	9,51	50	0,22
	Anthocephalus cadamba Mlq	4,76	25	0,10
	Sapindus rarak	4,46	12,5	0,06
	Mangifera indica	4,76	25	0,10
	Parkia speciosa Hassk	8,92	25	0,14
	Leucaena leucocephala	4,76	25	0,19
Shade coffee	*Gliricidia sepium*	**95,43**	**1287,5**	1,91
	Coffea canephora var.robusta	91,47	1187,5	1,94
	Albizzia falcataria	13,09	0	0,26

Plants under the shade coffee system was 33 families where in the coffee multistrata 26 families. Families which are common are Caryophyllaceae, Amaranthaceae, Asteraceae, and Commelinaceae. Species that dominate the two systems are *Ageratum conyzoides, Panicum* sp*., Achyranthes aspera, Synedrella* sp., *Synedrella nodiflora, Dendroenide, and Eupatorium riparium*.

DISCUSSION

Vegetation composition and diversity

Coffee management, will affect the composition and diversity of vegetation. Multistrata coffee has a higher species diversity (9 species), due to farmers' efforts to increase their income, so the dominance of *Gliricidia* sp. as shade trees was replaced with tree species having economic value, such as avocado and durian.

There are 2 types of coffe plantation in our site, ie, shade coffee plantations with *Gliricidia sp*. and shade coffee plantations with a mixture of *Gliricidia* sp., timber trees and fruit trees. This condition is different from the coffee area at Sumberjaya Lampung, which has a coffee plantation shade with three groups, namely, (1) shade coffee farm with fruit trees, (2) shade coffee plantations with woody trees, and (3) coffee plantations with *Erythrina subumbrans* or *Gliricidia* sp. (Budidarsono and Wijaya, 2004).

Shannon diversity index is very sensitive to see the dominance of a species (Bone et al., 1997), this means that the low diversity in shade coffee systems associated with high population of individuals *C. arabica* and *Gliricidia* sp. Index diversity is said low when smaller than 2, medium (2-4), and high (> 4) (Barbour et al., 1987).

Species richness are also changing due to management changes where the diversity has increased from 3 to 9 species. Understorey diversity is reduced from 64 species (shade coffee) to 55 species (coffee multistrata). This occurs because the reduction of light entering the bottom so the plant ability to compete taken the light is diminished.

Vegetation tructure

Diameter size distribution (dbh) are often used to show the vegetation structure of a land use (Khan et al., 1987). Diameter size distribution patterns show that stands in plot area largely consists of species with a relatively young age, although still found larger diameter on multistrata coffee. Coffee abundance has declined with the increasing population of non coffee species, either timber trees nor fruit trees.

Vegetation structure in the coffee multistrata is more complex due to various types of vegetation with different strata. Vegetation height was less than 20 m, except in the coffee multistrata there were height vegetation more than 30 m. Therefore, stratification type is C, although coffee multistrata has type A. Both systems may not find the B type. This indicates that the system multistrata coffee has more to resemble the structure of forest vegetation due to the multi-layered canopy.

Coffee structure

Diameter classes of the coffee plant on both systems was similir. Most of the coffee plant has a height between 1.5-2 m (88-90%), and only a small portion which has a height> 2.5 m (1-

4%). Diameter class 2 <dbh <10 cm was 46% (coffee multistrata) and 34% (shade coffee). Similar diameter distribution for both of landuse type occur because: (1) Coffee management intensity (frequency of weeding), and (2) land use; are relatively similar. Population density on multistrata coffee was higher, so there is competition nutrients uptake finally inhibit plant growth. Coffee is still young too, so that farmers are still concentrate on new plants rather than maintain the population structure of coffee.

Coffee sp. on multistrata system was lower (34%) than shade coffee (45%), and the Gliricidia sp. As shade trees was higher (50%) compared to coffee as a main crop (33-44%). This is due to farmers perception that the coffee takes time to grow, so they diversify with more economical crops such as timber and fruit crops.

CONCLUSION

Multistrata coffee and shade coffee has a relatively similar vegetation structure, but not so with the abundance and composition of stands and lower plants. Coffee multistrata more diverse species, although the basal area and canopy closure did not differ because of young age. Therefore, in the long term management plan should be a balanced composition between the percentage of the population and the necessary shade to coffee production is maintained.

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Effect of Higher Density Planting on Coffee Establishment and Growth in Nigeria

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SUMMARY

A field study was carried out at the Cocoa Research Institute of Nigeria (CRIN), Uhonmora Sub-station, Edo State to determine the effect of higher density planting on establishment and growth of *Coffea canephora* Pierre ex Froehner between 2007 and 2009. The treatments evaluated were four planting densities of 1.5m x 1.5m (4,444 stands/ha), 2.0 m x 2.0 m (2,500 stands/ha), 2.5 m x 2.5 m (1,600 stands/ha) and the control of 3.0 m x 3.0 m (1,111 stands/ha). The experiment was Randomized Complete Block Design (RCBD). The treatments were replicated three times. Morphological data of height, girth and leaf area were taken at monthly interval. Survival count was taken in the first dry season after field establishment. The data collected were analysed using ANOVA and LSD was used to separate the means that were significant. Overall results on growth parameter of height, girth and leaf area showed that higher density of 4,444 stands/ha (1.5 m x 1.5 m) and 2,500 stands/ha (2.0 m x 2.0 m) were superior to other treatments. The plant survival rate was above 95% for all the treatments. These results indicate possibility of maximisation of available land by coffee farmers and possibly higher yield, returns from coffee farms.

INTRODUCTION

The present planting distance of 3.1 m apart with plant population of 1040 per hectare adopted by coffee farmers in Nigeria may not be the optimum spacing for highest coffee berry yield. Each country has adopted a certain spacing which has become more or less traditional spacing of 3.0 m x 3.0 m (1111 trees/ha), 3.0 x 2.5 m (1330 trees/ha), 3.0 x 1.7m (1960 trees/ha) and 2.5 X 2.0 m (2000 trees/ha) have been used for coffee Canephora in some places. For coffee Arabica 3.0 x 3.0 (1111 trees/ha) was used as former East African standard, while 2.74 m x 2.74 m (1329 trees/ha) and 2.74 x 1.37 m (2658 trees/ha) respectively are recently common East African standard, Rene (1992). The density of planting also varies considerably depending on the pruning system that is adopted. It is important to have 5000-7000 stems with productive branches per hectare in order to improve productivity and production at early stage which could not be obtained in a plant population of less than 2000 trees per hectare (Rene 1992). A result of high yield with close spacing has been reported at Colombia for cocoa. Other beverage crop like coffee planted at 2500 per hectare that yielded 1905 kg dry beans per hectare (Gutierrez 1981).

Also, a yield of 5000 kg dry beans per hectare was reported for same crop at Tawau in Sarah Malaysia due to high density planting (Wintgens 1991). Presently, there are competitions on land for housing and industrial development thereby reducing the land area for coffee production in Nigeria. There is need therefore to optimally maximise the utilization of the available land for coffee production. Therefore, this study was initiated in 2007 to determine the optimum density for establishment and growth of coffee in Nigeria.

Table 1. Plant height (cm) of coffee 36 months after transplanting.

	Months after transplanting											
***Treatment**	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**27**	**30**	**33**	**36**
1.5 m apart	41.0	51.7	56.6	64.5	73.8	82.1	91.4	105.6	114.3	125.0	137.1	149.5
2.0 m "	41.4	50.8	57.8	61.7	68.4	75.3	83.5	94.1	102.8	111.5	121.6	132.3
2.5 m "	40.5	51.6	55.9	60.2	66.5	72.2	78.7	89.3	97.4	104.6	112.3	119.7
3.0 m "	40.9	54.5	56.4	60.4	66.9	73.1	79.5	90.4	97.6	105.1	113.2	120.0
Mean	41.0	52.2	56.7	62.0	68.9	75.7	83.3	94.9	103.0	111.6	112.1	130.40
LSD (P=0.05)	0.51	2.32	1.11	3.39	4.63	6.20	8.00	10.30	10.91	13.10	15.83	19.34

** Legend.*

Table 2. Stem diameter (cm) of coffee 36 months after transplanting.

	Months after transplanting											
***Treatment**	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**27**	**30**	**33**	**36**
1.5 m apart	0.93	1.01	1.13	1.48	1.53	1.59	1.64	1.68	1.73	1.76	1.78	1.81
2.0 m "	0.94	0.97	1.08	1.41	1.44	1.48	1.53	1.56	1.60	1.63	1.64	1.66
2.5 m "	0.72	0.83	0.91	1.06	1.10	1.13	1.17	1.20	1.24	1.26	1.27	1.29
3.0 m "	0.79	0.85	1.02	1.22	1.25	1.28	1.32	1.34	1.38	1.40	1.41	1.43
Mean	0.85	0.91	1.04	1.29	1.33	1.37	1.41	1.44	1.49	1.51	1.52	1.55
LSD (P=0.05)	0.50	0.12	0.13	0.26	0.26	0.28	0.29	0.30	0.30	0.31	0.31	0.32

** Legend.*

Table 3. Leaf area (cm^2) coffee 36 months after transplanting.

	Months after transplanting											
***Treatment**	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**27**	**30**	**33**	**36**
1.5 m apart	0.93	1.01	1.13	1.48	1.53	1.59	1.64	1.68	1.73	1.76	1.78	1.81
2.0 m "	0.94	0.97	1.08	1.41	1.44	1.48	1.543	24	1.60	1.63	1.64	1.66
2.5 m "	0.72	0.83	0.91	1.06	1.10	1.13	1.17	1.20	1.24	1.26	1.27	1.29
3.0 m "	0.79	0.85	1.02	1.22	1.25	1.28	1.32	1.34	1.38	1.40	1.41	1.43
Mean	0.85	0.1	1.04	1.29	1.33	1.37	1.41	1.44	1.49	1.51	1.52	1.55
LSD (P=0.05)	0.50	0.12	0.13	0.26	0.26	0.28	0.29	0.30	0.30	0.31	0.31	0.32

**Legend: 1.5 m apart = planting distance 1.5 m x 1.5 m; 2.0 m apart = planting distance 2.0 m x 2.0 m; 2.5 m apart = planting distance 2.5 m x 2.5 m; 3.0 m apart = planting distance 3.0 m x 3.0 m*

MATERIALS AND METHODS

The experiment was carried out at Cocoa Research Institute of Nigeria (CRIN), Uhonmora Substation, Edo State-a derived savannah zone, on latitude 6°50'N and longitude 5°50 E with an altitude of about 140 m above sea level. The coffee seedlings were obtained from the substation nursery.

The experimental design was a randomized complete block with four treatments in three replicates. These treatments were; spacing of 1.5 m x 1.5 m (4444 plants/ha), 2.0 m x 2.0 m (2500 plants/ha), 2.5 m x 2.5 m (1600 plants/ha) and the control 3.0 m x 3.0 m (1111 plants/ha). Parameters considered on monthly basis were plant height, stem diameter, leaf area and canopy spread. Survival count at the first dry season of field establishment. Data collected were subjected to statistical analysis of Variance (ANOVA) and LSD was used to separate the means that were significant.

RESULTS AND DISCUSSION

The result of survival count carried out the first dry season after transplanting gave a survival rate of over 95% in all the treatments without any significance different between them. Table 1-3 showed results obtained on the morphological parameters of all the treatments. There were no significance different observed in plant height, plant girth and leaf area during the first six months of field establishment. However, the 1.5 m x 1.5 m spacing was significantly higher ($P< 0.05$) in plant height than other treatment. It was closely followed by 2.0 m x 2.0 m spacing. There were no significance difference between 2.5 m x 2.5 m and 3.0 m x 3.0 m spacings. The same trend was observed in stem diameter except 3.0 m x 3.0 m spacing that was higher than 2.5 m spacing in all the months without any significance difference between them. On the leaf area, the 1.5 m x 1.5 m spacing was highest and significantly higher ($P< 0.05$) than 3.0 m x 3.0 m spacing but not significantly higher than 2.0 m and 2.5 m spacings in almost all the months.

These morphological results of better growth in closer spacing than wider spacing might have been due to optimum utilisation of soil nutrient status with more plant population than scattered plant population with the spacing occupied by weed that compete with the coffee for nutrients. This agreed with the recent common farming practices adopted in East Africa for closer spacing of 2.74m x 1.37 m (2658 trees/ha) to traditional spacing of 3.0 m x 3.0 m (1111 trees/ha) (Rene, 1992). The result was also in agreement with earlier report of good performance in cocoa, a beverage crop like coffee planted at higher density of 5000 trees/ha in Malaysia (Wintgens, 1991). Also, for cocoa in Colombia, the closer spacing with plant population of 2500 trees/ha have been found to give better performance than wider spacing as reported by Gutierrez (1981). Therefore, the closer spacings of 1.5 m x 1.5m and 2.0 m x 2.0m that gave better performance than wider spacings could be recommended to coffee farmer in Nigeria for higher berry yield which should be supported by regular pruning practices when they are matured in the field.

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Evaluation of Coffee Intercropped With Rice and Plantain at Early Stage of Field Establishment in Nigeria

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SUMMARY

An intercropping experiment involving coffee (sole), coffee/rice, coffee/plantain and coffee/rice/plantain was carried out between 2007 and 2008 at the Cocoa Research Institute of Nigeria (CRIN) Uhonmora Substation, Edo State situated in a derived Guinea Savanna agro-ecological zone of Nigeria. The experiment was a Randomized Complete Block Design (RCB) with above mentioned treatment and replicated three times. The spacing used for coffee and plantain was 3m apart respectively while rice was sown 30 cm apart. Morphological parameters on plant height, girth, leaf area and canopy score were taken on coffee monthly while the survival count were taken after two months of field establishment. Yields of the component crops were also collected at maturity. Data collected were subjected to statistical analysis of variance and LSD used to separate the means that were significant.

Result obtained showed 98% survival without any significant different between the treatments. On vegetative growth coffee/rice and coffee/plantain was significantly higher ($p<0.05$) than coffee sole and coffee/rice/plantain in plant girth and leaf area but not significantly higher in all the months. Plant height however did not follow the same trend as height in coffee sole was slightly higher than coffee/rice. However, the difference was not significant. But coffee/plantain was still significantly higher ($P<0.05$) than coffee/rice/ plantain. The least was recorded in coffee/rice/plantain intercrop.

Grain and bunch yields from rice and plantain respectively in the intercrops compare favourable well to what obtain from coffee sole. From the result obtained, it could be concluded that there was no deleterious effect on growth when rice and plantain were intercropped with coffee. Therefore coffee/rice, and plantain/rice intercropped with better performance could be recommended to coffee farmers in Nigeria rather than sole planting of coffee.

INTRODUCTION

Farming system involves harnessing resources as dictated by the environment. Monoculture is alien to Nigerian agriculture. Multiple cropping systems is the common practice among the farmers so as to benefit from the advantages derivable from the practices which according to Herrera and Hardwood (1973); Okigbo and Greenland (1975), include higher yield returns, insurance against crop failure, more efficient use of available nutrients, improved environmental factors, reduction in weed pests and diseases incidence, and efficient use of labour.

Intercropping is the practice of planting two or more crops simultaneously on the same piece of land and is mostly practiced among the various forms of multiple cropping. Intercropping had been reported to increase crop diversity, biological stability of the ecosystem and labour efficiency, Okigbo (1977). Oil palm, coffee, cocoa and kola have been successfully intercropped with their tree and arable crops (Ofoli and Lucas, 1988; Okpala, Jose and Lucas, 1989 and Famaye, 2005). Intercropping in coffee that was carried out to provide foods and incomes to farmers in Nigeria is usually carried out at early stage of field establishment before they close canopy and during rehabilitation of the old farms (Famaye, 2000). Suitable intercropping system have been achieved in coffee with arable crops like cassava, maize, cocoyam, sweet potato, okra and pepper (Famaye, 2003, 2005 and Okelana, 1982) but none yet on rice. Despite the various advantages derivable from rice as one of the choice staple food eaten by all and sundry in Nigeria, efforts have not been taken to intercrop it with coffee. Rice had been reported to be successfully grown as cover crop in young coffee, cacao, citrus and rubber trees in Japan, Brazil, Cote'Divore and Thailand (CTA 1993). Following the world food crisis in which Nigeria experienced high cost in rice as a result of short supply from exporting nations few years ago, it now becomes imperative for coffee farmers to intercrop with rice. Apart from food, they will equally get adequate income from rice at the early stage of field establishment when coffee has not started bearing berry to improve their livelihood. Therefore, the objective of this study is to evaluate the performance of coffee when intercropped with rice and plantain at early stage of field establishment.

MATERIALS AND METHODS

This study was carried out in Cocoa Research Institute of Nigeria (CRIN), Uhonmora Substation 6°50' N, 5°50' E) (utilsol) with altitude of about 140M above sea level in the year 2007 and 2008. Uhonmora is located in the derived savanna zone of Nigeria. The ripe berries of *Coffea canephora* (robusta coffee) was obtained from the substation's plot and the coffee seedlings were raised in the nursery. Plantain suckers were also obtained from one of the station's plot. While local rice variety was obtained from Ekpoma market, a community noted for Ekpoma local rice production in Edo State, Nigeria.

Land preparation was done and all trash and trees were removed. The experimental design was randomized complete block (RCBD) with four treatments comprising of coffee sole, coffee/rice, coffee/plantain and coffee/rice/plantain replicated three times. Parameters considered were physical and chemical properties of the soil at the beginning of the experiment, survival count, as well as plant height, stem diameter, and leaf area. Yield of rice (grain) and plantain (bunch) were also collected. The data collected were subjected to statistical analysis of variance and LSD was used to separate the means that were significant.

RESULTS AND DISCUSSION

The result obtained on the physical and chemical properties of the study location is shown in Table 1. The soil pH of 5.3 was adequate for coffee as earlier reported (Sylvian, 1958). On survival count carried out after the first dry season of transplanting over 98% was obtained in all the treatments without any significant difference ($P < 0.05$) between them. Tables 2, 3 and 4 show the plant height, stem diameter and leaf area of coffee in the different intercrops. The plant height was higher in coffee/plantain closely followed by coffee sole than other treatments with the least recorded in coffee/rice/plantain. On plant diameter and leaf area, the highest was coffee/plantain followed by coffee/rice which was a change in trend compared to the plant height of coffee sole. There was no significant different between coffee/rice and coffee/plantain intercrops. However, there were significant different ($P < 0.05$) between them

and coffee/rice/plantain. The good growth performance recorded in these two intercrops indicated that there were no deleterious effects of intercropping coffee with rice and plantain at early stage of field establishment. This agreed with earlier work reported on beneficial effect of intercropping some food crops with coffee, (Famaye, 2000, 2003 and 2005 and Okelana, 1982), cocoa, oil palm and kola (Adenikinju, 1980, 1986, Ofoli and Lucas, 1988; Okpala Jose and Lucas, 1989). In Thailand, Japan, Brazil and Cote 'Divore, rice have been successfully grown as cover crop with young coffee, cocoa, citrus and rubber trees (CTA, 1993).

The average yield of rice (grain) and plantain (bunch) is shown in Table 5. The yield obtained in the intercrops were as high as their sole crops. This affirm beneficial effect on food production other than better morphological growth recorded due to intercrops as earlier reported as advantage in intercropping (Herrera and Harwood 1973, Okigbo and Greenbad, 1975 and Okigbo, 1977). Therefore, coffee/rice and coffee/plantain intercrops which are of good plant growth and food production that reduce problem on food crisis could be recommended to coffee farmers at juvenile stage in Nigeria to boost rice production instead of sole planting.

Table 1. Soil physical and chemical properties of the Experimental site at the beginning of the experiment.

Soil Properties	**Uhonmora**
pH (H_20)	5.3
% Organic carbon	0.83
% Total Nitrogen	0.08
Available P (mg/kg) soil	7.16
Exchangeable K (c mol/kg) soil	0.06
Exchangeable Ca (c mol/kg) soil	2.70
Exchangeable Mg (c mol/kg) soil	0.05
Exchangeable Na (c mol/kg) soil	0.02
% Sand	78.6
% Silt	9.3
% Clay	12.1
Soil classification	Ultisol

Table 2. Mean plant height (cm) of coffee intercropped with rice and plantain, months after transplanting (Sept. '07 – Sept. 2008).

Treatment	S	O	N	D	J	F	M	A	M	J	J	A	S
Coffee sole	24.8	28.4	36.4	39.4	44.3	49.4	50.2	53.1	55.2	56.6	58.3	60.9	62.5
Coffee/rice	20.6	26.9	32.6	36.5	40.7	45.3	47.9	50.8	52.0	54.7	57.9	60.7	63.6
Coffee/Plantain	24.5	27.6	36.2	39.7	45.9	50.5	52.5	57.3	60.7	62.9	65.4	67.5	69.8
Coffee/rice/plantain	19.6	24.9	30.2	32.8	34.7	35.4	36.9	38.8	41.7	42.4	43.6	43.9	44.5
Mean	22.4	27.0	33.6	37.1	41.4	45.2	46.9	50	52.4	1.15	56.3	58.3	60.1
LSD(P=0.05)	3.67	2.06	3.83	4.42	6.84	9.47	9.52	10.96	11.00	11.82	12.59	13.88	15.0

Table 3. Mean plant diameter (cm) of coffee intercropped with rice and plantain, months after transplanting (Sept. '07 – Sept. '08).

Treatment	S	O	N	D	J	F	M	A	M	J	J	A	S
Coffee sole	0.55	0.67	0.77	0.85	0.93	0.97	0.99	1.04	1.08	1.1	1.1	1.2	1.2
Coffee/rice	0.48	0.59	0.72	0.83	0.91	0.99	1.05	1.09	1.12	1.19	1.20	1.23	1.28
Coffee/Plantain	0.50	0.63	0.83	0.90	1.05	1.09	1.14	1.18	1.23	1.29	1.36	1.40	1.51
Coffee/rice/plantain	0.42	0.58	0.63	0.77	0.80	0.84	0.89	0.92	0.97	1.01	1.03	1.06	1.10
Mean	0.49	0.62	0.74	0.84	0.92	0.97	1.02	1.06	11	1.15	1.10	1.23	1.28
LSD(P=0.05)	0.74	0.05	0.12	0.07	0.14	0.14	0.14	0.15	0.15	0.16	0.19	0.19	0.23

Table 4. Mean leaf area (cm) of coffee intercropped with rice plantain, months after transplanting (Sept '07 – Sept. 2008).

Treatment	S	O	N	D	J	F	M	A	M	J
Coffee sole	121.52	129.30	139.33	165.33	191.00	197.33	202.00	209.20	213.31	217.16
Coffee/rice	120.88	130.42	143.67	187.67	193.67	205.67	209.01	212.01	219.67	225.42
Coffee/Plantain	126.23	133.65	149.00	198.00	210.33	221.67	229.34	235.35	243.65	248.93
Coffee/rice/plantain	122.23	126.35	131.67	257.67	274.67	287.33	192.0	196.46	198.00	201.20
Mean	122.71	129.93	140.92	177.17	192.42	203	208.09	213.34	218.66	223.18
LSD(P=0.05)	3.32	4.16	10.09	25.96	20.12	20.02	21.76	22.32	26.15	27.41

Table 5. Average yield of rice and plantain in t/ha on intercrops with coffee.

Treatment	Yield t/ha	
	Grain	Bunch
Sole rice	2.0	-
Sole plantain	-	62.0
Coffee/rice	2.0	-
Coffee/plantain	-	61.9
Coffee/rice/plantain	1.9	61.5
Mean	1.97	61.80

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Coffee Sector Efficiency and Equity: Lesson Learned from a Comparative Commodity Chain Analysis of Costa Rican and Kenyan Coffee Sectors

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SUMMARY

Coffee sector is an important sector for small farmers in most of the producing countries, where it plays an important role in national economies. Despite the renowned quality of their production and the high price fetched by their coffee, some of the countries exhibit a pattern of declining production. Two of them, Costa Rica and Kenya, are presented here as case study for comparative purpose.

The poster presents an analysis of the structure and the functioning of the commodity chain of coffee in Costa Rica and Kenya using available secondary data and genuine data of direct interview of stakeholders of the 2 commodity chain. We especially compare their efficiency, their pattern of income distribution and their impact on the small farmer access to market.

The comparison showed that Kenya and Costa Rica have both a predominant smallholder coffee farmers sector. But they are enforcing 2 different types of regulation to facilitate farmers' access to market: Kenya chose an auction system while Costa Rica implemented a margin regulated commodity chain. The structure and governance of the 2 systems led to differential margin received by farmers. The difference in the level of cooperatives development appears as a key factor in the functioning of the commodity chain and small farmers access to market.

Based on these results, we finally propose some specific key recommendations to improve small farmers access to market in both countries.

INTRODUCTION

Coffee sector is an important sector for small farmers in producing countries. Following the deregulation of coffee market, scholars analysed the recomposition of global coffee chain at international level. They especially highlight the asymmetries of power and income distribution between producing and consuming countries (Fitter and Kaplinsky, 2001; Gereffi et al., 2005). More recently, specific impact of standards in governance of the Global Value Chains has been investigated (Ponte, 2002; Ponte, 2004). Nevertheless, few studies analysed the effects of national institutional framework on efficiency and income distribution within and between coffee commodity chains of producing countries.

Kenya and Costa Rica propose singular characteristics to analyse the effect of institutional framework on efficiency and income distribution among the coffee commodity chain. Both are fine Arabica coffee producers, but their production sectors appear to greatly differ in terms

of efficiency: whereas Costa Rica coffee farmers are more than 10 times less than in Kenya and crop 40% less land, they produced nearly as twice as Kenya producers in volume and generated 60% more income. (Table 1; 2007 taken as reference).

Table 1. Costa Rican and Kenyan coffee sector (2007 taken as reference).

	Kenya	Costa Rica
Producers (000)	**500**	**52**
Area (000 ha)	**163**	**97**
Production (000 tons)	**50**	**95**
Income (M. USD)	**158**	**247**

Aiming at explaining such pattern, the poster presents an analysis of the structure and the functioning of the commodity chain in both countries, comparing their efficiency, their income distribution and their impact on smallholder coffee farmers' access to market.

MATERIAL AND METHODS

The work is mainly based on information from secondary data: official statistics from the regulating institutions of Costa Rica (ICAFE) and Kenya (Coffee Board) and official publications (Coffee Act of Kenya). Other source of information includes ICO (International Coffee Organization) and NCE (Nairobi Coffee Exchange).

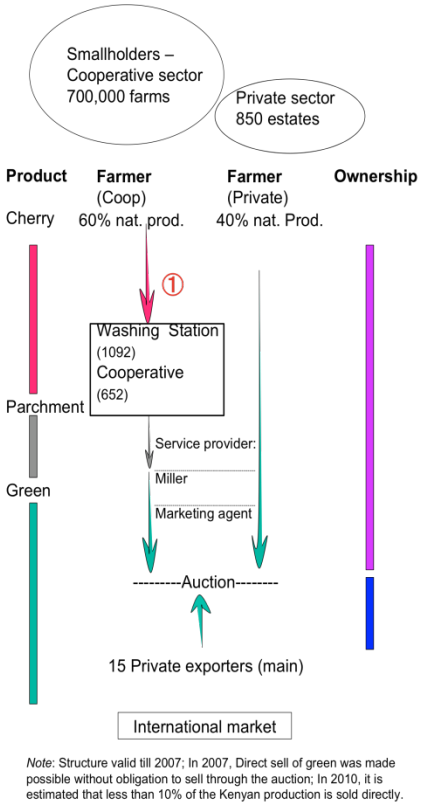

Figure 1. Structure and organization of the Kenyan coffee value chain prior 2007.

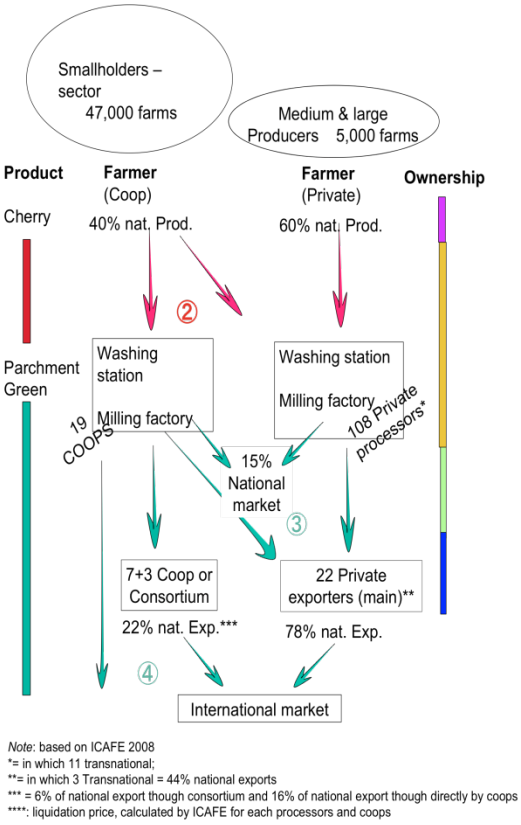

Figure 2. Structure and organization of the Costa Rican coffee value chain.

RESULTS

In Kenya, small coffee farmers are members of cooperatives to which they provide (not sell) their cherry①. In Costa Rica, farmers sell their cherry to the washing station of their choice. The competition for cherry is high between cooperative and private sector ②. There is no national market for coffee in Kenya as it exists and develops in Costa Rica ③, providing more market opportunity. In Costa Rica, the cooperatives have integrated the export function and they compete with the private exporters ④.

According to coffee act, Kenyan farmers should receive at least 75% of the auction price. In Costa Rica, ICAFE implements a mechanism of regulation of the distribution of the margin within the commodity chain ('liquidation price') and finally receives 91% for the same (Figure 3). Although higher internal costs and margin for processing and trading function, Costa Rican system guarantees a fairer price to farmers and a better reward of quality (Figure 3).

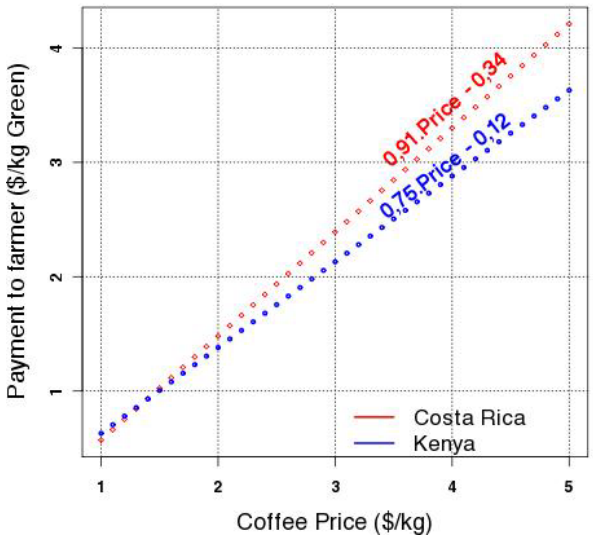

Figure 3. Coffee value chain efficiency in Costa Rica and Kenya Costa Rica: real price; Kenya: auction price.

CONCLUSION

Kenyan and Costa Rican coffee value chain are based on different philosophy to protect farmers' interests: in Kenya, the auction system aims to protect farmer but there's no competition within the chain and the cooperative efficiency is not stimulated. On the opposite, in Costa Rica, farmers' protection is achieved through a powerful mechanism implemented by ICAFE ('liquidation price') which enables competition within the chain leading to greater return to farmers and higher yield. Since 2007, the Kenyan value chain has been reorganized, cooperatives being incited to compete with traders for direct sells. But the competition between washing stations is still avoided. From the social point of view, this organization may have preserved the stability of the coffee production sector in Kenya. Its market efficiency is not demonstrated yet as the move to direct sells option is currently still limited.

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This study is part of the CAFNET Project: Connecting, enhancing and sustaining environmental services and market values of coffee agroforestry in Central America, East Africa and India

Financed by European Commission

Program on Environment in Developing Countries

Status of Coffee Production and Marketing in Ghana

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SUMMARY

Using structured survey instruments and in-depth interviews, this study was carried out with the main aim of determining the current state of coffee production and marketing in major growing regions and finding out ways of possibly reviving the industry. Interviews with major stakeholders were carried out between February and March 2009 in nine districts in four regions of Ghana. A total of 335 farmers from eight districts in the Brong-Ahafo, Eastern, Western and Volta regions of Ghana, were randomly selected and interviewed on one-on-one basis between May and April 2009. Most major growing areas are now concentrated along the southern borders of Ghana due to higher prices as well as foreign currency exchange advantage that farmers had. The knowledge and information system for coffee has also broken down hence most farmers are not aware and have not adopted good farm management practices. The study also showed that the state of coffee cultivation is far from the desired. If farmers are assured of a ready market and the prices offered is attractive, farmers are ready to invest in coffee cultivation. To revive coffee sector in Ghana, market structures should be put in place just like the case of cocoa. With the launch of a new extension systems for cocoa, coffee education could be included to whip up farmers' knowledge and interest.

INTRODUCTION

Coffee was the crop of choice in the late 60s to early 70s when the price was generally higher and in some cases up to about one and half times higher than the price of cocoa. However due to ever dwindling world market prices, the Ghana Cocoa Board divested itself of both production and marketing of coffee and had by 1992 sold the coffee plantations to private operators and redeployed most of the staff from the plantations. The divestiture of the coffee plantations immensely affected the production and marketing of coffee in Ghana. However, positive trends in prices on the global coffee market over the past few years have rekindled hopes for domestic industry players including the COCOBOD. With a view therefore of revitalizing the coffee industry in Ghana, this study was carried out with the main aim of determining the current state of the crop in major growing regions and finding out ways of possibly reviving the industry.

OBJECTIVES

- To identify current production trends, marketing channels and agents and also identify major constraints in the chain.
- To characterize and document farmers' production practices (including systems of tenure, land preparation, fertilizer use, weeding and other management practices) of the major coffee production systems prevailing within the country.
- To assess economic and farm level risk considerations connected with farmer adoption of coffee production technologies.

- To identify production and marketing constraints and how these affect the prospects of a sustainable coffee industry in Ghana.

STUDY METHODS AND AREAS COVERED

To achieve the above objectives both primary and secondary data were collected. The primary data collection employed two different survey methods: informal and formal. The objective of the informal survey was to get first hand information about current areas of cultivation and constraints and help focus questionnaires administration in the formal survey on the important issues identified that require further investigation and quantification. Given the fact that the crop cultivation is no longer uniformly distributed throughout the 6 producing regions, the team first of all visited regional and/or district offices of other divisions of COCOBOD particularly those of the Cocoa Swollen Shoot and Virus control (CSSVDU) where the various officers were engaged in an informal dialogue concerning the cultivation and marketing of coffee in their areas of operation. Other key informants contacted were staff of the Ministry of Food and Agriculture (MOFA), Licensed Buying Companies (LBCs) and other marketing agents. The team then visited communities and/or farms identified as important coffee growing areas where individual or group of farmers were involved in informal discussions concerning their coffee cultivation and marketing practices and constraints. In all cases the interviews were facilitated by the use of a checklist or interview guide.

Following the findings of the informal survey, a questionnaire was designed, pretested and administered to farmers in all the important coffee growing areas in the country. Due to the dwindling numbers of farmers in some areas, the enumerators tried to interview as many farmers as possible who still had coffee farms (maintained or abandoned) as at the time of the survey (March-June, 2009). However, in areas of high concentration like Nkawkaw and Hohoe cocoa districts a complete list of all farmers were drawn and a proportional random sample of ten to twenty farmers drawn and interviewed giving a total sample size of 335. The responses were then analyzed using statistical methods.

RESULTS

Informal Survey

Results from the informal survey indicate that coffee cultivation in the country is now concentrated in clusters mostly along the borders. Given the current low levels of production it makes a lot of economic sense for cultivation and marketing activities to be concentrated at these locations as this helps to reduce cost of marketing. Lack of information on grower recommendations is one of the major constraints to coffee production in Ghana (Oppong et al., 2010).

Though the Cocoa Research Institute of Ghana (CRIG) has some technologies for coffee cultivation (Adu-Ampomah et al., 1993; Oppong et al., 2010) the generally low priority rating for the crop has meant low transfer and hence adoption of these technologies by farmers. Due to the few marketing agents currently engaged in the coffee trade producer prices seem to be uniform throughout the country and ranged between 25-30 Ghana Cedis per bag (GH₵ 1.45 is equivalent to $1) of 65 kg unhulled coffee during the time of the survey in February/March 2009. The price for 2008 ranged between 20 and 25 Ghana Cedis. The price of hulled coffee around Dorma Ahenkro which is determined by companies in Cote De Voire was around 400-500 francs per bag.

Formal Survey

General demographics of farmers

Generally most (88.4%) of the respondents were males, few farmers were within vibrant working age groups (20-39 years). Majority (87%) of the farmers were married whilst few (5.1%) were widowed. Fifty-five percent of the respondents had from six to ten people in their households. Generally, 75% of the respondents were natives but none of the farmers belonged to a farmers association.

Characteristics of coffee farms and some production practices amongst coffee farmers

The total area under coffee cultivation in Ghana, was about 13,300ha during the period 1970 - 1980 and reduced to about 3,200ha in 1985 (World Bank, 1990). The reduction in area could be attributed to lack of interest to cultivate coffee over the past decades due to decline in world market prices (Anim-Kwapong and Osei Bonsu, 2000). As at the time of survey, the total land area cultivated by the respondents was 3988.20acres (1595.28hectares) out of which 36.4% (1452.5acres) was cultivated to coffee and 63.6% (2535.7acres) cultivated to the other crops. On the average, respondents cultivated three fields per annum but mostly cultivated one of the three fields (87%) to coffee. Sixty- eight percent (68%) of respondents were owner operators. All respondents had destroyed part of their coffee farms ranging from 0.5 - 2 acres (77%) and 2.5- 5 acres (23%) with crops they considered more profitable compared to the cultivation of coffee. It was revealed that only few farmers (9%) applied fertilizer to their coffee fields. Majority (55%) weeded their farms twice in a year. One main factor that contributes to low coffee production in Ghana is the use of wrong or poor planting material (Anim-Kwapong and Adu-Ampomah, 2000).The study revealed that, most farmers (87%), planted by direct seeding from berries harvested from their own farm, 12% of farmers used cuttings, 0.6% who acquired already established farms through either inheritance or outright purchase could not tell the type of planting material on their farms and 0.3% used both seed and cuttings. Of the whole sample, 77.2% of farmers provided shade for their farms. The knowledge and information system for coffee has also broken down hence most farmers are not aware and have not adopted good farm management practices.

Coffee production trends, marketing agents and constraints

The total quantity of coffee sold in 2008 was 40140.79kg with an average price of Gh₵ 0.63 per kilogram (Table 1). Most (31%) coffee farmers sold their coffee to coffee buyers within their communities (Figure 1). Lack of market/or low producer price (52%) constitute the two most important constraint (Figure 2) followed by low access to information (16%).

Table 1. Price trends and quantities sold from 2005 to 2008.

Year	Sample size		Average quantity sold (Kg/acre)	Average prices (Gh.¢/Kg)	Total quantity sold (Kg)
2008	258	155.58	0.63	40140.79	
2007	261	138.36	0.53	36112.00	
2006	244	151.33	0.48	36925.38	
2005	220	134.84	0.41	29665.55	

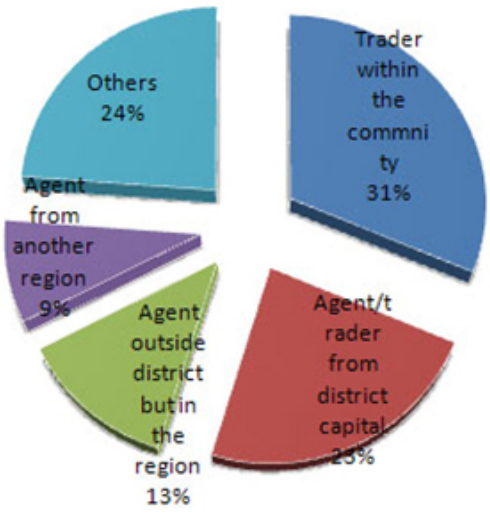

Figure 1. Marketing agents in the coffee industry.

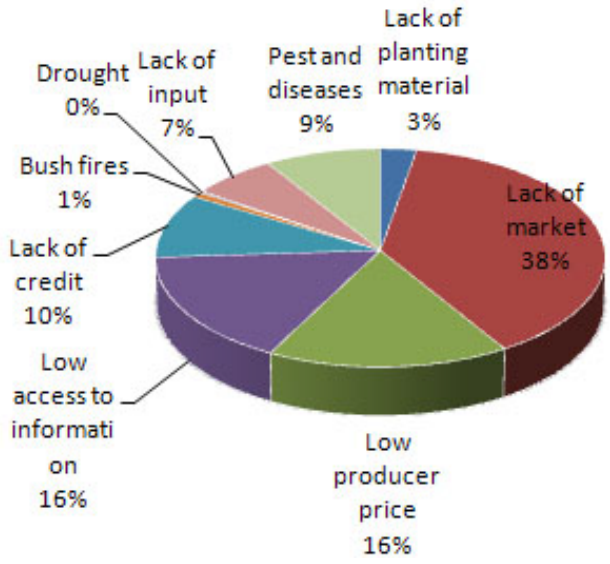

Figure 2. Constraints in the coffee industry.

CONCLUSIONS

It is concluded that, the state of coffee cultivation is far from the desired. Lack of proper marketing systems appears to be the main problem of farmers. To revive coffee sector in Ghana, market structures should be put in place just like the case of cocoa. With the launch of a new extension systems for cocoa, coffee education could be included to whip up farmers' interest.

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The Dynamics of Predacious Mites, *Euseuis kenyae*, (Acari: Phytoseiidae) Under Coffee Sprayed with Chlorpyrifos

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SUMMARY

The management of key or primary coffee insect pests involves heavy use of insecticides without considering their side effects on biological control agents such as the predacious phytoseiid mites that keeps minor or secondary pests to below economic injury levels. Under Kenyan coffee farming systems, management of primary pests such as Coffee Berry Borer (*Hypothenemus hampei* Ferrari) involves foliar application of chlorpyrifos. It is anticipated that use of chlorpyrifos is likely to negatively affect the populations of the biological control agents. To ascertain the effects of such product, study was conducted to investigate the possible long term effect of chlorpyrifos on predacious mites, *Euseuis kenyae* Swirski & Ragusa. This was carried out for a period of three years (2006-2008). The coffee blocks differently fertilized with inorganic and organic compound fertilizers, and chlorpyrifos sprayed against the Coffee Berry Borer, were used to assess the effect of this insecticide against the *E. kenyae*. The chlorpyrifos was sprayed twice in months of June/July for each year per coffee block for a period of three years. During this period, the E. *kenyae* populations fluctuated despite the chlorpyrifos application. They showed a progressively increasing tolerance or resistance to chlorpyrifos irrespective of different fertilizer regimes. The development of such resistance by the *E. kenyae* implies that such strains of mites can effectively be used to manage the secondary pests while chlorpyrifos controls the primary pests. Hence the potential of incorporating such strains of *E. kenyae* in an Integrated Pest Management programme under coffee farming systems.

INTRODUCTION

Heavy and indiscriminate use of pesticides such as insecticides to control coffee insect pests has been associated with a number of problems for instance pesticides resistance, environmental degradation, pests' upsurgence, natural enemies' elimination, and high cost of production. In Kenya, heavy uses of organophosphorous insecticides' sprays have been linked to an outbreak of Giant loopers, *Ascotis selenaria reciprocaria (*Walker) (Wheatly, 1964; Le Pelley, 1968). However, where they are prudently used, successes in management of pests such as coffee scale insects, *Coccus* spp. (Acland, 1971); Kenya mealybug, *Planococcus Kenyae* (Le Pelley) (Abasa, 1983) and Leafminers, *Leucoptera* spp. (Vega et al., 2007) by natural enemies have been reported.

Coffee insect pests host many natural enemies (Wheatly, 1964; Andrade, 1966; Le Pelley, 1968; Abasa and Mathenge, 1974; Kinuthia and Mwangi, 1986; Anonymous., 1991). In Kenya, the predacious phytoseiid mites are among the natural enemies found in coffee farming systems (El-Banhawy et al., 2009). The predacious mites are of economic importance (Parrott et al., 1906; Gilliant, 1935; Garman, 1948; Nesbitt, 1951; Pallini et al., 2008). They

control phytophagous mites and several small insects (McMurtry et al., 1970; Grout and Richards, 1994).

The majority of phytoseiid mites are facultative predators that feed on a wide range of prey including red spider mites, gall and rust mites and small insects. Other species feed on nematodes, fungal spores, pollen, honey dew and exudates from plants, but rarely on plant tissue **(**Zhang, 2003; Vega et al., 2007). However, factors such as hot-dry conditions (El-Banhawy, 1995), prey density and time of release (Chant, 1961; Sandness and McMurtry, 1970; Hairyappa and Kurkani, 1988; Zhang et al., 1992) and the great sensitivity of phytoseiids to most insecticides (McMurtry et al., 1970) limit their efficiency.

Agricultural sprays, especially the fungicides, insecticides and acaricides negatively affect the natural enemies of insect pests and mites' species. Most commonly used insecticides have a more or less broad spectrum activity and drastic effects on the predacious mites (Bartlet, 1964; Huffaker et al., 1969). As a result, many scientists have searched for selective pesticides that can control target pests but exhibiting low toxicity towards phytoseiids. These kinds of insecticides are rare because most products are designed and marketed on the basis of their wide spectrum action (Croft, 1972). Despite this, studies have shown that strains of phytoseiids can develop resistance particularly to organophosphorous compounds (Croft & Jeppson, 1970; Motoyama et al., 1970; Croft & Meyer, 1973; Croft and Stewart, 1973; Grande and Ingrassia, 1988). Such resistance may also be present on predacious mites occurring in coffee, particularly where some organophosphate compounds like Chlorpyrifos (Dursban 480 EC) are heavily sprayed and over a long period of time (Pers. observ.). Therefore, this work studied the dynamics of *E. kenyae* under coffee sprayed with Chlorpyrifos in Kenya.

METHODS

An experiment was laid out at Coffee Research Station (CRS), Ruiru in a main coffee block with mature trees of Arabica coffee hybrid, Ruiru 11 known to be resistant to two main coffee diseases; Coffee Berry Disease and Leaf Rust for three successive years (2006-2008). The trees had a planting of close spacing of 2M x 2M giving a total of 2500 trees per hectare. Agronomic practices such as pruning, liming, handling and weeding were carried out as recommended during the trial period. The main coffee block was sub-divided into three sub-blocks. Five rows were left between the sub- blocks as the guard rows. The three sub-blocks were differently applied with N.P.K 17:17:17, Organic fertilizer and N.P.K 22:6:12. In all the sub-blocks, Gypsum (Lime/calcium source) was applied at a nominal rate of 300 g/tree annually to improve the Calcium level and maintain PH level of between 4.4- 5.4.

In each sub-block, Chlorpyrifos (Dursban 480EC) and untreated (control) were applied as the treatments, each in a plot of 16 coffee trees. Using a Randomized Complete Block Design, each treatment was replicated four times in each sub-block. Two rows of coffee trees were left between the plots as guard rows.

Four coffee trees at the center of each plot were sampled fortnightly to monitor the population levels of predacious mites. The mites were dislodged from coffee trees using a beating stick for one minute, collected in a collecting board, counted and recorded.

RESULTS

Following application of Chlorpyrifos against the Coffee Berry Borer during the period 2006-2008 (two applications per year in June/July), the population of *E. kenyae* under coffee sub-block fertilized with NPK 17:17:17 remained low in the first five months (August – December 2006) before fluctuating in the first six months of 2007, with peaks in January and May and a depression in March 2007 (Figure 1). In 2007, insecticides application was done when the population was low (< 2 mites /sample) but the population remained low for only a month before fluctuating again with peaks in September and November and a depression in October 2007. The population remained low from December 2007 up to April 2008 when it started to rise again. Although application of the insecticides in June/ July 2008 was done at peak population, it resulted in only a transient (one month) population reduction with the population of the mites peaking at an even higher level in August before it started to oscillate again (Figure 1). Similar population trends occurred in all the other coffee sub-blocks under the different fertilization regimes but with subtle differences in the abundance of the mites (Figures 2 and 3). These population trends indicated a progressively increasing tolerance or resistance of *E. kenyae* to the insecticides. In all the sub- blocks, the population of mites was lower in plots sprayed with Chlorpyrifos as compared to the control.

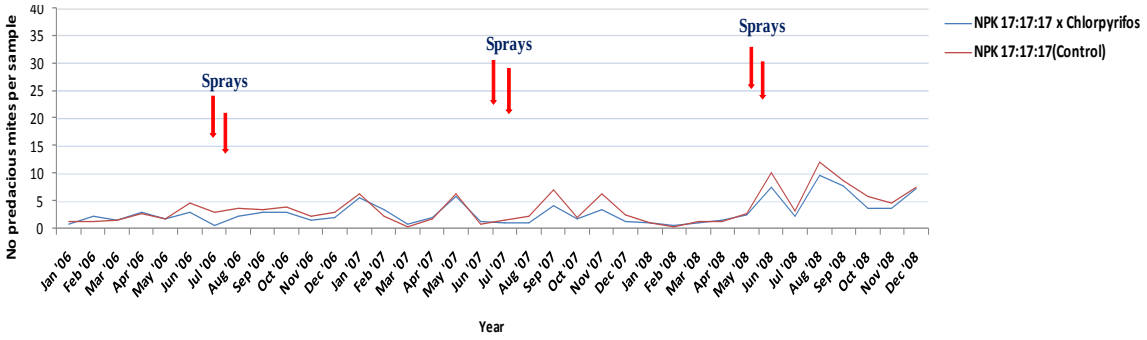

Figure 1. Population trends of *Euseius kenyae* on coffee under NPK 17:17:17 fertilizer following application of Chlorpyrifos.

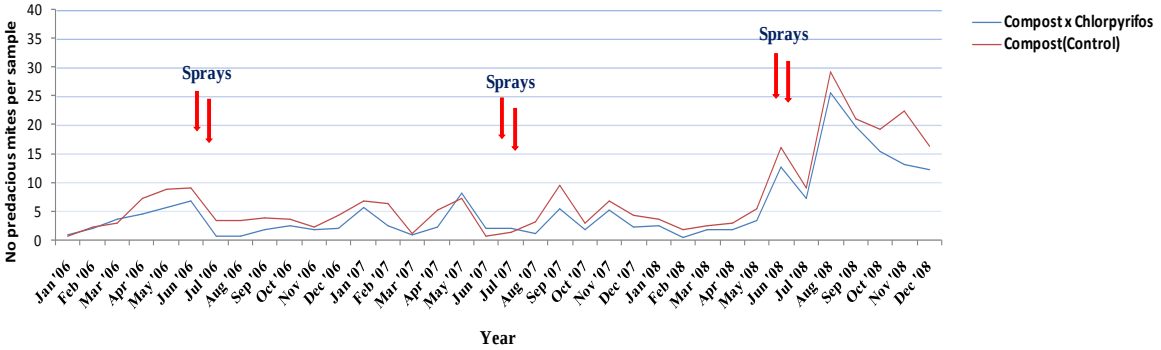

Figure 2. Population trends of *Euseius kenyae* on coffee under organic compost (NPK 0.8:O.2:1.0) following application of Chlorpyrifos.

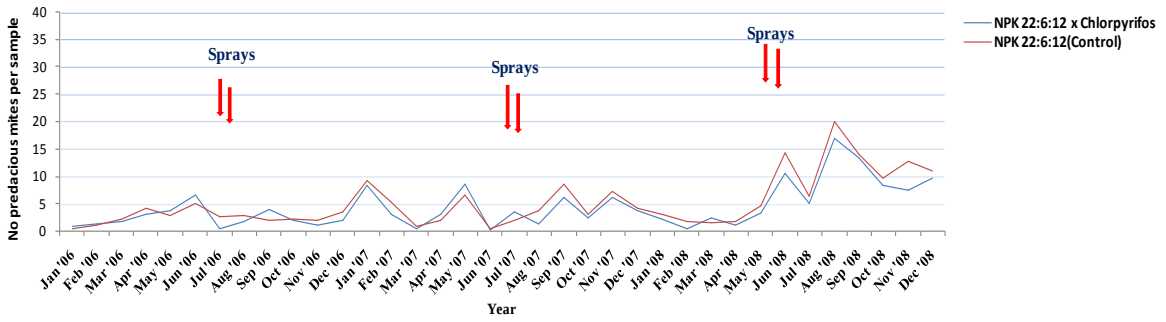

Figure 3. Population trends of *Euseius kenyae* on coffee under NPK 22:6:12 fertilizer following application of Chlorpyrifos.

DISCUSSION

Biological control is one of the options towards ecologically viable solutions in pest management. Normally predacious mites, control small insects such as thrips and contain them below economic injury levels. Field experiment was conducted to investigate the effect of Chlorpyrifos on predacious mites under different fertilizer regimes. The predacious mite, *E. kenyae* population during the start of this study was low especially in year 2006. This increased with time despite the application of chlorpyrifos. It was expected that as chlorpyrifos was sprayed, the population of predacious mites was rapidly to drop possibly to zero. This never occurred, meaning that the mites progressively acquired some resistant to chlorpyrifos. Under such a situation the survival of predacious mites was an advantage as they controlled secondary pests of coffee such as thrips and red spider mites. It is known that agricultural sprays (Pesticides) affect the natural enemies of phytophagous mites and other insect pests (Barlet, 1964; Patterson, 1966).This work confirmed these findings. Though this was the case, the predacious mites progressively developed resistant to Chlorpyrifos that led to increased number of mites with time hence control of secondary pests of coffee. Past studies have indicated that as a result of regular use of insecticides, species of predacious phytoseiid mites could develop resistance to insecticides. Such resistance has been detected from phytoseiid mites such as *Neoseiulus fallacis* (German), *Metaseiulus occidentalis* (Nesbitt), *Phytoseiulus persimilis* A. - H., *Amblyseius cydnodactylon* Shehata & Zaher (Motoyama et al., 1970; Croft and Meyer, 1973; Roush and Hoy, 1981; El- Banhawy et al., 2000).

The present investigations indicated that under coffee agro-ecosystems, resistance occurred on populations of *E. kenyae* after their exposure to Chlorpyrifos, a commonly used insecticide in management of key insect pests such as the Coffee Berry Borer.

ACKNOWLEDGMENTS

We greatly thank the Coffee Research Foundation for financial support. Our thanks also go to staff from 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.

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The Benefits and Limitations of Shade Practices in Kenya Coffee

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SUMMARY

In order to determine the benefits and limitations farmers experience when they include/incorporate trees within their coffee fields, a baseline survey was carried out in the major coffee growing areas in Kenya. The survey was conducted through farm/household visits and it covered 185 units. The units were selected through a multi-stage sampling approach. Data from the farms/households was adduced through structured interviews.

In all areas surveyed, it was evident that most farmers appreciated the use of shade in their coffee plantations. They cited several benefits derived from the use of shade trees such as provision of mulch, fuel wood, timber, adverse weather mitigation, inducing uniform/regular cropping as well as for aesthetic value. The limitations included lack of proper recommendations on the use of shade trees in terms of suitable species and management aspects such as, spacing and pruning. The results of the survey showed the need to carry out studies on potential shade trees to further assess their impact on coffee yield and quality and sustainability of the coffee agroforestry systems.

INTRODUCTION

Coffee is Kenya's third most important agricultural export commodity after tea and horticulture. In addition to its contribution to foreign earnings, it also provides livelihood for over 500,000 households. Coffee is native to the shaded forests of southern Ethiopia where it is believed to have evolved as an under-storey crop. Early plantations were shaded using over-storey trees in order to mimic its natural habitat since coffee was considered shade-obligatory (DaMatta, 2004). It been shown that coffee can perform well, even have higher yield, in full sun (Beer et al., 1998).

Shaded plantations provide diversified sources of income due to cash from sale of timber, fuel wood or fruits from the shade trees (Soto-Pinto et al. 2000). In addition, the shade/shelter trees enhance water shed services, promote biodiversity and CO_2 sequestration. Coffee grown in full sun, on the other hand, generally requires intensive management involving heavy application of inorganic fertilizers, to maximize yields. However, most coffee in Kenya is produced by small holder farmers who cannot afford to apply the required inputs. Even where the inputs are available, as in large estates, their indiscriminate use often leads to reduced plantation longevity and negative long-term environmental impacts.

About 75% of coffee in Kenya is grown by small scale farmers under rain-fed farming systems and un-shaded conditions. The addition and management of various intercrops such as annual food crops, shade/fruit trees may improve coffee farm sustainability and economic viability. This is achieved through production of food/tree products, improvement in coffee quality and provision of biodiversity and hence environmental conservation.

Recent observations point toward a growing number of farms being put under shade. While part of the reason is an effort towards improved environmental management, a lot more could be as a result of addressing other objectives such household energy needs, food security and income diversification. This survey was therefore carried out to assess farmers' perception regarding the use of shade trees in coffee in order to formulate strategies for promoting coffee agroforestry.

MATERIALS AND METHODS

The baseline field survey was undertaken, in the main coffee growing areas in Central, Eastern, Nyanza and Rift Valley provinces in Kenya (Table 1), between November 2008 and March 2009. The farm/household units, located in Upper Midland (UM) zones 1, 2 and 3 were selected through a multi-stage sampling approach. The areas were grouped into 7 clusters which were Meru, Embu/Kirinyaga, Nyeri, Murang'a, Machakos, Kiambu/Thika and West of Rift. The survey was conducted through farm/household visits and it covered 185 units which were distributed as shown below (Table 1).

Table 1. Distribution of sample units in the farm/household survey.

Cluster	**Sample size**			
	UM1	**UM2**	**UM3**	**Total**
Meru	12	12	12	**36**
Embu/Kirinyaga	12	12	12	**36**
Nyeri	6	6	6	**18**
Murang'a	4	12	-	**16**
Machakos	-	-	18	**18**
Kiambu/Thika	6	24	-	**30**
West of Rift	-	31	-	**31**
Total	**40**	**97**	**48**	**185**

Data from the farm/households were adduced structured interviews. The data gave insight into the prevalent shade practices and the benefits.

RESULTS AND DISCUSSION

Though coffee is traditionally grown in un-shaded condition in Kenya, it was observed that an increasing number of farms were being put under shade. A large number of farmers in Murang'a (75%), West of Rift (74.19%), Kiambu/Thika (70%) and Machakos (66.67%) practiced shade coffee farming. They showed a higher preference for exotic trees that ranged between 2.78 to 67.74% as compared to indigenous 0 to 37.5% (Table 1). In Embu/Kirinyaga and Nyeri clusters the use of shade trees was particularly low at 2.78 and 5.56% respectively.

Coffee has been mainly grown as a mono crop in full sun, under optimal conditions (cool temperatures, high humidity and adequate rainfall). These growing conditions often make shade seem unnecessary. These may explain the low of incidence of shade in areas such as Nyeri and Embu/Kirinyaga. However, it was observed from the survey that many farmers appreciated the benefits of establishing trees in their coffee fields.

Table 2. Prevalence, types and benefits of shade trees in Kenya coffee.

Cluster	% Practicing	% Type		% Reasons for Planting			
		Indigenous	Exotic	Adverse weather mitigation	Aesthetic value	Tree vigour improvement	Uniform cropping
Meru	33.33	5.56	2.78	2.78	0.00	0.00	0.00
Embu/ Kirinyaga	2.78	2.78	2.78	0.00	0.00	0.00	0.00
Nyeri	5.56	0.00	5.56	5.56	0.00	0.00	0.00
Murang'a	75.00	37.50	43.75	18.75	0.00	6.25	0.00
Kiambu/ Thika	70.00	20.00	60.00	3.33	0.00	16.67	10.00
Machakos	66.67	0.00	33.33	22.22	0.00	0.00	0.00
West of Rift	74.19	25.81	67.74	38.71	12.90	6.45	12.90

Table 3. Additional benefits of shade trees in Kenya coffee.

Cluster	% Mulch	% Firewood	% Extra income	% Construction materials
Meru	2.78	2.78	0.00	0.00
Embu/Kirinyaga	2.78	0.00	0.00	0.00
Nyeri	0.00	0.00	0.00	0.00
Murang'a	25.00	56.25	50.00	31.25
Kiambu/Thika	33.33	50.00	30.00	40.00
Machakos	38.89	27.78	16.67	5.56
West of Rift	16.13	67.74	9.68	32.26

The reasons given for planting shade trees included adverse weather mitigation, uniform cropping, tree vigour improvement and aesthetic value. Farmers in Murang'a (18.75%), Machakos (22.22%) and West of Rift (38.71%) cited adverse weather mitigation as a reason for planting shade trees. In Kiambu/Thika, 16.67% of farmers said that shade trees improved coffee tree vigour. In West of Rift, 12.9% of the farmers considered shade to contribute towards both uniform cropping and landscape beauty (Table 2). Other benefits of shade trees alluded to by farmers included provision of mulch, firewood, construction materials and as source of extra income (Table 3).

It has been observed in many situations that coffee can grow well without shade, sometimes producing higher yields than shaded coffee (Beer et al., 1998). Nonetheless, shade coffee systems have tremendous potential for biodiversity conservation of tropical plant and animal species due to their complex biophysical structure (Perfecto et al., 2005). It has also been demonstrated that agroforestry systems are among the most promising land uses for achieving both conservation goals and supporting livelihoods (Buck et. al., 1999; Huxley, 1999; Leakey, 1999).

The beneficial effects of shade trees may be crucial in many coffee producing areas in Kenya that are considered marginal for intensive production systems. The survey highlighted the need to undertake studies on the impact of shade on coffee yield and quality since little work (Kimemia, 2004) has been carried out on these factors. The selection of appropriate shade trees that address the areas of mulch, firewood, timber and extra income including the management of the trees should be the main focus.

ACKNOWLEDGEMENT

This study was supported mainly by the Quality Coffee Production and Commercialization Program (QCPCP) funded by the European Union. 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.

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Positive Effect of Bee Pollination on Coffee Production Is Highly Contingent of Irrigation in Coffee Agroforestry Landscape of Kodagu, Southern India

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SUMMARY

Agricultural productivity, particularly in the tropics, is at least partially dependent upon natural ecosystem services such as pollination, pest control, and water and soil conservation. While empirical studies have shown that the productivity of coffee is enhanced by insect pollination, rarely have management practices been included in such analyses. This omission means that the value of pollination services cannot be set within the range of management interventions available to farmers. Without this broader context, it is difficult to evaluate how farmers might respond to calls for managing land to secure pollination services. In Kodagu, a major coffee-growing region in southern India, we investigated the contribution that bee pollination makes to coffee production in the context of fertilization, irrigation and shade management practices as well as environmental variables.

We monitored coffee production on 10 coffee bushes from flowering to harvest in 123 sites located at least 1 km from one another. In each of these sites, bee observations were carried out when coffee flowered. Data on shade level and shade tree density were collected through field surveys, and management practices recorded through interviews with planters.

Our results show that even taking into account management decisions, bee abundance contributes more to coffee production in terms of number of berries harvested than other management practices, such as NPK fertilisation. Bee abundance, however, is highly contingent upon management actions, particularly irrigation, rather than the nature of the surrounding habitat matrix. Indeed, bee abundance at any one site was mainly driven by irrigation, which triggered asynchronous flowering and concentrated bee foraging. Raising awareness among coffee growers of the role of asynchronously irrigating can potentially contribute to improve quantitatively and qualitatively coffee production.

INTRODUCTION

Agricultural productivity, particularly in the tropics, is dependent upon largely unrecognized and undervalued natural ecosystem services – defined by the Millennium Ecosystem Assessment (2005) as an economic benefit that nature provides to people – such as pest control and insect pollination.

While empirical studies have shown that the productivity of coffee is enhanced by insect pollination, rarely have management practices been included in such analyses (Klein et al., 2002; Roubik, 2002; Klein et al., 2003a, c, b; De Marco and Coelho, 2004; Ricketts, 2004; Ricketts et al., 2004; Vergara and Badano, 2009). However, various management practices are implemented during the 10 to 11 month maturation period of coffee, the least one being fertilisation. Moreover, during the same maturation period, environmental conditions, such as rainfall, also influence coffee maturation. Therefore the positive effect of pollination at fruit set (i.e. 5 to 7 weeks after flowering) might be reduced by the effects of management interventions (Bos et al., 2007). Assessing the real impact of pollination on harvested berries in the broader context of farmer's management decisions might therefore leads to incentives for securing pollination services at the landscape scale.

In Kodagu, a major coffee-growing region in southern India, we investigated the contribution that bee pollination makes to coffee production in the context of fertilization, irrigation and shade management practices as well as environmental variables.

MATERIALS AND METHODS

We selected 113 sites located at least 1 km from one another and growing *Coffea robusta* trees between 20 and 40 years of age. In each of these sites, we collected data on coffee production, management practices and insect pollination.

Coffee production

We monitored coffee production on 10 coffee bushes per site, by selecting 5 branches in each tree, with each of the branch having 6 clusters. We counted the number of flowers and fruits on these 300 clusters per site at several time during one growing season (about 11 months): flowering time, five weeks after flowering (fruit set), before and after the monsoon, and finally at the harvest time.

Management practices and environmental variables

Data on shade level and shade tree density were collected through field surveys on two plots of 314 m^2 located around our coffee trees. Management practices, in particular fertilisation (NPK and lime), pruning regime, number of weedings per year, soil management practices (soil surface scrapping), were recorded through interviews with planters.

Pollination service

Between 3 and 8 simultaneous observations of 15 minutes each were carried in our sites on the day of flowering. During the observations, the number and species of the insect visitors were recorded.

Statistical analyses

Linear models were used and data transformed when necessary.

RESULTS AND DISCUSSION

Our results show that even taking into account management decisions, bee abundance contributes more to coffee production in terms of number of berries harvested than other

management practices, such as NPK fertilisation (Table 1, Figure 1). Bee abundance, however, is highly contingent upon management actions, particularly irrigation (Figure 2). Indeed, bee abundance at any one site was mainly driven by irrigation, which triggered asynchronous flowering and concentrated bee foraging. Raising awareness among coffee growers of the role of asynchronously irrigating can potentially contribute to improve quantitatively and qualitatively coffee production.

Table 1. Result from the linear model analysis showing the final model where only significant (or marginally significant) variables are remaining. The result show that lime, bee visitation, number of flowers and rain on the flowering day all have an impact on coffee production.

Coffee production	sign	F	p-value
Lime application	+	22.7	0.001
Bee visitation x Number of flowers	+	13.4	0.001
Rain on the flowering day	-	8.7	0.004
Density of native shade trees	+	1.9	0.06

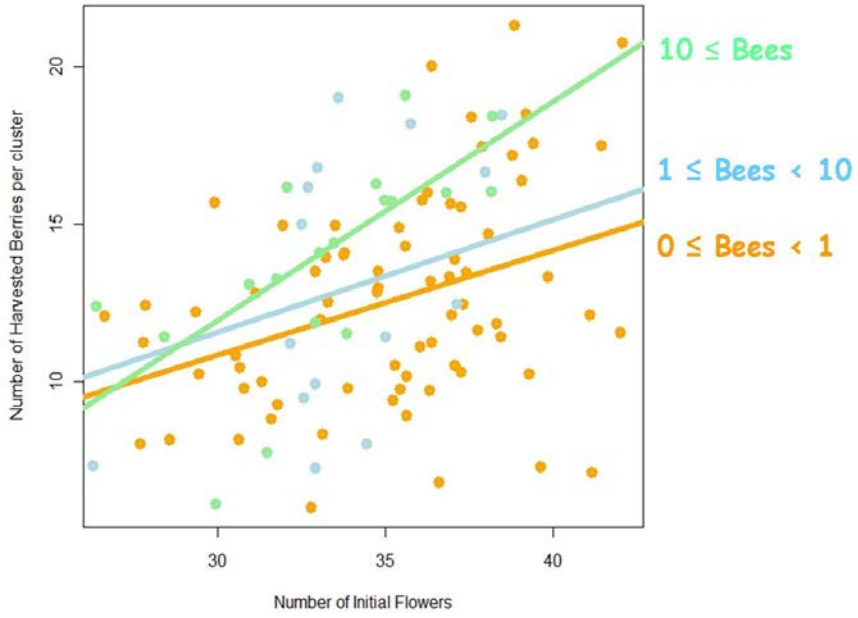

Figure 1. Plot of the number of harvested berries as a function of the number of initial flowers, showing the regression lines for sites which received low bee abundance (0≤ bees <1 individual per observation), medium bee abundance (1≤ bees <10 individuals per observation) and high bee abundance (10≤ bees per observation).

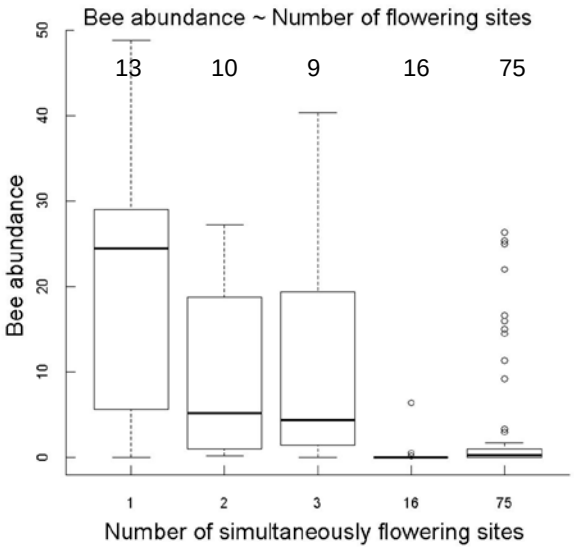

Figure 2. Bee abundance as a function of the number of plantations flowering on the same day. The numbers on top of the graph indicate how many sites flowered under such conditions: plantations flowering alone (under the category "1") numbered 13, those flowering simultaneously with one other plantation (under the category "2") totalled 10, while those flowering with two other plantations (under the category "3") were nine. All the plantations in the categories one to three were artificially irrigated. Four of the 16 co-flowering plantations (in the category "16") were irrigated; and the flowering in the 75 co- flowering plantations was stimulated by rainfall.

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Beverage Quality Potential of Bourbon Selections for Specialty Coffee Production in Brazil

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SUMMARY

Considering the high cup quality of Bourbon variety, this research aimed to evaluate the sensory profile of some Bourbon lines and Brazilian commercial varieties to verify if there are differences in their beverage quality. It was analyzed the beverage quality of 18 *Coffea arabica* genotypes including some commercial varieties, Caturra and Bourbon selections. Coffee samples were obtained by semi washed processing method and the sensory analyses for cup quality were done according to criteria adopted for specialty coffees. It was observed that Red Bourbon São João Batista, Yellow Bourbon Epamig and Red Catuaí IAC144 showed score lower than 80 points, being classified in the not specialty coffee category. All the remaining varieties were classified in the specialty coffee category, where Red Bourbon Procafé, Yellow Bourbon Betânia, Yellow Bourbon Castro, Yellow Bourbon Paixão, Yellow Bourbon IAC LCJ9, Yellow Bourbon Limoeiro, Yellow Bourbon Italiano, Yellow Bourbon Samambaia, Mundo Novo IAC 502, Mundo Novo IAC 4266 and Yellow Caturra IAC 476 presented score between 80-82 points and Yellow Bourbon Daterra, Yellow Bourbon Nogueira, Yellow Bourbon Trigo and Red Caturra IAC 477 presented score over 82 points. The predominant and subsidiary tastes and aromas, similar to chocolate, caramel, vanilla, floral, wine and fruits notes observed in some Yellow Bourbon and Red Caturra varieties that scored equal or over 82 out of 100 points confirm the high cup quality of these genotypes, indicating the possibility of genetic selection for beverage quality to attend the specialty coffee sector demand.

INTRODUCTION

The specialty coffee marked has been developed fast than commodity coffee market around the word and the Bourbon coffee is a requested variety at specialty coffee. It is generally claimed that Bourbon coffee enjoys a high intrinsic quality profile to the point of being considered as an international benchmark that any new variety should aim to reach in order to be considered as suited for the specialty coffee market. This justify the selection of new Bourbon varieties to supply the demand of specialty coffee sector for high quality and differentiated coffees. The first Brazilian Red Bourbon selection, named IAC662, was released by Instituto Agronômico de Campinas (IAC). During the 1940's were released several Yellow Bourbon selections, named IAC J2, IAC J9, IAC J10, IAC J19, IAC J20, IAC J22 and IAC J24, that showed yield potential 40% superior than Red Bourbon (Carvalho et al., 1957).

Beverage quality is an important attribute of coffee and contributed for price determination (Agwanda et al., 2003). It is very know that cup quality is influenced by large number of factors including genotype, environmental, processing and their interactions. Recent studies done by using the specific sensory methodologies have showed that Bourbon varieties have

frequently beverage quality better than other commercial varieties selected for the high yield, confirming the real genetic potential of the Bourbon types to produce high coffee quality. However, to get reliable information about beverage quality by genotype comparisons it is so important that all genotypes have been cultivated in the same site and have received the same post harvest processing to avoid possible interferences in the cup quality. To attend the recent needs by new Bourbon varieties in Brazil, the IAC restarted its Bourbon Breeding Program and at this time several Bourbon progenies are being studied in field trials to identify promissory genotypes to specialty coffee production in Brazilian conditions.

The beverage quality assessment done by trained coffee tasters and the prescribed sensory evaluation procedures method provides specific and reliable data about each coffee genotype and can be used successfully for screening in breeding selections to coffee quality improvement. In this research were done some specific studies about coffee quality focusing the sensory profile of some Bourbon lines comparing with commercial varieties aiming to identify promissory Bourbon genotypes for cup quality improvement and offer new perspectives to the specialty coffee production in Brazil.

MATERIAL AND METHODS

Study site and test varieties

The experiment was carried out at Recreio Estate Coffee, a recognized Brazilian farm by its high quality coffees in the Northwest Region of São Paulo State, Brazil. A total of eighteen *Coffea arabica* genotypes were used in this study (Table 1). Many of them are elite Bourbon genotypes that present high genetic potential for use in coffee breeding programs aiming the cup quality improvement, including two commercial varieties, Mundo Novo and Catuaí, which served as reference for quality and yield evaluation. Each of the genotype was represented by ten trees disposed in the field in a Randomized Complete Block Design with three replications.

Harvest and processing

Cherry samples were collected during the peak harvesting period of July-September, 2009. Ripe health fruits were harvested by hand from each of the experimental plot and processed using semi washed processing procedures, where the light and unripe fruits were removed out from the cherry fruit lot. The fruits were pulped leaving only the pulped coffee cherry (natural pulped coffee) and the parchment coffees were sun dried until the moisture content reached 11% (wb). After hulling the beans were graded by round perforations screens and for the sensory evaluation only 16, 17 and 18/64 inch screen size without defective beans were used.

Sensory evaluation

The sensory analyses were done according to the Specialty Coffee Association of America (SCAA) prescribed procedures. The beverage quality assessment was done by descriptive analysis of cup quality for each coffee sample from respective experimental plots. Roasting of green coffee beans was done to attain a light to light-medium roast (Agtron scale of approximately 58 on whole bean and 63 on ground, +/- 1 point) using a Probat laboratory roaster within 24 hours of evaluation and allowed to rest for at least eight hours. The roast was completed in no less than 8 minutes and no more than 12 minutes.

Table 1. *Coffea arabica* varieties studied in this research, São Sebastião da Grama, Brazil, 2009.

Reference	Varieties	Origin
1	Red Bourbon	Procafé - Varginha
2	Red Bourbon	Faz. São João Batista - Campos Altos
3	Yellow Bourbon	Epamig – Machado
4	Yellow Bourbon	Faz. Betânia – Santo Antonio do Amparo
5	Yellow Bourbon	Faz. Daterra - Patrocínio
6	Yellow Bourbon	Faz. Castro – Carmo de Minas
7	Yellow Bourbon	Faz. Nogueira – Carmo de Minas
8	Yellow Bourbon	Faz. Paixão – Carmo de Minas
9	Yellow Bourbon	Faz. Samambaia – Santo Antonio do Amparo
10	Yellow Bourbon IAC LCJ9	IAC - Campinas
11	Yellow Bourbon Italiano	Faz. Monte Alegre - Alfenas
12	Yellow Bourbon Trigo	Faz. Monte Alegre - Alfenas
13	Yellow Bourbon Limoeiro	Faz. Monte Alegre - Alfenas
14	Red Mundo Novo IAC 502/9	IAC - Campinas
15	Yellow Mundo Novo IAC 4266	IAC - Campinas
16	Red Catuaí IAC 144	IAC - Campinas
17	Yellow Caturra IAC 476	IAC - Campinas
18	Red Caturra IAC 477	IAC - Campinas

The samples were ground immediately prior to cupping, no more than 15 minutes before infusion with mineral pure water. Samples were weighed out to the predetermined ratio of 8,25 g of ground coffee per 150 ml of water (5,5% mass:volume). It was evaluated five cups per sample and the correspondent whole bean of each cup was ground after running a cleaning quantity of the sample through a laboratory grinder and then grinding each cup's batch individually into the cupping glasses, ensuring that the whole and consistent quantity of sample gets deposited into each cup. Sensory evaluation was performed by trained coffee tasters certified by SCAA according to the procedure described by Lingle (2001).

Ten sensory attributes including uniformity, sweetness, cleanliness and preference were assessed and rated on a 10-point scale. For the attributes fragrance/aroma, flavor, aftertaste and balance, 1=very poor and 10=outstanding, while for acidity 1=very flat and 10=outstanding and for body 1=very thin and 10=very bright. Other differentiated special attributes were also described. The data were organized and each coffee variety was analyzed by their cumulative sensory score, according to SCAA descriptions for specialty coffees.

RESULTS AND DISCUSSION

The results presented in Figure 1 showed that three varieties (Red Bourbon São João Batista, Yellow Bourbon Epamig and Red Catuaí IAC 144) presented cumulative score lower than 80 points, being classified in the not specialty coffee category. All the remaining varieties were classified in the specialty coffee category, where eleven varieties presented cumulative score between 80-82 points (Red Bourbon Procafé, Yellow Bourbon Betânia, Yellow Bourbon Castro, Yellow Bourbon Paixão, Yellow Bourbon IAC LCJ9, Yellow Bourbon Limoeiro, Yellow Bourbon Italiano, Yellow Bourbon Samambaia, Red Mundo Novo IAC 502, Yellow Mundo Novo IAC 4266 and Yellow Caturra IAC 476) and four varieties presented score over 82 points (Yellow Bourbon Daterra, Yellow Bourbon Nogueira, Yellow Bourbon Trigo and Red Caturra IAC 477).

In general, the Yellow Bourbon varieties showed beverage quality better than Red Bourbon and Red Catuaí varieties. The Red Caturra IAC 477 variety showed the highest cup quality reaching score over 83 out of 100 points in the SCAA scale. Considering that all coffees were obtained in the same environmental and processing conditions, under rigorous quality control in the post harvest procedures, it is supposed that the prominent differences in beverage quality could be attributed to the genetic effects, corroborating other results cited in literature. The predominant and subsidiary tastes and aromas, similar to chocolate, caramel, vanilla, floral, wine and fruits notes observed in some Yellow Bourbon and Red Caturra varieties that scored equal or over 82 out of 100 points confirm the high cup quality of these genotypes, indicating the possibility of genetic selection for beverage quality to attend the specialty coffee sector demand. Some of these genotypes presented real potential to be used in the future for genetic recombination and plant selection in breeding processes for coffee quality improvement.

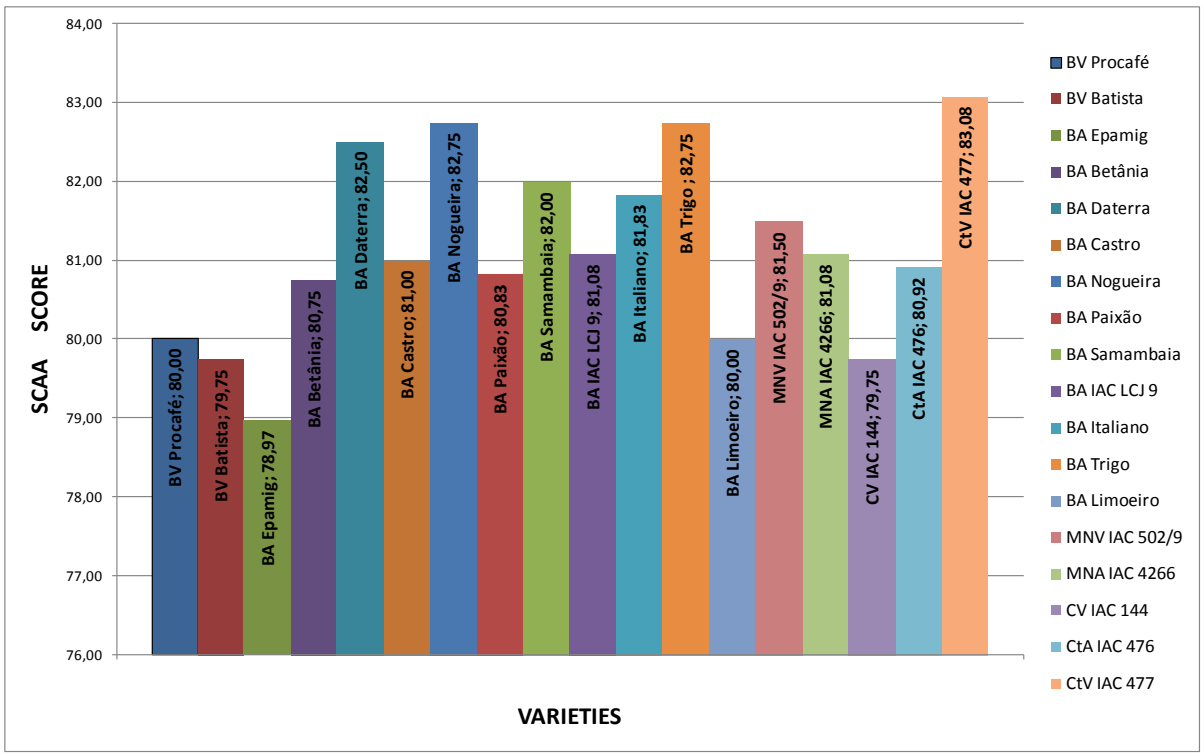

Figure 1. Cumulative SCAA score for eighteen *Coffea arabica* varieties, São Sebastião da Grama, Brazil, 2009.

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Validating the Agro-Ecological Systems of Coffee Land Evaluation in Tanzania Using the Parametric Approach

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SUMMARY

Land evaluation for coffee in Tanzania was compared between the Agro-ecological zoning system and parametric approach in Kasulu and Kibondo districts, Kigoma region. At 5% level, suitable categories showed significant variation between the two methods but the unsuitable category did not; implying that the change of method had a greater impact at the suitable range than the unsuitable range. The parametric method proved to be more detailed, and therefore more reliable, than the agro-ecological method, so it can be used with coffee.

INTRODUCTION

Land evaluation is a process whereby land is assessed for its potential or performance in relation to specific purposes. In Tanzania, coffee land evaluation was tried by Oosterom et al. (1998) based on the agro-ecological zonations of De Pauw (1984). An alternative qualitative land evaluation method, as suggested by Van Ranst (1995) is the parametric method which is common for annual crops (Behzad et al., 2009), but no record could be traced of it being used for perennial crops. The two were compared in this work, in terms of reliability.

MATERIALS AND METHODS

This work was conducted in Kasulu and Kibondo districts, Kigoma region. A total of 125 sites in 9 villages (Kasulu) and 98 sites in 8 villages (Kibondo) were surveyed according to FAO (1990) with physiographic and soil parameters studied. In method 1, the AEZ map with rainfall in isohyets was used as a base map. Data for temperature, soil type and moisture availability were compared to the requirements of coffee in a GIS environment. In method 2, climatic data were used on actual basis, and assigned ratings (R) which were used to calculate the climatic index (Ic) by the Square Root formula (Khiddir, 1986). Land index (I_L) was similarly calculated from ratings of climate, slope, drainage, flooding, soil depth and texture, CEC, SBC, OC and pH. The land was categorized as N (unsuitable) where $I_L \leq 25$; S3, S2 and S1 (marginal, moderate and suitable) where I_L ranged from 25-50, 51-75 and > 75 respectively.

Specific locations and their respective suitability categories according to Method 2 were overlaid onto the maps created by Method 1 as point themes to facilitate a visual comparison. Statistical comparison of the two methods was also done by using the Student's t-test under CoStat statistical package.

RESULTS AND DISCUSSION

In Kasulu, AEZ showed category S2 dominating at 86% of total land, followed by S3 and N (8 % and 6% respectively). In PA, categories were more widely distributed, with S2 reduced to 50% while the rest of surveyed sites are S3, N and S1 in a descending order.

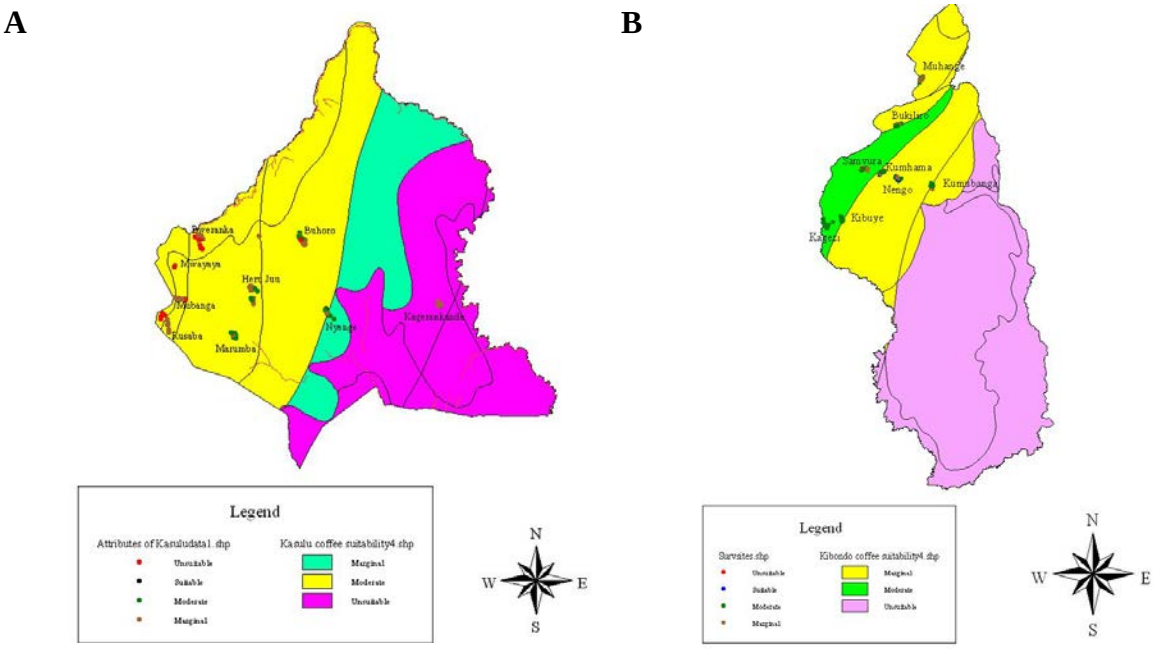

Figure 1. Caomparison of suitability categories, Kasulu (A) and Kibondo (B).

Table 1. Distribution of suitability classes in surveyed districts.

Method	District	Total sites surveyed	S1	S2	S3	N
Agro-ecological 1	Kasulu	125	0	106	10	8
	Kibondo	98	0	35	63	0
	Total	**223**	**0**	**141**	**73**	**8**
	Mean	*111.5*	*0*	*70.5*	*36.5*	*4*
Parametric 2	Kasulu	125	2	32	63	27
	Kibondo	98	9	65	23	1
	Total	**223**	**11**	**97**	**86**	**28**
	Mean	*111.5*	*5.5*	*48.5*	*43*	*14*

In Kibondo, AEZ showed only two categories S2 (36%) and S3 (64%). PA showed a wider range with dominance of S2 (67%) followed by S3, S1 and N in a descending order. The aggregation of sites into categories S2 and S3 by AEZ and a more even distribution of such sites by PA suggest that the latter is more detailed, and therefore more reliable.

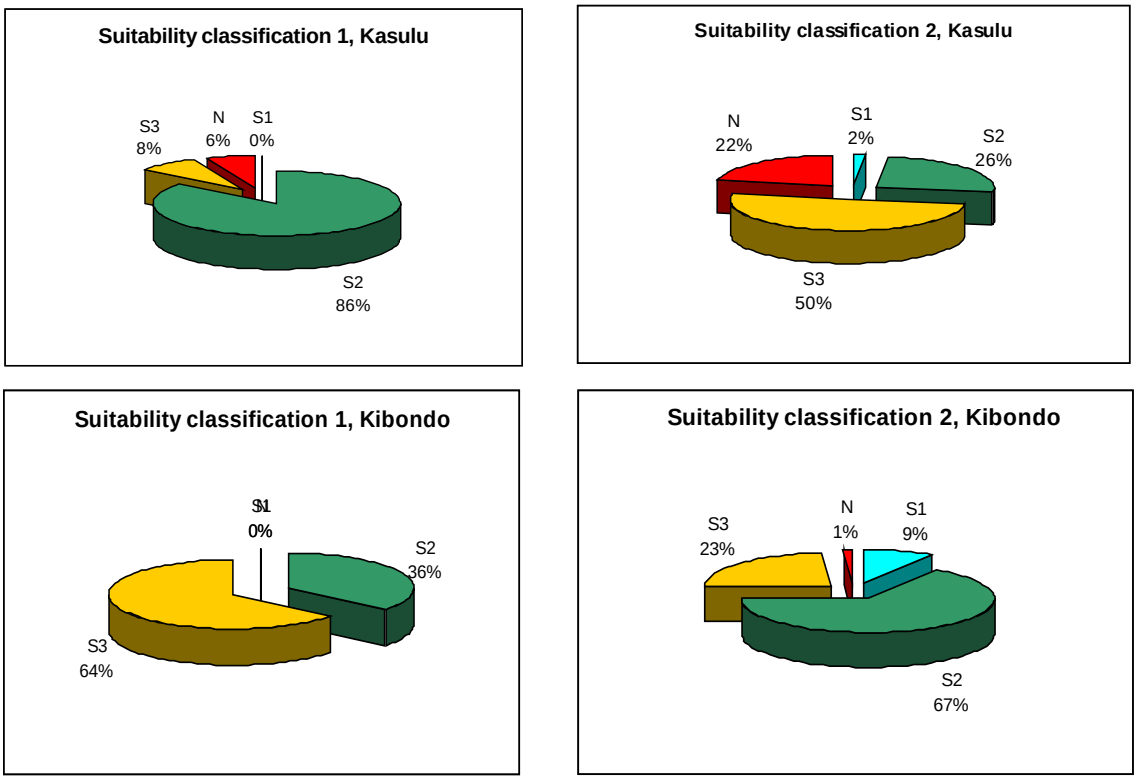

Figure 2.

Table 2. Statistical comparison ('t' test) between agro-ecological and parametric methods.

Category	S1	S2	S3	N
n (villages x methods)	34	34	34	34
Mean difference	-1.176471	5.5	3.1764706	-0.441176
SE mean difference	0.2212853	1.009977	0.876989	0.4511644
t	-5.316533	5.4456687	3.6220186	-0.977862
P (t = 0)	0.0000***	0.0000***	0.0010***	0.3353 ns

All the three suitable categories S1, S2 and S3 showed very highly significant differences between the two methods at 0.05 level, but the N category did not show any significance.

This means that the change of methodology had a greater impact at the suitable range than the unsuitable range.

CONCLUSION

This paper has shown that limitations to coffee production in Kasulu and Kibondo are mainly soil based, and, through adoption of GAP, the land will be improved to suitable and moderately suitable respectively.

The parametric method was used successfully in evaluating land for coffee production. It has proved to be more detailed, and therefore more reliable, than the agro-ecological method. It is hereby recommended for use in Tanzania with coffee and for trials in other perennials.

ACKNOWLEDGEMENT

We are grateful to the MAFSC through PADEP project for financially supporting this work. Special thanks to Messrs. JDJ Mbogoni and C.T. Shawa (ARI Mlingano), E. Mdoe and J.E.Kiwia (TaCRI Soil Fertility Lab) for their physical and intellectual support.

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Beverage Quality of Wild Ethiopian Arabica Coffee Accessions in Brazil

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SUMMARY

In this study the beverage quality of 74 samples of *Coffea arabica* genotypes including some wild coffee accessions from Ethiopia, India and Brazil was evaluated in Campinas, São Paulo State, Brazil. The objective of this study was to assess the cup quality to identify differentiated sensory attributes to coffee quality improvement in Brazil. Sensory analyses for cup quality were done according to criteria adopted for Specialty Coffee Association of America. The results showed that 51% of samples were classified like specialty coffee, where the highest cumulative score was over 85 out of 100 points for two samples and over 80 points for 38 samples. The predominant taste and aroma encountered in 39 samples permitted to classify some wild accessions in the exotic category, which distinguished them as exceptional cup quality coffees, revealing the high genetic potential of Ethiopian and Indian genotypes for the coffee beverage improvement in Brazil.

INTRODUCTION

Nowadays Brazil is the biggest coffee producer around the world and the Mundo Novo and Catuaí varieties released by Instituto Agronômico de Campinas (IAC) are predominant in all Brazilian coffee regions production, occupying at least 80% of the cultivated area. The little natural genetic variability encountered in original population derivates from Typica and Bourbon varieties has limited the selection of new coffee varieties with high beverage quality. To increase the genetic variability for quality in Brazil it was introduced several coffee varieties and accessions from Africa, India and Central America (Carvalho et al., 1962). Over 30 years of intensive studies with these accessions it was found several coffee types including many sources of resistance to leaf rust, sterile male and low caffeine content. The beverage/cup quality added to some bean physical characteristics are the most important aspects for the coffee price determination at market (Agwanda et al., 2003).

The sensory evaluation done by trained coffee tasters is essential to get information about each coffee genotype for use as a basis of selection in coffee breeding program to coffee quality improvement. It is very known that coffee quality is influenced by genotype, environmental, processing and their interactions. Than, to get reliable information about cup quality by genotype comparisons it is so important that all genotypes have been cultivated in the same site with the same post harvest processing to avoid interferences in the cup quality. In this research were evaluated and described the sensory profile of some wild Ethiopian and Indian accessions aiming to identify promissory genotypes for coffee quality improvement and to offer new perspectives to the *C. arabica* Breeding Program in Brazil.

MATERIAL AND METHODS

The study was carried out at Instituto Agronômico de Campinas, São Paulo State, Brazil, where a total of 74 *C. arabica* individual genotypes from Ethiopia, India and Brazil were evaluate. The Brazilian commercial varieties 'Catuaí Vermelho', 'Catuaí Amarelo' and 'Ouro Verde' were used as reference for quality comparisons. Each of the genotype was represented by one or more plants and cherry samples were collected during the peak harvesting period of July-September, 2009. Ripe health fruits were harvested in bulk by hand from each plant and processed individually using dry processing procedures where the coffee berries were sun dried until the moisture content reached 11% (wb). Hulled beans were classified in a screen up 14/64 inch (4.93 mm) round perforations and the beverage quality assessment was done by descriptive analysis.

Roasting of green coffee beans was done to attain a light to light-medium roast (Agtron scale of approximately 63 on ground bean, +/- 1 point) using a Probat laboratory roaster within 24 hours of evaluation and allowed to rest for at least eight hours. The roast was completed in no less than 8 minutes and no more than 12 minutes. The samples were ground immediately prior to cupping, no more than 15 minutes before infusion with mineral pure water. Samples were weighed out to the predetermined ratio of 8,25 g of ground coffee per 150 ml of water. It was evaluated five cups per sample and the correspondent whole bean of each cup was ground after running a cleansing quantity of the sample through a laboratory grinder and then grinding each cup's batch individually into the cupping glasses, ensuring that the whole and consistent quantity of sample gets deposited into each cup, according to the procedure described by Lingle (2001).

Ten sensory attributes including were assessed and rated on a 10-point scale. For the attributes fragrance/aroma, flavor, aftertaste and balance, 1 = very poor and 10 = outstanding while for acidity 1 = very flat and 10 = outstanding and body 1 = very thin and 10 = very bright. Other descriptors including uniformity, sweetness, cleanliness and preference were also described. Each genotype was analyzed and classified according SCAA cumulative score.

RESULTS AND DISCUSSION

The results showed that two of the analyzed samples (3%) were Excellent Specialty Coffees according to the SCAA scale, where the highest cumulative score was over 85 out of 100 points (Gojjan P1 and Kaffa P1). The others 38 analyzed samples (51%), including 5 Brazilian hybrids, one Indian and 32 Ethiopian accessions were Very Good Coffees", with cumulative score equal or over 80 points. The remaining 34 samples (46%) showed score lower than 80 points being classified like not specialty coffee (Table 1).

The predominant taste and aroma and subsidiary flavors encountered in 39 samples permitted to classify some wild accessions in the exotic category (citric, earthy, floral, fruity, spicy, leathery, cedarish and winey taste), which distinguished them as exceptional complexity and cup quality coffees, revealing the high genetic potential of Ethiopian and Indian genotypes for the coffee beverage improvement in Brazil.

Several genotypes and all reference commercial varieties (Catuaí Amarelo, Catuaí Vermelho and Ouro Verde) showed cumulative score lower than 80 points, indicating that these varieties could require specific environmental or processing conditions to express their potential for cup quality.

Table 1. SCAA scores for sensory analysis of *C. arabica* genotypes, Brazil, 2009.

Genotype #sample	Score	Status		Genotype #sample	Score	Status
Catuaí Vermelho 81 #5	78	CV		Kaffa #38	84	EA
Catuaí Vermelho 8 #6	78	CV		Kaffa #39	83	EA
Catuaí Amarelo 62 #4	77	CV		Kaffa #40	83	EA
Ouro Verde/6 #65	77	CV		Kaffa #41	83	EA
H97/27-3/16 #16	84	HL		Kaffa #42	82	EA
H15455-1/14 #14	83	HL		Kaffa #43	81	EA
Caturra x Geisha #7	82	HL		Kaffa #44	81	EA
S353 4/5 x BE5 #67	81	HL		Kaffa #45	81	EA
H15460-9 #15	80	HL		Kaffa #46	80	EA
832/1 (HT) #1	77	HL		Kaffa #47	80	EA
Blue Mountain x HT #3	77	HL		Kaffa #48	80	EA
Caturra x HT #9	77	HL		Kaffa #49	79	EA
S333 x Dilla & Alghe #66	77	HL		Kaffa #50	78	EA
Caturra x Geisha #8	76	HL		Kaffa #51	78	EA
Geisha #11	83	EA		Kaffa #52	78	EA
Geisha #12	80	EA		Kaffa #53	78	EA
Harar #17	84	EA		Kaffa #54	78	EA
Harar #18	83	EA		Kaffa #55	77	EA
Harar #19	81	EA		Kaffa #56	77	EA
Harar #20	78	EA		Kaffa #57	77	EA
Illubabor #21	84	EA		Kaffa #58	77	EA
Illubabor #22	83	EA		Kaffa #59	77	EA
Illubabor #23	83	EA		Kaffa #60	77	EA
Illubabor #24	82	EA		Kaffa #61	77	EA
Illubabor #25	82	EA		Kaffa #62	77	EA
Illubabor #26	82	EA		Kaffa #63	77	EA
Illubabor #27	82	EA		M7846 #64	81	EA
Illubabor #28	81	EA		Shoa #68	81	EA
Illubabor #29	81	EA		Shoa #69	81	EA
Illubabor #30	79	EA		Shoa #70	80	EA
Illubabor #31	78	EA		Shoa #71	79	EA
Illubabor #32	78	EA		Shoa #72	77	EA
Illubabor #33	77	EA		Shoa #73	77	EA
Illubabor #34	77	EA		Sidamo #74	82	EA
Illubabor #35	77	EA		Eritrea #10	82	EA
Kaffa #36	85	EA		Gojjan #13	86	EA
Kaffa #37	84	EA		BA10 #2	84	IA

Legend: CV=Commercial Variety; HL=Hybrid Line; EA=Ethiopian Accession; IA= Indian Accession

Considering that all coffee samples were obtained in the same environmental conditions and by same dry processing method, under rigorous quality control in the post harvest procedures, it is supposed that the differences in beverage quality could be attributed to genetic effects. Some accessions presented real potential for very high cup quality and could be used in the future for genetic recombination and selection in breeding programs.

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Microbiological Soil Characteristics of Areas Cultivated with Conilon Coffee under Conventional and Organic Management Systems

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SUMMARY

In Brazil Most of the *Coffea* sp. is cultivated by conventional methods. However, in recent years, organic farming is emerging and creating a new nich market for organic products. Organic agricultural products fetch higher prices and are safer for consumers, compared to conventional products. The objective of this study was to evaluate the effect of Conilon coffee (*Coffea canephora*) cultivated under conventional and organic management systems on microbiological characteristics of the soil, and an Atlantic Forest area was used as reference. Microbial biomass carbon, microbial biomass nitrogen, microbial activity, and metabolic quotient were determined at 0-10 cm and 10-20 cm soil depths in two seasons (summer and winter). The values of the studied attributes were generally higher on the top soil. In the summer the values of microbial biomass carbon and total nitrogen at 0-10 cm depth and, microbial biomass nitrogen at 0-10 and 10-20 cm depth were the same for the Atlantic Forest system and for the organic coffee system, but both were larger than conventional coffee. In the winter the highest values of Microbial activity, microbial biomass carbon, microbial biomass nitrogen and total nitrogen were found on the Atlantic Forest system. In addition, the highest values of metabolic quotient were observed under conventional coffee. Results indicate that the cultivation of coffee under organic management is more sustainable as compared to conventional system. Carbon from microbial biomass was the most important soil microbiological attribute in the clustering of the different management methods. Atlantic Forest soil, followed by organic coffee cultivation showed the best soil quality indices.

INTRODUCTION

In Brazil, most of the *Coffea* sp. is cultivated on conventional systems of production. However, in recent years, organic farming is emerging and creating a new niche market for organic products. Organic agricultural products fetch higher prices and are safer for consumers, compared to conventional products. However, there are limitations to organic production, like the difficulty to obtain the certification for organic farmers, the lack of professional assistance, research data on organic coffee cultivation and difficulties on chemical fertilizer replacement (Partelli et al., 2006). Nutrient availability or immobilization depends on soil microorganism dynamics, amount of vegetal residues and on the efficiency of

carbon utilization by the local microbiota (Baudoin et al., 2003). Microbial biomass respond rapidly to changes on organic inputs applied to the soil, determining the organic matter decomposition, mineralization and nutrient immobilization. The mineralization of organic products by microorganisms contributes for the increase in N contents, being considered of high relevance to support natural ecosystems (Conte et al., 2002).

Conventional agriculture provided progress to the agronomical sciences in terms of knowledge and yield in a short time. On the other hand, the misuse of these technologies has lead to soil and environmental degradation. The objective of this study was to evaluate the effect of Conilon coffee (*Coffea canephora*) cultivated under conventional and organic management systems on microbiological characteristics of the soil, and an Atlantic Forest area was used as reference.

MATERIAL AND METHODS

The experiment was conducted at Espírito Santo state in Brazil. The area was located at 80 m above sea level, at 18° South and 40° West. The climate is typically tropical with hot humid summers and winters with low precipitation. The annual average precipitation is around 1,200 mm, and temperatures around 12 °C in the coldest months and 34 °C in the hottest ones.

In this study it were evaluated two areas cultivated with *Coffea canephora* Pierre cv. Conilon, under conventional and organic management systems, and one area of Atlantic Forest, used as a reference. The soil was classified as sandy-clay for all experimental areas. The organic coffee area was certified at the beginning of its establishment as required by Brazilian law. The 8 year old plot was planted with vegetative stick at a density of approximately 20,000 orthotropic sticks per hectare. A 9 years old area under conventional coffee management was planted with vegetative sticks with approximately 20,000 sticks per hectare.

The areas of study were divided in 4 plots of approximately 5,000 m^2 each, representing 4 replications. Soil samples were collected (each composite sample was obtained from 12 subsamples) with a hand probe in January (summer) and July (winter), at 0-10 and 10-20cm depth. Microbial biomass carbon (MBC) was quantified by the fumigation-extraction method (Vance et al., 1987). To analyze the microbial biomass nitrogen (MBN), half of the samples were fumigated with chloroform (Wang et al., 2007). The rate of respiratory activity was obtained by Fluorescein Diacetate hydrolysis (FDA) as previously described by Das et al (Das et al., 2007).

Data from the management systems were submitted to the analysis of variance and mean comparisons were performed at the 5% level of probability by the Tukey test.

RESULTS AND DISCUSSION

Higher amounts of microbial biomass C and N (Table 1) were detected on the top soil, in agreement with many previous reports (Baudoin et al., 2003; Agnelli et al., 2004). This fact may be correlated with the higher content of total carbon and nitrogen (TC and TN) found on the surface soil layer. The highest values of metabolic quotient qCO_2 were also observed for this soil layer (Table 1), which may be related to a great use of energy to metabolize organic residues (Agnelli et al., 2004).

The indices indicating a better soil quality were found at the reference area of Atlantic Forest. This may be associated, among other factors, to the high quantity of total carbon (Table 1),

once the organic matter represents a complex system of substances influenced by the addition of organic materials of diverse nature and forms, and by their continuous transformation by biological, chemical and physical factors. Higher amounts of MBC were found in Atlantic Forest soil. The MBC may be stimulated by the continuous supply of diversified organic materials, with different degrees of decomposition susceptibility (Conte et al., 2002) originated from the native vegetative diversity.

The MBC was found in larger amount in coffee soil under organic management when compared to coffee under conventional soil management. This difference has resulted from the application of organic materials, which could have increased carbon and nitrogen contents in the soil (Table 1). According to Ouédraogo et al. (2007), enzymatic activity may also increase as well as nitrate reductase and glutamate sinthase in response to addition of nitrogen organic compounds.

The MBN was 20% lower at 10-20 cm depth compared to the top soil. Under conventional management, higher rates of qCO_2 were also observed compared to organic management system, indicating a low soil quality under conventional management. Similar results were reported by Maluche-Baretta et al. (2007), on study with apples trees (*M. domestica*) under conventional and organic management. The lower soil quality under conventional management may be related to the constant use of machinery (plough, chemical fertilization, etc), promoting soil disturbance, and affecting microbial population. This fact, associated with the lack of carbon addition and soil carbon consumption by microorganisms lead to the consumption of organic carbon and consequently to soil quality loss under conventional management. Additionally, the use of herbicides or other agrochemicals may also affect soil microbial population, reducing MBC, and increasing microbial activity and qCO_2 (Das et al., 2007).

Comparisons between conservative and conventional managements had demonstrated higher MBC content under no-tillage system, as well as under organic system when compared to conventional system management (Glover et al., 2000). Hence, the diversity and abundance of microorganisms, together with the absence of human disturbance makes possible the existence of higher contents of microbial biomass, demonstrating higher equilibrium of the soil microbiota in this environment. In complex agro-ecological systems, positive interactions among soil biota may promote improvement on soil structure and fertility, resulting in favorable environment for biological process takes place (Maluche-Baretta et al., 2007). Microbial carbon and nitrogen quotient was higher under forest soil and organic soils compared to coffee soil under conventional management (Table 1), corroborating similar results observed by Maluche-Baretta et al. (2007).

Table 1. Microbial activity (MA, μg of hydrolyzed FDA g soil^{-1} h^{-1}), microbial biomass carbon (MBC, μg C g^{-1} soil), microbial biomass nitrogen (MBN μg N g^{-1} soil), relation between MBC and MBN (MBC/MBN), total carbon (TC, g kg^{-1}), total nitrogen (TN, g kg^{-1}), relation between TC and TN (TC/TN), microbial quotient (MBC/TC and MBN/TN, (in percentage), and metabolic quotient (qCO_2, μg hydrolyzed FDA μg MBC h^{-1}), at different depths and harvesting times (January = summer and July = winter) under conventional and organic coffee and Atlantic Forest.

Treatment	Depth	MA	MBC	MBN	MBC/MBN	TC	TN	TC/TN	MBC/TC	MBN/TN	qCO_2
Conv. Jan. & Jul.	**0-20**	**136**	**80.6**	**13.5**	**7.09**	**6.23**	**0.83**	**7.49**	**1.32**	**1.62**	**2.97**
Mean standard error		7.2	13.6	2.14	1.2	0.5	0.03	0.5	0.21	0.23	0.7
Forest Jan. & Jul.	**0-20**	**257**	**199**	**48.8**	**4.17**	**12.7**	**1.33**	**9.39**	**1.77**	**3.8**	**1.45**
Mean standard error		9.94	15.9	3.69	0.25	1.49	0.07	0.85	0.17	0.38	0.15
Org. Jan. & Jul.	**0-20**	**172**	**103**	**47**	**2.32**	**7.28**	**1.04**	**6.81**	**1.53**	**4.74**	**2.31**
Mean standard error		11.5	15.9	6.75	0.25	0.81	0.07	0.54	0.22	0.72	0.38
Geral mean J & J	**0-10**	**200**	**160**	**40.5**	**5.25**	**11.5**	**1.2**	**9.47**	**1.44**	**3.29**	**1.53**
Mean standard error		11.1	15.9	5.19	0.77	1.05	0.07	0.54	0.12	0.4	0.14
Geral mean J & J	**10-20**	**177**	**95.5**	**32.4**	**3.81**	**5.92**	**0.93**	**6.32**	**1.64**	**3.48**	**2.95**
Mean standard error		14	12.9	4.48	0.59	0.44	0.04	0.35	0.2	0.53	0.49
Geral mean Jan.	**0-20**	**191**	**167**	**48.6**	**4.68**	**8.75**	**1.06**	**7.93**	**2.06**	**4.62**	**1.31**
Mean standard error		12	15.1	5.22	0.75	0.99	0.07	0.49	0.13	0.51	0.11
Geral mean Jul.	**0-20**	**186**	**87.9**	**24.2**	**4.37**	**8.72**	**1.07**	**7.87**	**1.02**	**2.16**	**3.17**
Mean standard error		13.6	12.1	2.96	0.64	0.99	0.06	0.62	0.12	0.22	0.47

ACKNOWLEDGEMENTS

The authors would like to thank the Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Universidade Federal do Espírito Santo (UFES), the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), the Instituto Capixaba de Pesquisa e Assistência Técnica (INCAPER) and to Embrapa Agrobiologia, for the financial and technical support and to Mr. Gerson Coser, for the experimental area.

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Climate Change Adaptation and Mitigation in the Kenyan Coffee Sector (Sangana PPP)

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SUMMARY

Coffee is a vulnerable crop to changes in climate. We are having trouble finding the right quantities and qualities for our markets (Philip Valentine, 2009).

Due to changing weather patterns coffee zones are already affected. Adaptation is key to securing production systems (Jeremy Haggar, 2009).

Unseasonal rains are changing affecting coffee flowering and therefore distorting our whole production cycle (Nahashon M. Nyaga, 2009).

While climate change is just one of numerous factors that may affect global coffee production, it is nonetheless likely to be one of the most important ones (ICO, 2010).

As these quotes reflect, climate change is impacting negatively on coffee production and affecting the various actors along the coffee value chain. **GTZ**, on behalf of the German Ministry for Economic Cooperation and Development, and **Sangana Commodities Ltd**. are implementing a three-year **Development Partnership** (Sangana PPP, 2010) between October 2008 and September 2011 in order to improve the Kenyan coffee sector's capacity to adapt to climate change and incorporate climate change mitigation where possible. The aim is to create a standard component which allows coffee producers to adapt their production to the changing climate and to create and use synergies between adaptation and mitigation means.

BACKGROUND

Climate change is affecting coffee production and the livelihoods of producers all over the world. Climate impact models predict that Kenya will suffer severe consequences from a warming of temperatures such as prolonged drought periods (Climate Change, 2007). One of the economic sectors most affected by these changes is agricultural production. Due to the gradual impoverishment of agricultural systems to a large extent caused by changes in climatic conditions, coffee production has declined, negatively affecting the incomes of coffee farmers. Taking Kenya as an example coffee production has declined from 892kg/ha in 1980 to 284 kg/ha in 2008 (Food and Agriculture Organization of the United Nations Statistical Database). Export volume has dropped from 2.1 million bags in 1987 to 0.9 million bags in 2007 (ICO, 2008). Climate change is not the only reason for this decline, but in recent years especially has contributed to it substantially.

The variables impacting most on coffee quantity and quality are temperature and precipitation.

Increasing temperature	Changing precipitation patterns
Reduced photosynthesis Increasing pest incidents Erosion provoked by prolonged droughts Change in planting periods reducing growing times and leading to smaller yields Move of current suitable production areas to higher altitudes 	Landslides and floods destroying infrastructure Due to lack of rain: coffee husk sticking to the bean hindering maturation Loss of soil, plants and homes through landslides caused by heavy rains and extreme weather events Reduced rainfall decreasing coffee quality, 150 mm per month needed during flowering and maturation Rains during harvest season reducing coffee quality by hindering the drying process Increasing strong winds causing the falling of coffee flowers and changing pollination

Figure 1. Variables impacting on coffee (Resume of different publications by CATIE, Colombia and Costa Rica; CIAT, Nicaragua and others; AdapCC 2008).

Greenhouse gases (GHG) are major contributors to climate change. Burning of fossil fuels and clearing of land are key contributors to increasing GHG emissions. Agricultural land use systems and the application of sustainable agricultural practices help to prevent climate change by storing these emissions within production systems. In this way, coffee producers can contribute to mitigation of climate change – even through small scale farming.

This is why the project proposes to work on climate change in the coffee sector with a focus on adaptation creating synergies to mitigation where possible.

THE PROJECT

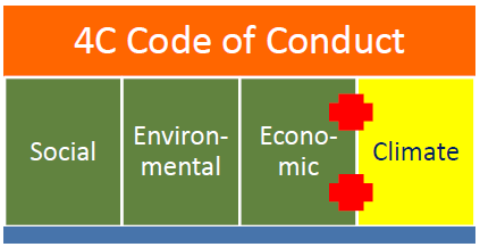

Figure 2.

Sangana Commodities Ltd., the Kenyan subsidiary of the ECOM Group, has joined forces with GTZ as they were already feeling the effects of climate change on their coffee supply chains with negative impacts on quantity and quality. Besides these main partners the project cooperates with further strategically important additional partners such as the **4C Association**, the **World Bank**, **Tchibo GmbH**, **Rainforest Alliance**, **Efico**, and **Sustainable Management Services Ltd**.

The aim is to support coffee producers to adapt their production to the changing climate and to create and use synergies between adaptation and mitigation means. This goal will be reached by developing an additional component to the existing 4C Code of Conduct taking into account climate aspects. The 4C Association already has three components (social, environmental, economic) in its Code of Conduct. The Sangana PPP develops an additional (add-on) voluntary component which means any coffee producer group opting for the 4C standard will have to gradually comply with the existing three components whereas they can opt to comply with the fourth one: the climate component. This climate component consists of agricultural practices for adaptation and mitigation, trainings for producers and verifiers, verification instruments and a climate data base and will be tested together with the Baragwi Farmers' Cooperative Society Ltd. as a pilot group.

THE ADD-ON CLIMATE MODULE

The add-on standard module to be developed within the framework of the Sangana PPP is aiming to take into account climate change adaptation and mitigation within the coffee production. This means it is aiming to a) ***enable coffee producers to adapt*** their production systems to changing climatic conditions and to b) ***promote adaptation options which at the same time have mitigation effects***, i.e. reducing or removing greenhouse gases.

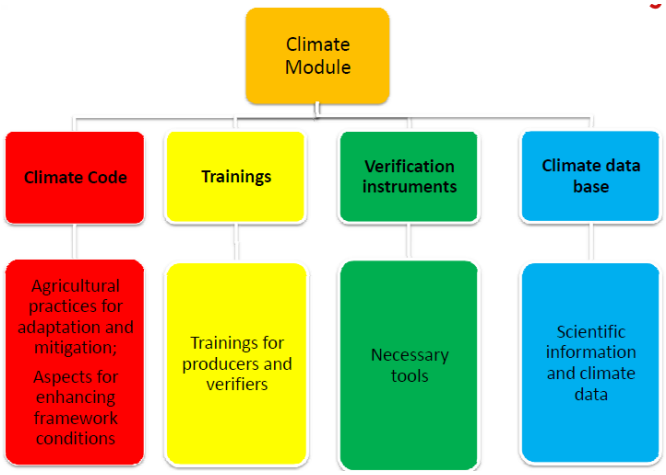

Figure 3.

The ***module*** is planned to consist of ***four main pillars***:

- A ***Climate Code*** stating principles and measurable indicators for adaptation and mitigation
- Sensitization and ***trainings*** for producers and verifiers
- ***Verification instruments***
- A ***climate database*** creating access for producers and verifiers to relevant climate information

The "heart" of the module is the Climate Code which defines principles, i.e. a desired status related to climate change adaptation and mitigation, as well as indicators which enable credible measurement of the principles. This ***code*** consists of ***four main components***:

- Enabling environment
- Natural Resource Management
- Soil and Crop Management
- Energy, GHG and carbon stocks

Example
Principle: Water resources are conserved and water harvesting mechanisms are explored.
Indicator: Water conservation and harvesting options have been explored; a water conservation plan has been developed and put in place.

Figure 4.

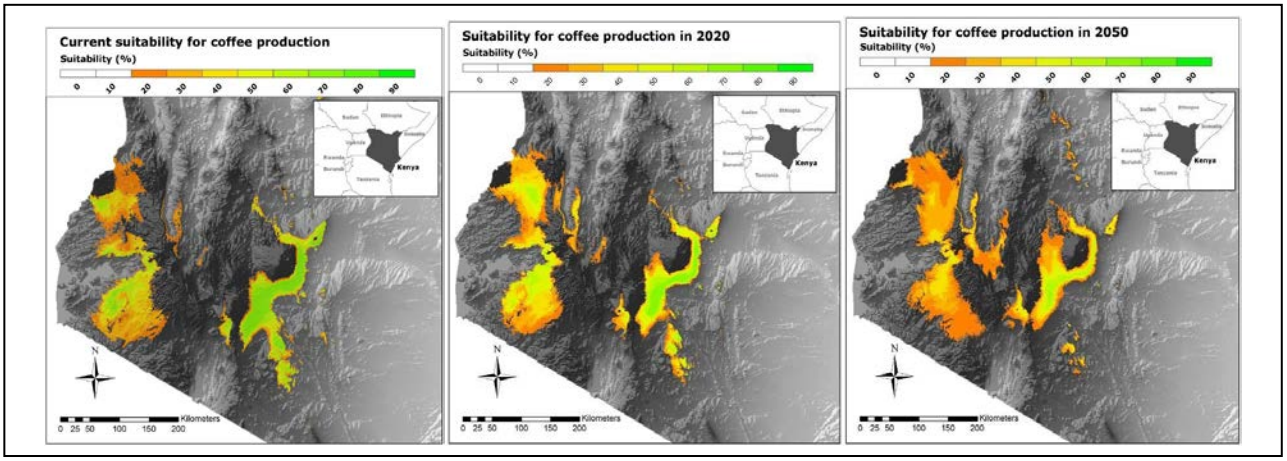

Figure 5. Suitability change of Kenya's coffee growing regions (International Centre for Tropical Agriculture (CIAT), Sangana Commodities Ltd., GTZ, January 2010). By 2050 Kenya will have less seasonality in its climate and maximum mean temperature is predicted to increase to 31.2 °C (currently 28.6 °C), minimum mean temperature increases to 12 °C (currently 9.8 °C). Coffee currently grown at 1300 masl will suffer most under climate changes by 2050 whereas coffee at 2200 masl will benefit most. The optimal coffee producing zones will shift from currently 1600 masl to higher altitudes at 1700masl. In general the suitability of the coffee regions will decrease. Current suitability for coffee production is between 50 to 70%, by 2050 suitability is predicted between 30 to 60%.

THE PROGRESS

Since project start in October 2008 several milestones have been reached. The project started working with the KOMOTHAI Coffee Farmers' Cooperative Society Ltd. as pilot group. The World Bank's BioCarbon Fund was developing a carbon finance project together with KOMOTHAI focusing on soil organic carbon and the Sangana PPP was developed in order to support the producers in the implementation on the ground. Within this framework the Sustainable Agricultural Land Management carbon methodology was developed and presented to the Voluntary Carbon Standard for approval. The methodology has undergone first validation and is waiting for second validation. For KOMOTHAI the carbon as well as the socio-economic baseline has been developed. Trainings for producers on good agricultural practices have been designed and implemented. Climate specific sensitization meetings have

been held and climate risks and vulnerabilities of the pilot group have been identified. At the same time the climate code has been developed and the project elaborated future climate scenarios for Kenya's coffee growing regions.

Since October 2009 the project is collaborating with a project by Efico, the Rainforest Alliance, Anacafé and the University del Valle of Guatemala aiming at designing an additional voluntary climate module to the standard of the Sustainable Agriculture Network (SAN). Due to the clear overlaps in objectives the two projects are working together to harmonize their approaches and results. Following the strategy of the 4C Association, the 4C climate module is likely to serve as a stepping stone for verification under the SAN climate module.

In May 2010 the PPP started working with a new pilot group, the Baragwi Farmers' Cooperative Society Ltd. Baragwi is undergoing certification under the SAN standard and is at the same time receiving sensitization and training measures for the 4C climate module. This will allow testing the stepping stone approach in the long run. The Sangana PPP developed several trainings and participatory workshops. Building on experience and lessons learned from a Public-Private Partnership between GTZ and Cafédirect plc called "Adaptation for Smallholders to Climate Change" (AdapCC: www.adapcc.org) the Sangana PPP came up with a 2-day participatory workshop in order to analyze climate vulnerabilities and risks of coffee smallholder organizations. Furthermore, AdapCC delivered the base for developing a manual for training trainers on climate change adaptation in the Kenyan coffee sector. In August 2010 the Sangana PPP tested a 3-day on-farm carbon monitoring training which is currently being further defined. The project is also looking into developing a coffee specific GHG calculator together with Sustainable Food Lab. For the rest of 2010 the verification of the 4C climate module will be looked at. The idea is to combine the GHG calculator with further verification aspects on the adaptation issues covered in the climate code. Therefore each verification of the climate module, which shall be carried out together with normal verification under the 4C standards, will also deliver indications on emission hotspots and changes of emissions caused and GHG stocks sequestered in the coffee ecosystem.

By the end of 2010 a first test verification under the 4C climate module is scheduled with Baragwi and a final verification is planned for by July 2011.

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Resume of different publications by CATIE, Colombia and Costa Rica; CIAT, Nicaragua and others; AdapCC 2008

Until mid 2010 Development Partnerships were called Public-Private Partnership, the present project is called Sangana PPP

Standardization of Optimum Planting Densities and Training Methods for Dwarf Arabica Genotypes Grown under Shaded Canopy

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SUMMARY

A field experiment was conducted during 1996-2009 at CCRI research farm in split-split plot design for standardization of optimum planting densities and training methods for two dwarf arabica genotypes viz; S.4634 and Cauvery.

Among the two varieties, irrespective of variations in planting densities and topping heights, the S. 4634 recorded significantly higher yield of 1182 kg Clean Coffee ha^{-1} when compared to Cauvery (969 kg cc ha^{-1}) over 10 cropping seasons.

Among the planting densities, the coffee planted at closer spacing of 5 X 5' (4000 plants/ha) recorded significantly higher yield for ten years (1264 kg cc ha^{-1}) as compared to other spacings of 6X5' (3333 plants/ha) and 6X6' spacing (2777 plants/ha) (1063 and 900 kg ha^{-1} respectively).

With respect to training methods the pooled data over the 10 years indicated that the coffee plants trained on single stem and topped at two tiers (2.5' and 5') recorded significantly superior yield (1125 kg cc ha^{-1}) as compared to plants trained on single stem and topped either at single tier (3.5') or at three tiers (2.5', 4.0' and 5.0') which recorded 1057 and 1047 kg ha^{-1} respectively.

The interaction effects between spacing and topping was found to be non-significant in both varieties (S.4634 and Cauvery). The results revealed that a plant population of 4000 plants/ ha and training on single stem with two tier topping is ideal for dwarf Arabica varieties under natural shade grown conditions in India.

INTRODUCTION

In India mainly two varieties of coffees are grown. They are *Coffea arabica* and *Coffea robusta*. Both these coffees are grown under mixed shade and managed under single stem method of bush management. Under the single stem method of plant management, the important operations involved are training and pruning.

Training basically refers to allowing the plant to have a single stem or multiple stems. In India single stem method of training is followed. Under this method vertically growing main stem is cut two inches above the node at the prescribed topping height. After topping to prescribed height one of the top most primary branches is cut beyond the basal node to prevent splitting of main stem due to crop load.

The recommended topping heights for the two varieties of coffee are as follows.

- Tall arabica varieties
- First tier – 2.5 ft
- Second tier – 4.5 to 5.0 ft
- Dwarf arabicas – 3.5 ft
- Robusta – 3.5 to 4.0 ft

While pruning, refers to removal of old unproductive branches, criss cross branches, lean, lanky and whippy wood, diseased and damaged branches as well branches growing towards main stem and ground.

With advent of the newer genotypes S.4634 released by the Central Coffee Research Institute (CCRI), Chikmaglur one of the prime objective is to standardize the agronomic practices such as right spacing and topping /capping height in comparison with the standard recommended practices of the ruling varieties (Cauvery).

Keeping this in view on the subject of standardizing the right management practices for the newer variety the following experiment was taken which is discussed as follows.

MATERIAL AND METHODS

A field study was conducted during 1996 at CCRI farm located at an altitude of 900 m above mean sea level, 13° 21'00'' N Latitudes and 75° 25' 45'' Longitude, under cool humid climate with mean annual rainfall of about 2792 mm. Soils of the study location is traditionally red non-gravelly clay with surface texture being sandy clay loam and taxonomically placed under Mollic Kandiudalfs. The soil characteristics of the site are shown in Table 1.

The experiment was laid out during the year 1996-97 in NMS-2 block of Central Coffee Research Institute farm by replanting the existing old Arabica in double split plot design where varieties was taken as main plot, spacing as sub plot and topping height as sub-sub plot. The details of the experiment includes two varieties viz., V1- Cauvery and V2- Hemavathy (S.4634); three spacing S1 = 5'x5', S2 = 6'x5' and S3 = 6'x6' and three topping heights T1 = single tier at 3.5'; T2 = 2-tier topping at 2.5' and 5'; T3 = 3-tier topping at 2.5', 4' and 5'.

The main objective of an experiment is to evaluate the performance of new Arabica genotype Hemavathy (S.4634) in comparison with the semi tall Arabica genotype variety Cauvery and also to standardize the spacing and topping height for new genotype Hemavathy (S4634) .

Table 1. Soil characteristics of the location.

Sand (%)	Silt (%)	Clay (%)	Texture	BD (M g M^{-3})	pH	OC (%)	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})
56	15.05	28.95	Scl	1.05	5.8	1.8	90	357

At the time of planting the seedlings were planted as per the treatment under different spacing S1 = 5'x5', S2 = 6'x5' and S3 = 6'x6'.

The different tier of topping treatments was imposed as the plants grew well and sturdy. The first treatment of topping T1- single tier topping was imposed when the plant completed three years (1999), the second treatment of topping T2- two-tier was imposed when the plants were at sixth year (2002-03) and subsequently the third treatment T3- Three - tier topping was imposed when the plants were at ninth year (2006-07).

Except for the imposition of treatments, both the varieties were grown as per the standard package of practices for coffee production in India (Anon, 1996).

RESULTS AND DISCUSSION

The results revealed that spacing had a significant impact on coffee yield during all the years of the experiment, except during the year 2007-08 which was non significant from the Table 2.

Among both the cultivars of Arabica, Hemavathi (V1) and Cauvery (V2), the mean yield recorded over the years was statistically significant in Hemavati variety (1182 kg ha^{-1}) than Cauvery (969 kg ha^{-1}). Except during and after the year of imposing topping treatment (1999-2000) (2002-03) and (2007-08) non significant results were recorded, this may be due to the shock undergone by the plant during topping treatment imposition.

Accordingly, highest and lowest mean clean coffee yield of 1264 and 900 kg ha^{-1} were recorded at closer spacing of (5' X5') and widest spacing (6'X6') respectively. Coffee yield tend to increase linearly with population and over years and most of the cropping season.

Moreover the effects of training methods on coffee yield were also considerable. When the coffee plant was toped for the first tier during 1999-2000 which was a general treatment of first tier for the plants under the experiment the results were not significant. Only after imposition of the treatments T2 = 2-tier topping at 2.5' and 5'; T3 = 3-tier topping at 2.5', 4' and 5' during 2002-03 and 2006-07 the treatment effects registered significant differences over the capped treatments across the years. Significant difference in crop yield over the different topping treatments was observed due to crop years, where highest yields were recorded during the year 2007/08 and 2008/09 under second tier topping (1185 and 1125 kg ha^{-1}) respectively.

The combined effect of different plant densities (spacing) and topping was statistically non-significant. More yield advantage was noticed as population density increases from 2777 to 4000 plants ha^{-1}.

The above results can be corroborated with the findings of Kufa Taye et al. (2001) and Baldwin (2007) where in, the yields of arabica coffees under different population densities respond significantly. Further, the yield responses is remarkable to closer spacing in different crop season and the yield increase with increasing plant population densities. Such impact of close spacing on coffee yield performances under different spacing or population densities is largely associated with the prevailing climatic factors that determined the rate of vegetative growth which is most suitable in India for growing Arabica coffee in close spacing.

Table 2. Coffee yield (Kg ha^{-1}) as influenced by varieties, planting densities and training methods in dwarf Arabica genotypes grown under shaded canopy during 1999 to 2009.

Treatments	1999-2000	2000-2001	2001-2002	2002-2003	2003-2004	2004-2005	2005-2006	2006-2007	2007-2008	2008-2009	Pooled
Varieties											
V1	1167	842	1496	442	1436	943.7	1595	1486	1146	1267	1182
V2	1198	745	1098	472	1141	789.8	1162	1085	1127	874	969
CD @ 5%	NS	NS	389	NS	159	NS	274	94	NS	NS	142
Planting densities / Spacing											
S1	905	940	1811	574	1757	961	1710	1524	1157	1302	1264
S2	1387	801	1201	106	1196	945	1257	1212	1189	1036	1063
S3	1255	639	879	391	914	694	1169	1121	1064	873	900
CD @ 5%	79	31	330	78	111	143	204	175	NS	223	47
Topping height											
T1	1282	790	1250	578	1669	837	1015	1268	900	980	1057
T2	1185	804	1385	404	1096	940	1637	1382	1229	1185	1125
T3	1080	786	1256	389	1102	823	1484	1207	1280	1045	1045
CD @ 5%	78	NS	NS	42	71	NS	213	NS	124	120	47

Note: Varieties- V1= Hemavathi; V2 = Cauvery; Planting densities - S1= 5X5 feet; S2= 6X5 feet; S3= 6X6 feet; Topping height – T1= 3.51 (single tier) T2 = 2.51 and 51 (two tier); T3 = 2.51, 4.01 and 5.51 (three tier)

CONCLUSION

The study indicated that irrespective of the variety, planting at closer spacing of (5' X 5') with plant population of 4000 plants/ ha and training on single stem with two tier at 2.5' and 5' recorded significantly superior yield and indicating long lasting efficiency of close spacing with two tier system of topping in India for Arabica varieties.

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Study of Spacing and Fertilizer Requirement on Compact Coffee Varieties in Tanzania

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SUMMARY

A study was conducted to evaluate spacing and fertilizer requirements on new compact coffee varieties released by the Tanzania Coffee Research Institute (TaCRI). It was done in a split plot design, treatments were replicated four times; with 4 different spacing as main plot (2.0 m x 1.5 m, 2.0 m x 1.0 m, 2.0 m x 1.25 m and 1.5 m x 1.0 m) and 4 different fertilizer regimes as sub-plot (75 g of NPK 20:10:10 (recommended rate per tree) 112.5 g, 150 g, and 37.5 g of the same + 1 tin FYM) the latter being superimposed onto the trial during the second year after planting. Coffee yield (kg ha^{-1} parchment) and economic returns were characteristics evaluated. The analysis of variance showed significant variability in yield for different spacing used ($p < 0.05$), coffee planted at 1.5 m x 1.0m and 2.0 m x 1.0 m yielded significantly higher than coffee planted at 2.0 m x 1.25 m and 2.0 m x 1.5 m. There was no significant variation ($p > 0.05$) for different fertilizer levels used. The economic analysis showed substantial profit in the early harvest year ensuring an early large return to the investment costs. Based on the results obtained the new compact coffee varieties can be planted at high density to increase yield, with reduced costs of production which are desirable factors for the sustainability of coffee sector in Tanzania. These are preliminary findings, more data will be collected in the coming seasons to consolidate these results.

INTRODUCTION

Coffee contributes to around 23% of the total export earnings with over 450,000 smallholders depend on this crop for income in Tanzania (Chimilila et al., 2008). In the past years the Tanzanian coffee farmers used to grow traditional tall coffee varieties N39, KP162, KP 423 and H.66. These are susceptible to coffee berry disease (CBD) and coffee leaf rust (CLR) which are mainly managed by chemical means, using inorganic fungicides (copper-based fungicides) as well as host plant resistance (Waller, 1982). But the use of inorganic fungicides is very expensive especially to smallholder resource-poor farmers and is also not environmentally friendly (Teri et al., 2004). Recently TaCRI has released nine improved coffee varieties with high yielding potential and resistance to CBD and CLR; efforts have started to multiply and distribute them quickly through clonal seedlings or grafting (Nzallawahe et al., 2004) so as to replace those traditional varieties in as short time as possible.

Further to the already released varieties, TaCRI is currently developing compact coffee varieties these are intermediate stature plants with durable resistance to CBD and CLR and yield better than the traditional varieties while retaining the exceptionally good beverage quality the traditional varieties are renowned for (TaCRI 2008). Another advantage of the new varieties is an optimum density (more plants per unit area) hence higher yield especially in the early harvest years (Njoroge, 1991; van der Vossen and Walyaro, 1980). Agronomic

recommended packages to go with those compact coffee varieties are not yet in place. There is a need to develop suitable recommendations which will make coffee farming using these new compact varieties cost effective, ensuring high production per unit area. Therefore the objectives of the present study were: - **(**1) To determine N.P.K fertilizer requirement for different spacing of coffee. (2) To determine the suitable spacing. (3) To determine the cost-benefit analysis of the compact varieties.

MATERIALS AND METHODS

An experiment was carried out during 2005-2009 at TaCRI in a split plot design; with 4 different spacing as main treatments and 4 different fertilizer regimes as sub-treatments, the latter being superimposed onto the trial during the second year after planting.

Table 1. Main plot and Sub-plot treatments.

S/No.	Spacing (main plot):	NPK fertilizer levels (sub-plot)
1	S1. 2.0m x 1.5m planting density 3,333tree/ha	F1: 75 g of NPK 20:10:10 (recommended/tree)
2	S2. 2.0m x 1.25m planting density 4,000tree/ha	F2: 112.5 g of the same
3	S3. 2.0m x 1.0m planting density 5,000tree/ha	F3: 150g of the same
4	S4. 1.5m x 1.0m planting density 6,667tree/ha	F4: 37.5g of the same + 1 tin FYM

DATA COLLECTION AND ANALYSIS

Coffee yield (parchment) data in T/ha and income against cost of inputs were collected. The collected data were analyzed using GENSTAT software.

RESULTS AND DISCUSSION

Effects of spacing and fertilizer levels

Mean coffee yield at the second year after transplanting shows a significant difference ($p < 0.05$) for different spacing used but no significant difference ($p > 0.05$) for different fertilizer level (Appendix 1).

Plant density had a positive effect on yield/unit area with those planted at higher density resulting in an increased yield (Figure 1). The best density guarantees optimum light interception, and this has been associated with coffee tree populations that produce complete ground cover (Kuguru et al., 1978). A density of 5000 trees/ha was identified as a biological optimum for arabica coffee by Browning and Fisher (1976), which also conform to these results. However the difference in yield between 5000 tree/ha and 6,667 trees/ha was not statistically significant although there was a higher yield at the higher density. Therefore a population of 5,000 could be optimum depending on the management of the field.

Soil test from this area before establishment indicates optimum N, P and K with a pH of 6.2 However, from the small difference in yield between the higher and lower doses of NPK fertilizers (Figure 1), it is advisable to apply a recommended dose or half recommended dose

plus one tin of FYM to protect the trees from exhaustion as compact varieties a heavy bearer. Data presented here are only for the second year of harvesting therefore more observations will be made in the coming seasons.

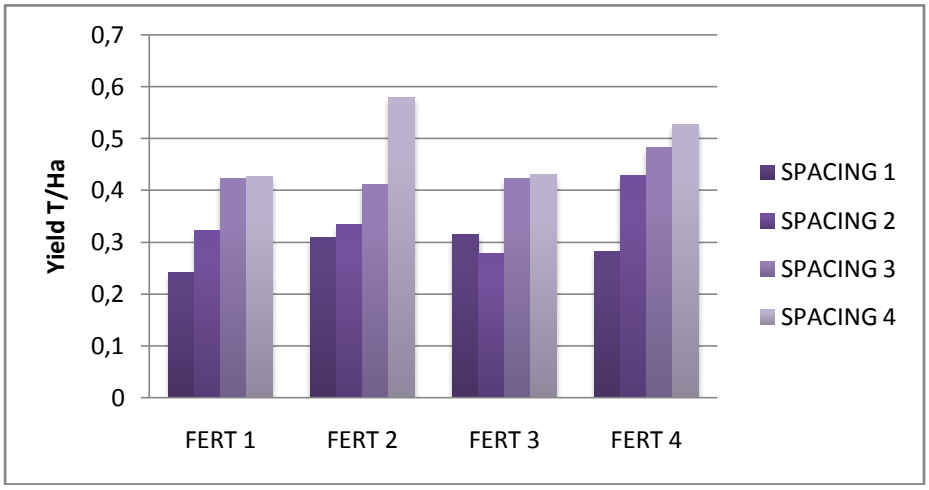

Figure 1. Yield in T/Ha for different spacing and fertilizer levels.

Income against cost of inputs

Studies on use of NPK fertilizers for different spacing of compact coffee varieties shows that the total variable cost in first year was $511. Whereas the total variable costs in the second year was $961, Total revenue was $ 2,500. This gives a gross margin of $1,539 for the second year. These findings showed substantial profit in early harvest years ensuring an early large return to the investment costs (Appendix 2).

CONCLUSIONS

The results obtained on the use of different spacing on compact coffee varieties showed that there is higher yield at the higher density, but a density of 5,000trees/ha can be optimum for compact coffee varieties. However more work still needs to be done to confirm the above findings as the data presented here are only for the second year of harvesting.

Until the second year of harvesting, application of different levels of NPK fertilizers did not significantly affect the yields of clean coffee; therefore a recommended dose can be used or half a recommended dose + 1tin of FYM. More data on various characters of soil will be accumulated and processed to confirm this result.

ACKNOWLEDGEMENT

The authors are grateful to the Tanzania coffee growers and European Union for the financial support. Also to Mr. Harrison Monyo for his interest in the work and supervising field activities which makes this work possible.

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Appendix 1. Summary statistics for different Spacing and Fertilizer regimes.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	3	0.0016172	0.0005391	0.36	
rep.spacing_1 stratum					
spacing_1	3	0.0250172	0.0083391	5.63	0.019
Residual	9	0.0133391	0.0014821	2.19	
rep.spacing_1.fert_1 stratum					
fert_1	3	0.0036797	0.0012266	1.81	0.163
spacing_1.fert_1	9	0.0044766	0.0004974	0.73	0.676
Residual	36	0.0244187 0.0006783			
Total	63	0.0725484			

Appendix 2. Cost-Benefits analysis for year 1 and year 2.

Activity	Unit	Quantity	Year 1	Year 2
Land preparation	Man days	50	61	
Diesel	Lts	20	17	
Sisal twine	Kg	6	9	
Material preparation	Man days	50	61	
FYM	Trailer		86	64
Planting	Man days	75	91	
NPK	Bags		75	386
Managing trial				
Roundup herbicide	Lts		17	46
Slashing	Man days		24	
Insecticide (Selecron)	Lts		13	89
CAN 27%N	Bags	2	36	
Weeding	Man days		36	
Polyfeed 19:19:19	Kg		9	26
Chemical and fertilizer application	Man days		12	45
Pruning	Man days	50		90
Irrigation	Man days	30		54
Picking	Man days	90		161
Total costs in USD ($)			**511**	**961**
Production per tree (Kg)				0.5
Production per ha (Kg)				3,333
Price per Kg of parchment in USD **(**$)			1.5	1.5
Total revenue ($)			**0**	**2,500**
Gross margin ($)			**-511**	**1,539**

Integrating *Cedrela odorata* Trees into Robusta Coffee Production in Ghana as a Diversification Strategy – Establishment Phase

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SUMMARY

The initial growth of clonal Robusta coffee (*Coffea canephora* Pierre ex Froehner) grown in association with *Cedrela odorata*, a commercial timber species, was assessed in a field experiment in Ghana. The objectives of the trial were to evaluate *C. odorata* as shade for coffee and eventually assess its potential in generating additional income for coffee producers. The experimental treatments consisted of planting *C. odorata* stumps at densities of 434 plants ha^{-1} (T1); 434 plants ha^{-1} to be thinned to 217 plants ha^{-1} in the 8^{th} year (T2); 217 plants ha^{-1} (T3); 434 plants ha^{-1} to be thinned to 108 plants ha^{-1} in the 8^{th} year (T4); or 192 plants ha^{-1} (T5). *Gliricidia sepium* cuttings, planted at 192 plants ha^{-1} but to be thinned to 48 plants in the 4^{th} year served as the control (T6). The *C. odorata* and *G. sepium* were planted one year ahead of the coffee clones. The coffee clones were planted at a density of 1736 plants ha^{-1} in all the treatment plots. The experiment was designed as randomized complete block with 6 treatments and 4 replicates. The coffee plants did not show significant differences ($P \leq 0.05$) in girth and height between the treatments after 26 months in the field. There were no significant differences between the treatments in the number of laterals and length of laterals of the coffee plants at 26 months after transplanting. Percentage transmitted light recorded in the treatments at 38 months after transplanting *C. odorata* did not show significant differences even though less light was transmitted in Treatments 1 and 2 which had higher densities of *C. odorata*. Initial data from this trial indicate that Robusta coffee can be successfully established in association with *C. odorata* trees but the plant density that could be grown with coffee to guarantee optimum yield would be determined after data collection in the reproductive phase.

INTRODUCTION

Robusta coffee was introduced into Ghana in the mid-eighteenth century and was cultivated in smallholdings at some specific locations in the south eastern part of Ghana. Coffee generally thrives well in the forest zones of Ghana as well as areas classified as marginal for the cultivation of *Theobroma cacao*, the major economic tree crop in Ghana. In its efforts to diversify the sources of revenue, the government of Ghana instituted several programmes to promote coffee cultivation between the late seventies and the mid nineties culminating in the establishment of some large plantations and many smallholdings. The collapse of coffee prices between 1999 and 2004 however reversed the successes chalked and most farmers either abandoned their coffee farms or converted them to other crops.

However, the current dynamics in the supply and demand structure in the world coffee trade which offer some hope for sustained price stability over a considerable period should be motivational enough for the rehabilitation or replanting of abandoned farms. In order to entice farmers back into coffee production, several strategies that can buffer then against future price

fluctuations have been suggested (Greenhalgh et al., 2006) and among them is diversification to generate additional income from alternative plants while maintaining the coffee trees. Greenhalgh et al. (2006) grouped the options available for coffee farmers as *vertical* or horizontal diversification. Vertical diversification was described as measures that aim at moving the producer up to capture a higher proportion of the value chain which includes increased productivity and overall returns from the land. This implies that complementary income could be generated by farmers through the introduction of new crops/plants without eliminating the coffee trees. The same authors described horizontal diversification as investment in alternative crops or products including non-agricultural products.

Vertical diversification, specifically introduction of other plants in coffee cultivation is one of the options that could be conveniently considered for adoption by coffee farmers in Ghana. *C. odorata* is one of several timber species that could be integrated into coffee cultivation to provide shade and alternative source of income for coffee farmers.

In Ghana, it is recommended that coffee is cultivated under light and medium shade for optimum growth and yield (Amoah et al., 1999). Apart from some timbers species, *G. sepium* is the main fast growing tree that has been recommended for the provision of shade for coffee in Ghana and some biological benefits of this association has been reported (Anim-Kwapong et al., 1999). *C. odorata* is a fast growing high value timber tree that could be exploited for commercial purposes from as early as fifteen years after cultivation (Lamb, 1968). It has for a long time been used as an avenue tree in South America, Africa and the West Indies and has been reported as a useful shade tree in coffee plantations in South America (FAO 1986). This paper reports on the initial establishment phase of integrating *C. odorata* into coffee cultivation in Ghana.

MATERIALS AND METHODS

The following treatments were compared on a 1.4 hectare plot at the Cocoa Research Institute of Ghana substation at Afosu.

- Treatment 1 – Clonal Robusta coffee planted with *C. odorata* at a density of 434 plants ha^{-1}.
- Treatment 2 – Clonal Robusta coffee planted with *C. odorata* at a density of 434 plants ha^{-1} to be thinned to 217 plants ha^{-1} in the 8^{th} year.
- Treatment 3 – Clonal Robusta coffee planted with *C. odorata* at a density of 217 plants ha^{-1}.
- Treatment 4 – Clonal Robusta coffee planted with *C. odorata* at a density of 434 plants ha^{-1} to be thinned to 108 plants ha^{-1} in the 8^{th} year.
- Treatment 5 – Clonal Robusta coffee planted with *C. odorata* at a density of 192 plants ha^{-1}.
- Treatment 6 – Clonal Robusta coffee planted with *G. sepium* at a density of 192 plants ha^{-1} to be thinned to 48 plants ha^{-1} in the 4^{th} year (Control).

The *C. odorata* stumps and *G. sepium* cuttings were planted one year ahead of the coffee clones in July 2007. Plantains were planted in the entire plot at a density of 1736 plants ha^{-1} in September 2007. Each plot size measured 24 m x 24 m containing 100 coffee plants. The experiment was designed as randomized complete block with 4 replicates.

Data recorded during the establishment phase of the trial included the fertility status of the soil at the beginning of the trial and at yearly intervals, growth of the coffee and *Cedrela* plants, mortality of the coffee plants, incidence of pests and diseases on both the coffee and *Cedrela* plants as well as light interception.

RESULTS AND DISCUSSION

The coffee plants did not show significant girth and height differences between the treatments, 6 months after transplanting. Significant differences ($P < 0.05$) in the girth of the coffee plants were however recorded at 9 months after transplanting but these differences in girth disappeared at 15, 22 and 26 months after transplanting (Figures 1 and 2). Similarly, there were no significant differences ($P \leq 0.05$) in the number of laterals and length of laterals of the coffee plants at 26 months after transplanting (Figures 3 and 4). There were no significant differences in the girth and height of the *C. odorata* plants at 18, 24, 30 and 38 months after planting (Figures 5 and 6). Percentage light transmitted through the canopies of the *C. odorata* and *G. sepium* plants, recorded at 38 months after planting was lower in Treatments 1 and 2 than the other treatments but the differences were not significant ($P \leq 0.05$) (Figure 7). The differences in the projected crown area of the *C. odorata* plants were also not significant at 38 months after transplanting (Figure 8).

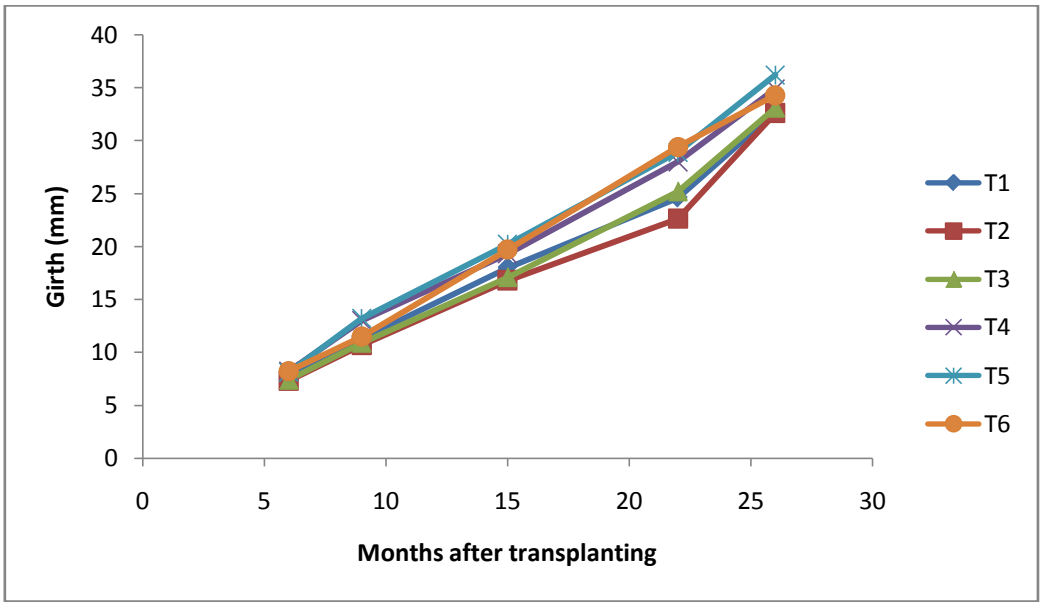

Figure 1. Effect of treatment on girth (mm) of coffee plants.

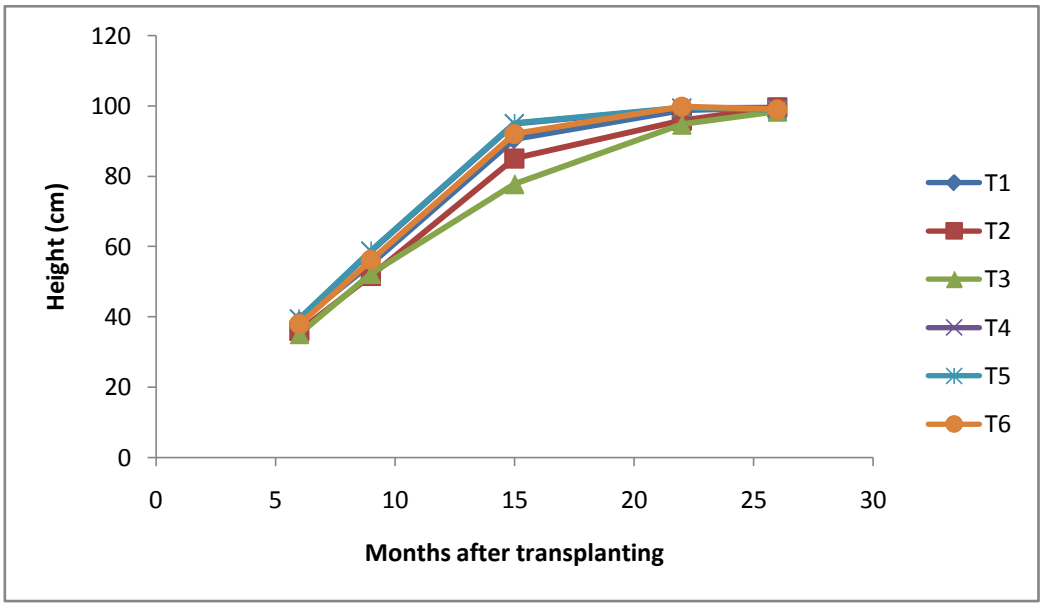

Figure 2. Effect of treatments on height (cm) of coffee plants.

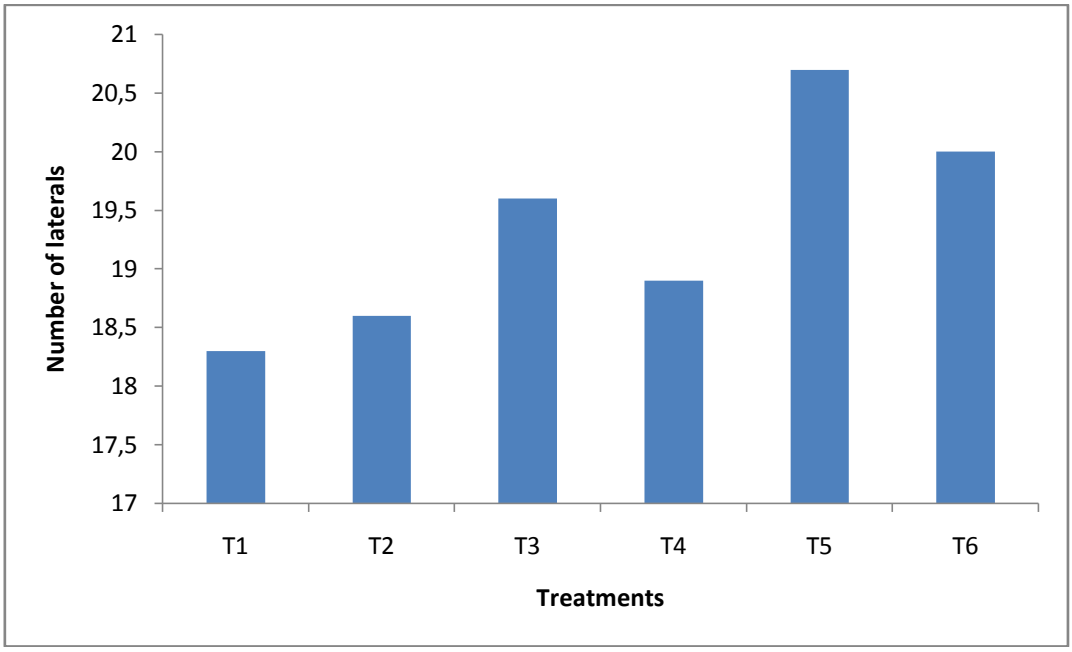

Figure 3. Number of laterals/ coffee plants at 26 months after transplanting.

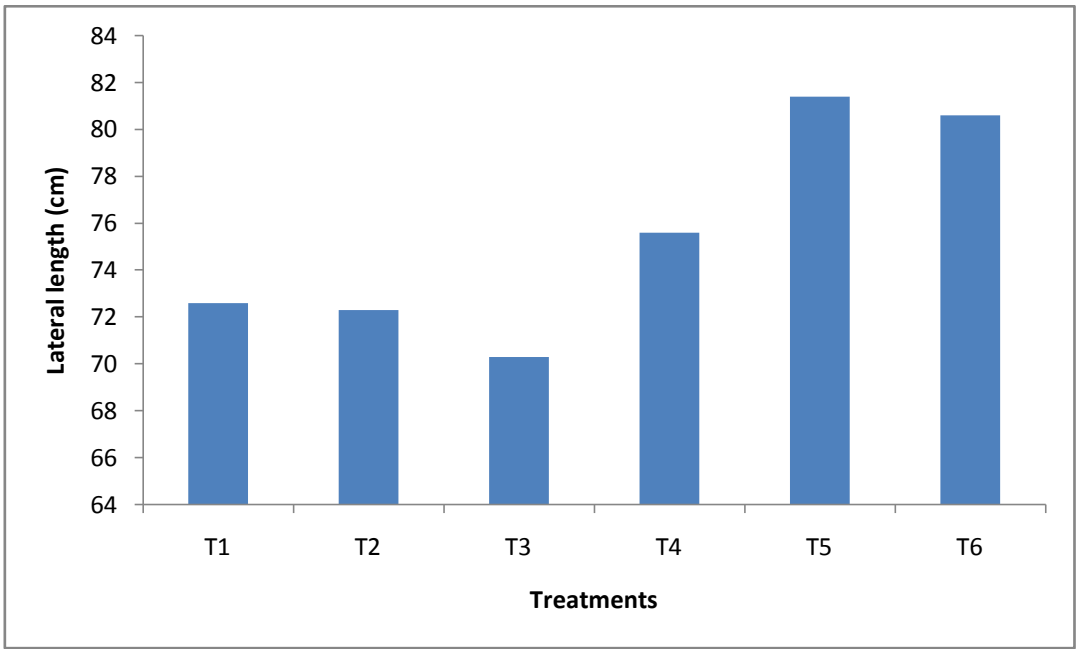

Figure 4. Mean of length (cm) of longest lateral /coffee plant at 26 months after transplanting.

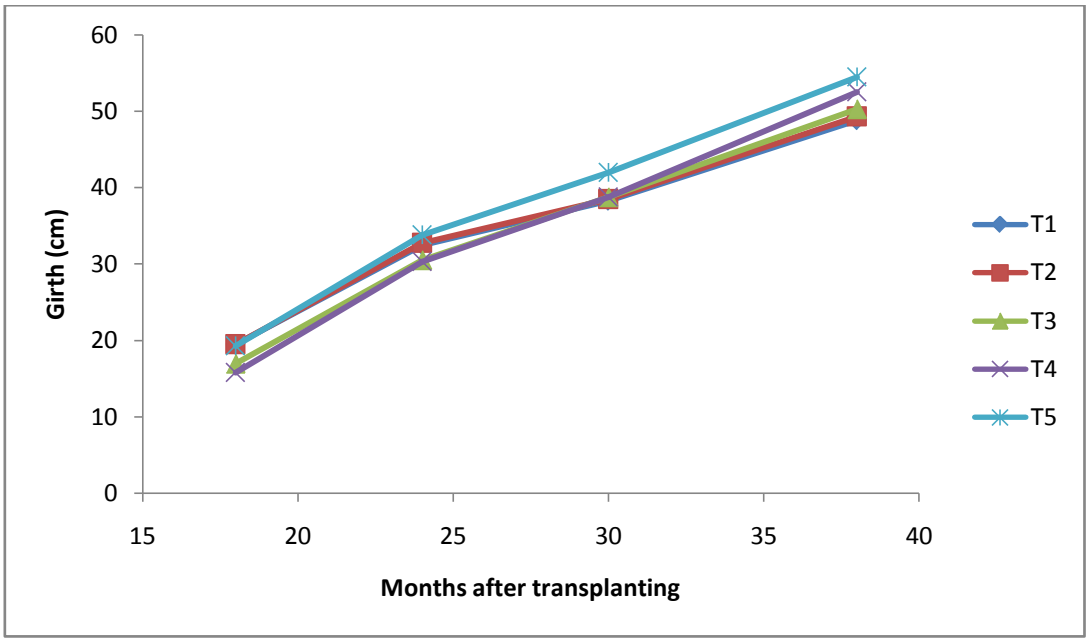

Figure 5. Effect of treatment on girth (measured at 1.3m above ground level) of *C. Odorata* plants.

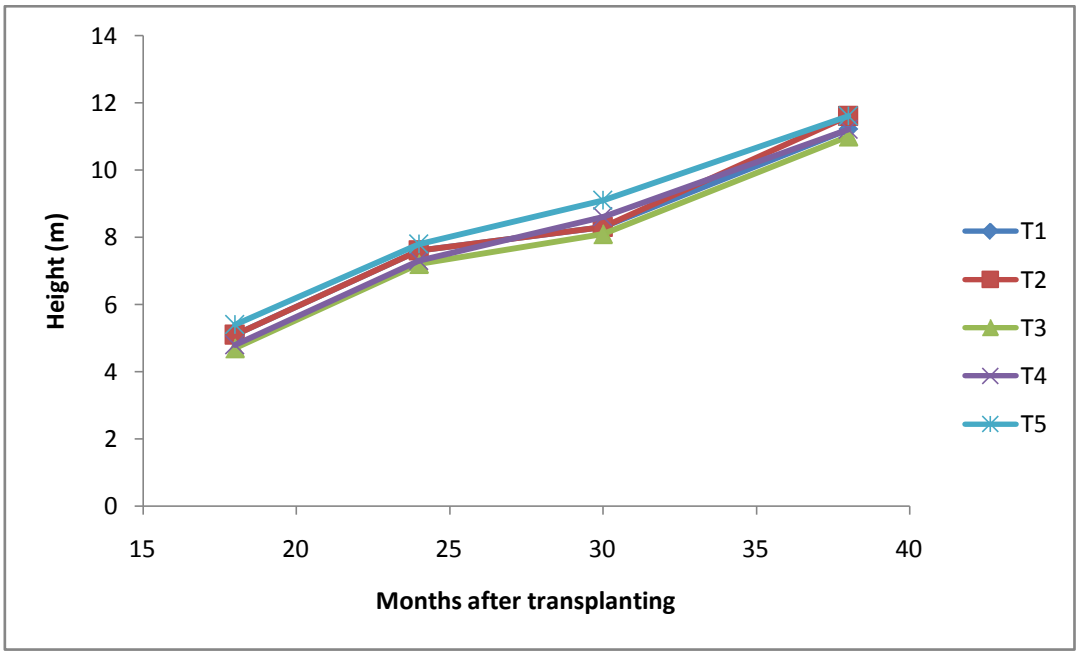

Figure 6. Effect of treatments on height (m) of C. odorata plants.

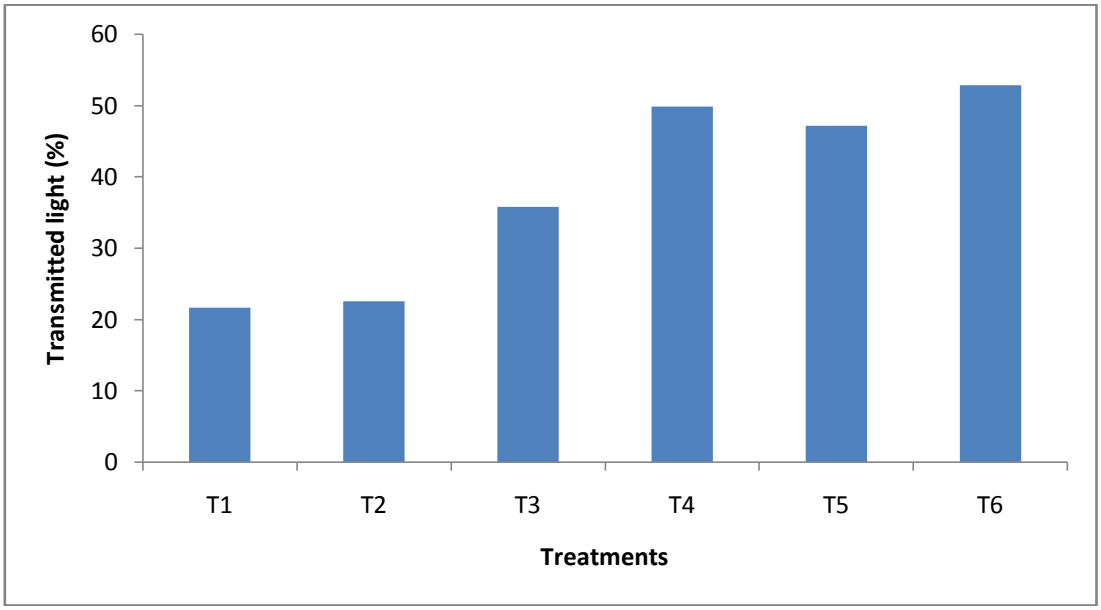

Figure 7. Effect of treatment on percentage transmitted light in experimental plots.

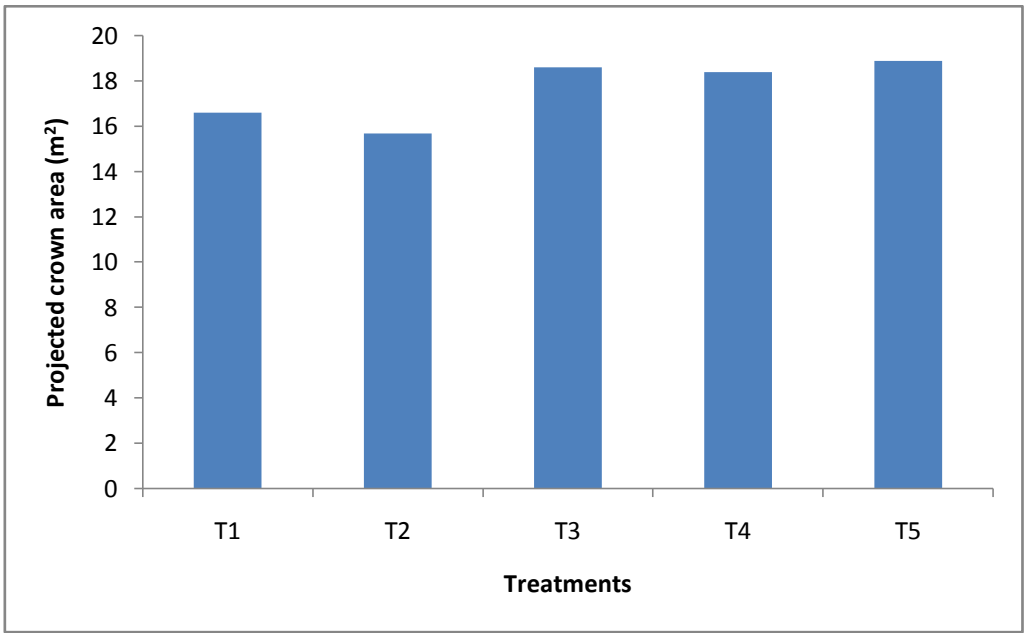

Figure 8. Projected crown area of *C. odorata* trees as at 38 months after transplanting.

Due to the prolonged dry season usually experienced in most of the coffee growing areas in Ghana, it is recommended that Robusta coffee is grown under light to medium shade (Amoah et al., 1999). With the exception of *G. sepium*, no other fast growing tree has been used for the provision of shade for coffee in Ghana. The lack of significant differences in the vegetative growth of the coffee plants grown in association with *C. odorata* as compared to that grown with *G. sepium* (Control) during the initial 26 months of establishment of the coffee plants suggest that *C. odorata* could be grown to provide shade for young coffee plants. The transmission of lower percentage light in treatments 1 and 2 could be attributed to the high density of *C. odorata* in the two treatments but this did not adversely affect the vegetative growth of the coffee plants. Although the vegetative growth of the coffee plants was not adversely affected by the different densities of *C. odorata* during the initial stages of establishment, the plant density of *C. odorata* that will be required for optimum growth and yield of Robusta coffee will only be known after yield data have been collected during the reproductive phase.

CONCLUSION

Cedrela odorata could be planted to provide shade for young Robusta coffee plants without adverse effects on the vegetative growth of the coffee plants. However the plant density of *C. odorata* required for optimum growth and yield of Robusta coffee will be determined after data collection in the reproductive phase.

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Population Dynamics of The Coffee Leaf Miner, *Leucoptera meyricki* (Ghesq.) (Lepidoptera: Lyonetiidae) in a Block of Unsprayed Coffee in Chipinge, Zimbabwe

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SUMMARY

The population dynamics of the coffee leaf miner, *Leucoptera meyricki* (Ghesquire) and its parasitoids was investigated in an experimental plot at the Coffee Research Station (Chipinge, SE Zimbabwe) between 1990 and 1996 by counting the number of parasitoid wasps and leaf miner moths emerging from mined coffee leaves in the laboratory. There were no consistent patterns in peaks of both leaf miner moths and its parasitoids. The number of leaf miner moths emerging at a given time ranged from 12 to 708 whereas that of total parasitoids ranged from 8 to 612. Four parasitoids were constantly present during the study period but the most abundant was *Parahormius leucopterae*. The parasitoids, *Chrysonotomyia ritchei* and *Cirrospilus variegatus* had closer correlations of 0.5804 and 0.5688 ($p<0.05$) respectively with leaf miner moths than *Pediobus coffeicola* (r= 0.3503) ($p<0.05$). There were very weak correlations between leaf miner moths, parasitoids and key weather factors. Leaf miner could possibly be managed by augmentation and conservation of the hymenopteran parasitoids.

INTRODUCTION

In Zimbabwe, the three most important insect pests are coffee leaf miner – *Leucoptera meyricki* (Ghesquire), antestia bug – *Antestiopsis orbitalis bechuana* (Kirkaldy) and white stem borer – *Monochamus leuconotus* (Pascoe) (Kutywayo, 1989).

The leaf miner is present throughout the year with many generations per given season because of its short life cycle (Kutywayo, 1989; Le Pelley, 1968). The full life cycle takes between 38 and 62 days depending on temperature (Crowe, 1964). Currently, the pest is controlled by chemical means involving spray and soil application of mainly organophosphate and carbamate insecticides.

Hymenopteran parasitoids play an important role in regulating pest infestations (Viggiani, 2000). Twelve hymenopteran larval and pupal parasitoids of the coffee leaf miner have been recorded in Zimbabwe (Kutywayo, 1989). Of these parasitoids, three were considered important *(Parahormius leucopterae*, *Cirrospilus variegatus* and *Chrysonotomyia ritchiei).* However, their relative abundance and population dynamics in relation to the population of leaf miner have not been determined in Zimbabwe and Southern Africa.

The objective of the study was to determine aspects of the population dynamics of the parasitoids in relation to the population dynamics of the leaf miner with the aim of improving biological or integrated control of this important pest. Such information will be important in devising integrated management strategies for the coffee leaf miner.

MATERIALS AND METHODS

The studies were done at the Coffee Research Station (Latitude: 20°13' 57" S. Longitude: 32° 38' 51" E., Altitude 1132 m.a.s.l.), Chipinge, Zimbabwe.

Weekly sampling of mined coffee leaves was done from a block of mature coffee (1,2 Hectares) where no insecticides were applied. Twelve leaves were collected at random from each tree, 4 from the bottom, 4 from the middle and 4 from the top levels of the canopy. Collected leaves were sealed in brown paper bags open at the top and leading into polythene bags that were sealed at the top so that insects were unable to escape. The bags were opened after 6 weeks and examined for adult leaf miner moths and parasitoids. The total number of ecclosed moths, parasitoids as well as the total number of individual parasitoid species were recorded on a weekly basis. These were used to calculate the monthly totals. All the ecclosed insects were preserved for identification. Specimens were sent to the International Institute of Entomology (IIE) and Plant Protection Research Institute, Harare for identification.

From this information, the relative seasonal abundance of *L. meyricki*, total parasitoids as well as the abundance of each individual species was then graphically determined. Monthly rainfall totals and mean monthly temperatures were correlated with the incidence of *L. meyricki* in an attempt to quantify the relationship between environmental variables and pest dynamics. Data collection was done between March 1990 and December 1996.

RESULTS AND DISCUSSION

Leaf miner moths

Major peaks of over 600 were in 1990, 1991 and 1993 (Figure 1). Populations were less than 400 moths per bag in 1992, 1994, 1995 and 1996 and were at their lowest in April 1995.

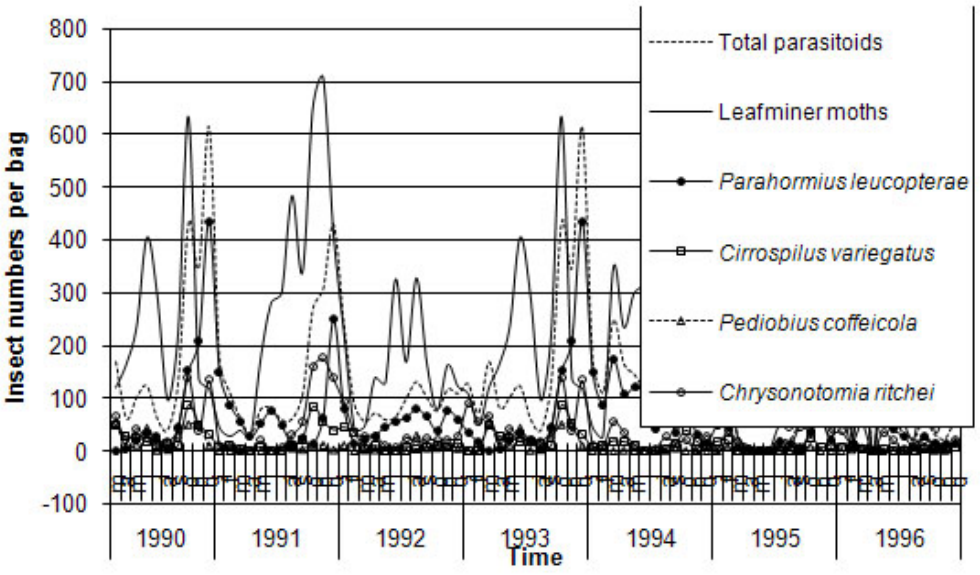

Figure 1. Population dynamics of the coffee leafminer and its parasitoids in Chipinge, Zimbabwe.

The population peaks of leaf miner moths observed in this study compare well with studies done by suction trapping in Zimbabwe (Plant Protection Research Institute, 1975). The major peaks coincide with the dry periods in Zimbabwe (Figure 2).

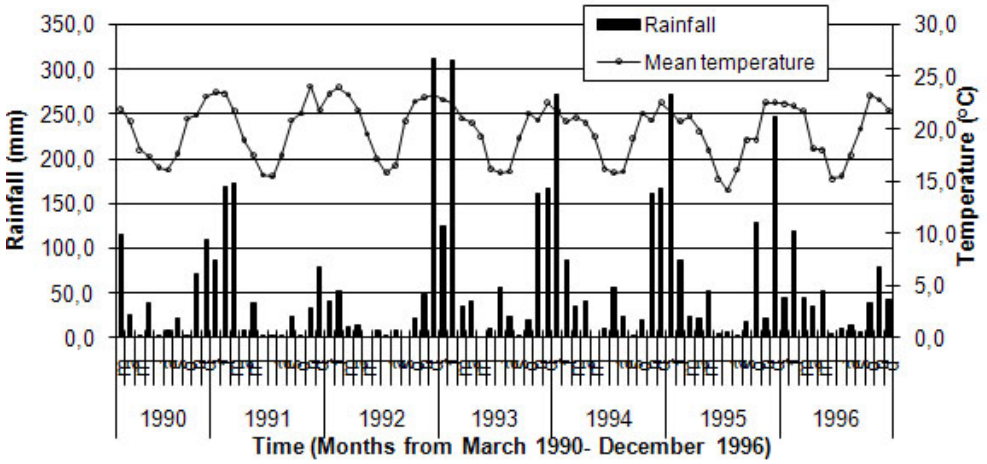

Figure 2. Rainfall and temperature pattern at coffee research station, Chipinge, Zimbabwe from 1990 to 1996.

This coincides with observations by Gallardo-Covas (1992) in Puerto Rico where peaks of *Leucoptera coffeella* occurred during the dry season. Tapley (1962) found that high larval mortality was associated with wet conditions due to water flooding the mines. Le Pelley (1968) also reports that heavy rains and high winds may kill the moths since they are frail. These factors together may explain the low leaf miner numbers observed during the wet months. However, there were very weak correlations between the leaf miner moths and these weather factors in this study.

There was a weak correlation between the moths and rainfall (r= -0.3403) (p<0.05) (Table 1).

Table 1. Correlation of leaf miner and its parasitoids with rainfall and temperature.

Variable 1	Variable 2	r
Leafminer moths	Temperature	**-0.1137ns**
	Rainfall	**-0.3403***
Total parasitoids	Temperature	**0.3459***
	Rainfall	**0.1264ns**
Pediobus coffeicola	Temperature	**0.0444ns**
	Rainfall	**-0.0746ns**
Chrysonotomia ritchei	Temperature	**0.3958***
	Rainfall	**0.0205ns**
Cirrospilus variegatus	Temperature	**0.3365***
	Rainfall	**0.0063ns**
Parahormius leucopterae	Temperature	**0.2377***
	Rainfall	**0.1961ns**

*ns = not significant at p<0.05; * = significant at p<0.05.*

Parasitoids

Peak populations of parasitoids coincided with the main pest peaks, with *Parahormius leucopterae* being the most abundant parasitoid (Figure 1). However, it had weak correlation with leaf miner moths. *Cirrospilus variegatus* was the second most abundant parasitoid and had better correlation of 0.5688 (p<0.05) (Table 2). Peak numbers of parasitoids lagged slightly behind those of leaf miner moths and at times coincided with those of leaf miner moths. The factors leading to this type of relationship need to be examined. In addition, the inter-specific competition among the four parasitoid species will need to be properly studied in order to map out a strategy for increasing abundance of the two most important parasitoids, *Chrysonotomyia ritchei* and *Cirrospilus variegatus* for integrated management of the leaf miner.

Table 2. Correlation of leaf miner with its parasitoids.

Variable 1	Variable 2	r
Total parasitoids	Leafminer moths	0.4277*
Pediobus coffeicola		0.3503*
Chrysonotomia ritchei		0.5804**
Cirrospilus variegatus		0.5688**
Parahormius leucopterae		0.1575ns

*ns = not significant at p<0.05; * = significant at p<0.05; ** = significant at p<0.01.*

The other parasitoids, *Pediobus coffeicola* and *Chrysonotomyia ritchei* with significant correlations of 0.3503 and 0.5804 respectively remained at very low levels during the study. There were also weak correlations between the parasitoids and the weather factors (Table 1).

Kutywayo (1995) examined the potential for biological control of the coffee leaf miner, *L. meyricki* and concluded that the most feasible options were augmentation of the parasitoid, *P.leucopterae* and conservation. The study confirmed the importance of *P. leucopterae* as a key parasitoid of the coffee leaf miner in Zimbabwe. Augmentation could be done by periodic release of mass reared parasitoids. A method for mass rearing leaf miners was developed by Katiyar (1968). This could be used to rear the host for augmentative releases. Augmentation would be highly desirable since the impact is anticipated within a very short time unlike in classical biological control where it may take years (Overholt, 1997).

Another approach to improving the effectiveness of the leaf miner parasitoids would be to practise conservation measures such as the use of habitat manipulation to enhance the native natural enemies (Gurr and Wratten, 1999). Such measures would include the provision of the agents' requirements for nectar, pollen, moderated microclimate or alternative hosts. Therefore, it is important to increase diversity in coffee plantations by introducing some annual crops such as soya beans and sugar beans through strip- and inter-cropping plants to act as alternative hosts for parasitoids.

Outbreaks of coffee leaf miner in East Africa were linked to the use of persistent insecticides such as dieldrin that caused high mortality of the parasitoids and allowed populations of the leaf miner to increase (Tapley, 1961; Bess, 1964). In Zimbabwe parathion is the most common insecticide used for spraying against leaf miner as it is less persistent, but despite

timely spraying, the leaf miner remains a problem, probably due to continued decimation of the parasitoids by the sprays. There is a need to determine the proper timing of sprays that will have a minimum impact on parasitoid population levels.

This control by parasitoids could be supplemented with the use of a biopesticide such as an entomopathogenic nematode. LeBeck et al. (1993) reported on the successful use of an entomopathogenic nematode *Steinernema carpocapsae* (Rhabditida: Steinernematidae) on the serpentine leafminer, *Liriomyza trifolii* (Diptera: Agromyzidae). The entomopathogenic nematode entered the leafmine through the oviposition puncture created by the female during egg laying, or through any unnatural tear in the mine surface. This could also be applied in the control of Leucoptera *meyricki* targeting the spray at the end of the rainy season when moths are low and a lot of mines are active. *Steinernema sp.* have been isolated from the coffee white stem borer, *Monochamus leuconotus* (Coleoptera: Cerambycidae) in Chipinge, Zimbabwe (Kutywayo, unpublished). Work should be carried out to investigate the possibility of using entomopathogenic nematodes as biological control agents. The use of biological control for coffee leaf miner is the most feasible option in view of the natural abundance of the parasitoids as reported in this study and the potential of using a biopesticide. Biological control is a better option since it does not have adverse effects on the environment.

The relationship between the peaks of moths and parasitoids with environmental factors such as temperature, relative humidity and rainfall needs to be thoroughly analysed since this is important in devising pest management strategies. In terms of habitat manipulation, work needs to be done to identify suitable plants that will provide nectar at the required time and will not result in lower yields and lead to increased infestation by other pests and/or compete with the crop for nutrients and water. There is also need to explore the potential of using a bio-pesticide in the control of the coffee leaf miner.

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Effect of Organic Nursery Media on Coffee Seed Germination and Initial Growth

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SUMMARY

A study was conducted to evaluate the effect of 5 different nursery media on the germination and initial growth of 3 coffee varieties at Coffee Research Station in Zimbabwe. Organic media treatments gave significantly higher ($p<0.05$) coffee seed germination over the unamended control except for composted ash which actually inhibited germination. Yellow Catuai coffee variety had significantly higher ($p<0.05$) seed germination followed by SL28 while Catimor F6 had the least. The earliest germination were recorded in composted ash which took 4.21 weeks to germinate followed by the unamended control which took 5.27 weeks to germinate. In terms of varieties, Catimor F6 germinated earlier (mean germination time of 5.01 weeks) than other varieties while SL28 was the slowest to germinate with a mean germination time of 6.37 weeks. Cattle manure produced significantly superior ($p<0.05$) vegetative growth and biomass accumulation in coffee seedlings than the rest of the treatments. Yellow Catuai developed more in terms of number of leaves, girth and height when growing in organic media with Catimor F6 was the least. The nutrient source and genotype interaction was not significant in all cases ($p>0.05$). The study concluded that organic nutrient sources are essential in coffee nursery establishment.

INTRODUCTION

According to estimates from ICO, there are between 25 and 30 million coffee farmers in the world, the majority being smallholders in Africa, Asia and Latin America providing about 70% of the world coffee supplies (Baker et al., 2001). Coffee is a legal and unique source of income for these smallholder farmers' livelihoods. However, research has rarely been oriented towards the needs and circumstances of this group of farmers, but rather on an agribusiness approach (Baker et al., 2001).

When many of these farmers decide to expand or replant, they buy coffee seedlings from centralised nurseries. With the high risk of bringing diseases and pests into their farms and lack of funds to buy and transport the seedlings, farmer's-own-nurseries are an important aspect of the sustainability of the smallholder coffee industry.

With increasing costs of inorganic fertilizers in recent years, scientific interests have turned towards the evaluation of organic fertilizers based on locally available resources including composts, animal manures and green manures (Lekasi et al., 2001; Boswell-Brown, 2007; Njoroge et al., 1990). The ability of manures to provide cheaper, locally available, convenient and slow released nutrients especially nitrogen, phosphorous and potassium has since been established.

Amujoyegbe et al. (2007) reported the use of several organic materials such as cow dung, poultry droppings, refuse compost and farm yard manure as soil amendments suitable for increasing crop production particularly among subsistence farmers in West Africa. Organic manures such as cattle and poultry manure may improve the soil structure, aeration and slowly release nutrients, which support root development leading to better quality plants and higher yields (EL-Magd et al., 2006). Organic manure also plays a direct role in plant growth as a source of macro and micronutrients in available forms and improving the physiological properties of the soil. Issues with organic nutrient sources are mainly those of slow mineralization, which may not coincide with peak crop nutrient demand and being hosts to pathogens and pests (Lekasi et al., 2001; Boswell-Brown, 2007; Njoroge et al., 1990; Amujoyegbe et al., 2007; EL-Magd et al., 2006).

Promotion of coffee smallholders through developing flexible and adaptable production technologies is very important. This is because of the role of coffee in providing rural employment and in preserving the environment where it is grown (Baker et al., 2001). In Zimbabwe, recommendations for nursery establishment are to mix virgin forest soils with Compound S ($7N:21:P_2O_5:7K_2O_5$). However, smallholder farmers do not often afford or access fertilizers (Boswell-Brown, 2007; Njoroge et al., 1990; Amujoyegbe et al., 2007; EL-Magd et al., 2006; Logan and Biscoe, 1987) There is thus need to find alternative nutrient sources for nursery establishment that are locally available, cheap and are competitive in producing healthy coffee seedlings for a vibrant coffee sector.

MATERIALS AND METHODS

The study was done at the Coffee Research Institute in Zimbabwe. The trial was a 5 x 3 factorial design of 5 media treatments and 3 coffee varieties replicated 3 times and laid out in randomized complete blocks (RCBD). The media treatments were un-amended soil, Poultry Manure, Cattle Manure, Humus and Composted Ash and the varietal treatments were Catimor F6, Yellow Catuai and SL28. The media treatments were crushed and passed through a 5mm sieve and mixed with forest soil in the ratio 1:1. Three coffee seeds were sown directly into each pot and these were later thinned to 1 after germination assessments. The number of germinated seeds was recorded weekly and seeds were considered to have germinated when the plumule appeared above the soil level. Germinations in composted ash were very poor such that no measurements of stem thickness (using vernier callipers 5cm above soil level), number of leaves and plant height (using a 30cm rule) were taken. Destructive sampling of 3 plants per treatment was done when the seedlings were 6 months old to measure biomass. Shoot dry matter was determined when shoots above soil surface and roots were oven dried for 8hrs at 70 °C.

Mean germination time (MGT) was computed using the method standardized by Ellis and Roberts (1980) based on the formula

$$MGT = \frac{\sum(D - 0.5)n}{\sum n}$$

where D is the duration of germination (weeks), n is the number of seeds germinating per week and 0.5 is a correction factor for germination which could occur before the assessment date. Data sets were analyzed for variance between treatments (ANOVA) using Genstat 10 (Lawes Agricultural Trust 2006) and means were separated using the Duncan's Multiple Range Test (DMRT) in MSTAT C when differences were significant. Simple Linear Regression was also done using Genstat 10.

RESULTS AND DISCUSSION

Effect of organic media on germination of coffee seeds

Coffee seed germination under organic media treatments were significantly different ($p<0.05$, Table 1). The time it took coffee seed to germinate in the organic nursery media had a highly significant positive influence on germination success ($p<0.05$, $y=21.84-85.4x$, $r^2=0.87$).

Table 1. Germination of coffee seeds in organic media after 12 weeks.

Treatment	Germination	% Germination	MGT (weeks)
Control	18.17b	40.37	5.47
Poultry Manure	21.83a	48.52	6.73
Cattle Manure	23.83a	52.96	6.06
Humus	25.83a	57.41	6.49
Composted Ash	0.67c	1.48	4.21
p	0.002		

**Means followed by different letters are significantly different according to Duncan's Multiple Range Test ($p\leq0.05$).*

Germination variation between varieties

Yellow Catuai had the highest coffee seed germination in organic media, while Catimor F6 seeds were the earliest to germinate ($p<0.05$, Table 2).

Table 2. Germination of different coffee varieties after 12 weeks.

Treatment	Germination	% Germinations	MGT(Weeks)
Catimor F6	9.8b	36.30	5.01
Yellow Catuai	24.3a	90.00	5.51
SL28	20.4a	78.52	6.37
p	0.006		

**Means followed by different letters are significantly different according to Duncan's Multiple Range Test ($p\leq0.05$).*

Effect of organic media treatments on growth

Use of different organic sources of nutrition as nursery media for coffee resulted in significant differences in number of leaves developed ($p<0.05$, Table 3). Using cattle manure as organic media produced the highest number of leaves, biggest leaf area, tallest coffee plants and the thickest coffee stems.

Table 3. Effect of nursery media on number and size of leaves.

Treatment	No. of leaves	Leaf Area (cm^2)	Height (cm)	Stem thickness (mm)
Control	5.86b	7.7b	6.57b	2.41
Poultry Manure	8.84a	35.3a	10.67a	3.17
Cattle Manure	10.47a	42.0a	13.47a	3.19
Humus	8.93a	31.1a	12.74a	3.00
p	0.003	0.001	0.003	0.057

**Means followed by different letters are significantly different according to Duncan Multiple Range Test ($p \leq 0.05$).*

Response of different coffee varieties under organic amendments

Yellow Catuai developed the highest mean number of leaves with 9.8 leaves when growing under organic media which was closely followed by SL28 which developed an average 8.9 leaves ($p<0.05$, Table 4). Yellow Catuai developed thicker stems under organic media followed by SL28.

Table 4. Vegetative growth of different coffee varieties under organic amendments.

Variety	No. of leaves	Leaf area (cm^2)	Stem thickness (mm)	Height (cm)
Catimor F6	6.9b	27.1	2.50b	8.8b
Yellow Catuai	9.8a	30.7	3.33a	12.5a
SL28	8.9ab	29.3	3.00a	11.3ab
p	0.008	0.745	0.025	0.023

**Means followed by different letters are significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).*

Effects of organic media on shoot and root weights

Table 5. Effect of media treatments on dry mass and moisture content.

Treatment	Stem & leaf dry mass (g)	Stem & leaf moisture content (g)	Root dry mass (g)	Root moisture content (g)
Control	1.30b	2.88b	0.61b	1.24b
Poultry Manure	5.02a	8.64a	1.99a	2.34b
Cattle Manure	6.20a	12.01a	2.55a	4.10a
Humus	4.55a	9.68a	1.89a	4.96a
p	0.003	0.007	0.015	0.002

**Means followed by different letters are significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).*

Application of organic amendments significantly affected stem and leaf dry mass ($p<0.05$, Table 5). Use of cattle manure produced the heaviest coffee stems and leaves followed by poultry manure.

Shoot and root weights of different varieties under organic media

There were no significant differences in stem and leaf dry mass, root dry mass, stem and leaf moisture content and in root moisture content between coffee varieties ($p>0.05$, Table 6).

Table 6. Effect of organic amendments on stem, leaf and root dry mass and moisture content of different coffee varieties.

Treatment	Stem & leaf dry mass (g)	Stem & leaf moisture content (g)	Root dry mass (g)	Root moisture content (g)*
Catimor F6	2.64	5.18	5.74	0.44
Yellow Catuai	3.05	6.80	7.73	0.79
SL28	2.61	5.05	6.25	0.64
p	0.567	0.111	0.333	0.603

**Data on root moisture content was transformed using log base$_e$ to homogenise variance.*

Coffee seed under composted ash germinated four weeks after sowing. Previous studies reported germination four weeks after sowing when the endocarp was removed (Osei-Bonsu et al., 1989). This suggests that different organic media influence germination while coffee varieties may also vary in responding to the stimulations in both time and outcomes. Poultry manure and cattle manure may have resulted in better germinations as they provided necessary conditions for germination; well aerated, moisture retention and higher temperatures than the unamended control. Contrary, the fine texture of composted ash, even at 1:1 with soil may have been detrimental to coffee seed germination because of causing oxygen deficit and excessive soaking due to poor drainage.

EL-Magd et al. (2006) evaluated organic nutrient sources for broccoli and realised the high performance of cattle manure over other organic amendments in growth characteristics, which concur with the findings of this study. Studies of crop varieties' response to nutrient application in crops such as maize (Amujoyegbe et al., 2007), broccoli (EL-Magd et al., 2006), apples (Reganold et al., 2001) and coffee (Osorio et al., 2002) correspond to findings of this study that plant genotypes differ in their response not only to nutrient sources but also to other growth conditions.

The study has demonstrated that enhanced use of manures can be a basis for strengthening crop production by resource-constrained farmers. While the role of inorganic fertilisers in crop production remains important, complimentary benefits of using manures need to be extended especially in small-scale agriculture where crop-livestock synergies are cultural and economic fundamentals.

ACKNOWLEDGEMENTS

The authors express their appreciation to Messrs G. Masasi and P. Chidoko for their role in the implementation of this research project.

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Selected Agroclimatic Factors Determining Water Stress of Coffee Plants

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SUMMARY

Study on the selected agroclimatic factors affecting water stress on coffee plants (by statistical approach) has been carried out on Alluvial plain with seasonal tropical climate of D type rainfal according to Schmidt and Ferguson classification with 45 m elevation above sea level. Observation was done by survey method on the plot of fertilization trial on coffee plants in Kaliwining Experimental Station, Indonesian Coffee and Cocoa Research Institute. Water stress of the plants was observed from the turgidities of the plants whereas its meteorological condition, including rainfall, maximum and minimum temperatures, relative humidity, radiation, and wind velocity, was observed at local meteorological station in the station. Soil samples were taken from the depth of 0-100 cm for assessing soil moisture condition that were observed periodically as those of observation on growth parameters, during period of observation from dry season to rainy season. Other parameter observed related to water stress of plants was tanspiration. Assessment of environmental factors affecting water stress of coffee plants used statistical approach for characterizing the factors dominantly affecting water stress of plants, that was multiple regression. The results showed the following figures. Relative humidity (atmospheeric evaporative demand) showed to be the environmental factor dominantly affecting water stress of coffee plants beside soil moisture condition, and wind velocity. Relative humidity also showed to be the most significant environmental factor influencing transpiration beside soil water condition, although their effects were much less significant than those on water stress of the plant. Clones of the coffee plants have different sensitivities or tolerance to water stress that was shown by the significant levels of the environmental factors affecting water stress of the plants. Among 4 clones observed, *viz.* BP-288, BP-409, BP-42, BP-358, it was shown that coffee clone of BP-358 was most sensitive/responsive to agroclimatic factor change compared to the other three clones. Multiple linier regression analysis may be usefull to asses the environmental factors affecting plant water stress.

INTRODUCTION

Water stress resulted from inbalance (the lag) between transpiration and absorption in dry season (Shaw and Laing, 1966; Kumar and Tieszen, 1980; Carr, 2001) is problems in coffee at seasonal tropical regions, and the degree of stress increases with the duration of drought. However, a period of water shortage (water stress) is apparently essential for breaking the dormancy of flower buds and flowering when stimulated then by rain or irrigation (Alvim, 1960; Carr, 2001). Different responses of coffee plants to drought were reported by Carr (2001) that included partial closure of stomata to minimize transpiration at high irradiances.

Kramer (1969) explained that the main environmental factors influencing transpiration rate were sunshine intensity, water vapor pressure and atmospheric temperature, wind, and water supply to roots; whereas in a controlled environment growth room Pallas et al. (1965) found that radiant energy, relative humidity, and soil moisture tension had marked effects on transpiration rates.

Objective of the study is to identify the factors determining water stress of coffee, a case on alluvial plain.

MATERIALS AND METHODS

Observation was carried out in Kaliwining Experimental Station, Indonesian Coffee and Cocoa Research Institute in Jember, East Java, on Alluvial plain of 45 m asl. altitude with seasonal tropical climate of D rainfall type according to Schmidt & Ferguson (1951), from dry season to rainy season. Observation was carried out by survey method in the plot of N fertilization trial, at the same rate of N application (100 g Urea/tree/year) and 6 replicates, on Robusta coffee plants of 12 year old, clones of BP-288, BP-42, BP-409 and BP-358.

Water stress of the plants was observed from their relative turgidities as measured by Machlis and Torrey (1956) and Shaw and Laing (1966). Whereas its meteorological condition, including rainfall, maximum and minimum temperatures, relative humidity, radiation, and wind velocity, were observed at local meteorological station in the station. Soil samples were taken from the depth of 0-100 cm for assessing soil moisture condition (presented in mm/m) that were observed periodically (two week intervals) at the same time as those of observation on growth parameters, during period of observation from dry season to rainy season. Other parameter observed related to water stress of plants was tanspiration, in $mg/cm^2/s$ or $mg/m^2/s$, measured from the weight of water loss per leaf per day by wrapping single leaves in plastic bags and tied with rubber tie each in 24 hours, as presented in details in a different paper. Meteorological data was performed in two week intervals, *i.e.* accumulation of rain for rainfall, average of daily radiation for solar radiation, averages of maximum and minimum air temperatures for T-max and T-min, and average of daily wind velocity for wind velocity.

Evaluation on agroclimatic factors most responsible to plant water stress and transpiration was performed using a statistical approach by multiple regression analysis.

RESULTS AND DISCUSSIONS

The results showed the following figures. Relative humidity (atmospheeric evaporative demand) showed to be the environmental factor dominantly affecting water stress of coffee plants beside soil moisture condition, and wind velocity (Table 1). By excluding non significant agroclimatic factors in steps and considering their interdependency; then, performing multiple regression analysis to quadratic level to fit agroclimatic factors most significantly affecting plant turgidity, it is found the results as presented in Table 2. However, when relative turgidity data of the four clones are evaluated in a whole it is found that in general relative humidity and soil moisture content are the agroclimatic factors most important in controlling plant water stress (Table 3).

Table 1. Multiple linier regression analysis of relative turgidity on agroclimatic factors.

Variable	BP-288		BP-409		BP-42		BP-358	
	Coefficient	**P-value**	**Coefficient**	**P-value**	**Coefficient**	**P-value**	**Coefficient**	**P-value**
Intercept	146,7946442	0,405106	122,9160959	0,013155	182,9632793	0,047237	83,26205603	0,005491
Rainfall,mm	-0,038165516	0,690599	-0,003386566	0,294986	0,096254008	0,059743	-0,023426975	0,012997
T-max,^{o}C	1,584758499	0,676979	-0,508517746	0,083105	-0,114191067	0,803149	1,238301488	0,009702
T-min,^{o}C	0,123068046	0,873209	0,014527911	0,494534	-0,443811931	0,10861	0,195476332	0,013146
Relativ humidity,%	-1,039735106	0,463933	-0,444433376	0,031113	-1,462581987	0,050553	-0,445692703	0,008777
Radiation,%	-0,240251686	0,555616	-0,062950214	0,067575	0,218060514	0,103908	-0,126886788	0,009512
Wind velocity,km/h	-4,691734435	0,840131	12,45117739	0,021892	11,89889053	0,121214	-1,179372778	0,065134
Soil moisture,mm/m	-0,009112897	0,819147	0,043725232	0,010642	0,034262544	0,072406	0,01791443	0,007343
R^2-regression	0,650281147	0,9065	0,9998502	0,024931	0,998925984	0,066703	0,999969045	0,011334

Table 2. Agroclimatic factors that most significantly fit affecting plant turgidity and the patterns.

Variables	BP-288		BP-409		BP-42		BP-358	
	Coefficient	**P-value**	**Coefficient**	**P-value**	**Coefficient**	**P-value**	**Coefficient**	**P-value**
Intercept	-639,038	0,051736	42,102873	7,79E-10	-268,62768	0,03144534	77,25248	3,79E-11
Rainfall^2	-	-	-	-	0,00590217	6,1117E-08	-	-
T-max	-	-	-0,0198693	0,077804	-	-	-	-
Relative humidity	17,76434	0,027442	-	-	7,03644971	0,01901321	-	-
Radiation	-0,721 99	0,000282	-	-	-	-	-	-
Relative humidity^2	-0,10524	0,022894	0,00601077	1,39E-11	-	-	-	-
Radiation^2	0,004933	0,000438	-	-	-	-	-	-
Soil moisture	-	-	0,0003773	0,026633	-	-	0,017942	0,001913
Soil moisture^2	-	-	-	-	-0,0397874	0,01883081	-	-
R^2-regression	0,980631	0,001111	0,99998017	2,02E-11	0,999684	3,63E-09	0,768734	0,001913

Table 3. Agroclimatic factors most responsible to relative plant turgidity of the coffee plants.

Agroclimatic factors	Coefficient	P-value	Coefficient	P-value
Intercept	-505,753869	0,395417	100,0461407	1,11E-16
Relative humidity (%)	13,5742317	0,309864	-	-
Soil moisture content (mm/m)	0,01999988	0,005217	0,021897936	0,001722
Relative humidity^2	-0,07892754	0.291305	-0,003028569	0,001427
R^2-regression	0,37050628	0,001791	0,34956027	0,0008278

Table 4. Agroclimatic factors most responsible for transpiration of the coffee plants.

Agroclimatic factors	Coefficient	P-value
Intercept	-0,004476753	0,991117103
Rainfall	-0,002659025	0,000225359
Solar radiation	0,013348974	0,005717464
Rainfall^2	9,78737E-06	1,64508E-05
Relative humidity^2	0,00014604	0,001162558
Solar radiation^2	-9,20973E-05	0,02257379
R^2-regression	0,528697603	0,000258545

Relative humidity also shows to be the most significant environmental factor influencing transpiration beside soil water condition, although their effects are much less significant than those on water stress of the plant. However, further analysis to quadratic level it is found that in general agroclimatic factors most responsible for the plant transpiration are solar radiation, relative humidity, and rainfall (Table 4). As a note, the plot of plants under study was managed under leguminous shading trees and windbreakers.

Clones of the coffee plants have different sensitivities or tolerance to water stress that is shown by the signicant levels of the environmental factors affecting water stress of the plants. Among 4 clones observed, *viz.* BP-288, BP-409, BP-42, BP-358, it is shown that coffee clone of BP-358 is most responsive to agroclimatic factor change compared to the other three clones.

Multiple linier regression analysis may be usefull to asses the environmental factors affecting plant water stress; however, the analysis may perform and fit more significantly the agroclimatic factors most responsible to water stress and transpiration of the plants when it is performed to quadratic level.

CONCLUSIONS

From this study, it may be drawn conclusions as follows. In general, relative humidity and soil moisture content are the agroclimatic factors most important in controlling plant water stress; whereas agroclimatic factors most responsible for the plant transpiration are solar radiation, relative humidity, and rainfall. Clones of the coffee plants have different responses to water stress performed by the significant levels of the environmental factors significantly affecting plant turgidity.

ACKNOWLEDGEMENTS

The authors wish to thank Ms. Alifah and Mr. Nurkholis for excellent technical works, and staffs of Soil and Agroclimate Division and Meteorological Station, Indonesian Coffee and Cocoa Research Institute, for meteorological data observation.

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Influence of Arbuscular Mycorrhizal Inoculation and Phosphate Fertilizer Types on the Growth of Coffee Seedlings in Two Soil Types in Nigeria

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SUMMARY

Greenhouse studies were carried out to evaluate the effect of phosphate fertilizer types and Arbuscular Mycorrhizal fungi inoculation (AMF) on the growth of coffee seedlings in two soils of types in Nigeria. The factorial experiment involved two types of P-fertilizers (Single Super Phosphate - SSP and Sokoto Rock Phosphate - SRP) and AMF inoculation. The P-fertilizers were applied at 30 kg P_20_5/ha and a control while the AMF was applied at two levels (with and without). The six treatment combinations were applied to two-month old coffee seedlings grown in the two soils. The experimental design was a CRD with three replications and data on growth of coffee were taken on monthly basis for six months. In Mambilla soil, the height and stem diameter of coffee seedlings were significantly ($p<0.05$) higher as a result of application of SSP and AMF inoculation compared with SRP and AMF inoculation or the control at 6MAT. The number of leaves and leaf area were significantly ($p<0.05$) improved due to application of SSP and SRP with or without AMF inoculation in Mambilla soil while in Ibadan soil, AMF inoculation resulted in consistent and significant ($p< 005$) improvement on the leaf area of coffee seedlings. The soil available P was significantly ($p<0.05$) enhanced in the two soils due to application of SSP with or without AMF inoculation. Similarly, the root colonization of coffee seedlings at 6 MAT was significantly ($p<0.05$) higher as a result of AMF inoculation without P-fertilizer application compared with others treatments. SRP had a comparable effect with SSP on the growth of coffee seedlings especially under AMF inoculation.

INTRODUCTION

Coffee production in Nigeria is fast declining due to neglect, abandonment of farms, inconsistent governmental policies, soil fertility degradation, poor pricing, pest and diseases among others. Most soils upon which coffee is grown are generally poor in nutrients such as nitrogen and phosphorus (Ibiremo et al., 2002). The wide spread deficiency of phosphorus in most soils in Nigeria is due to fixation. The application of inorganic phosphate fertilizers such as SSP, TSP, NPK etc over a long period of time result in negative environmental consequences (Zainol et al., 1993). The potential of Arbuscular mycorrhizal fungi (AMF) in enhancing crop production is well recognized (Siquerra et al., 1998). Arbuscular mycorrhizal fungi (AMF) readily form association with coffee roots and specifically mycorrhizal coffee seedlings grow much faster, exhibit improved nutrition and gave higher yields than those without at the nursery stage. Presently, there is paucity of information on the effect of Arbuscular mycorrhizal inoculation and phosphate fertilizers on the growth of coffee seedlings in Nigeria hence this study.

MATERIALS AND METHODS

The study was conducted in the greenhouse of Cocoa Research Institute of Nigeria, Ibadan between late 2007 and early 2008. The soil of Ibadan used for the study was Ferric Luvisols and Ibadan series while that of Mambilla soil was Nitrosols. Top soils (0-30 cm depth) were collected at the coffee plantations at Ibadan and Mambilla Substations. The soils were air-dried and sieved using 2 mm sieve. The factorial experiment had three phosphate fertilizer types namely: Single superphosphate (SSP), Sokoto rock phosphate (SRP) and no P application (control). Each of Phosphate fertilizers (SSP and SRP) was applied at rate equivalent to 30kg P_2O_5/ha. The second factor was inoculation with Arbuscular mycorrhizal fungi (*Glomus clarum* Nicolsen Shenk) at two levels (with and without). Seedlings inoculated with mycorrhiza were done with 20g of the fungus containing spores, hyphae and roots of the culture plant. The experiment was laid out in a complete randomized design with four replicates. Data on height, stem diameter, number of leaves and leaf area were collected on monthly basis. Mycorrhizal root infection was estimated using the grid-line intersect method according to Giovanetti and Mosse (1980). SRP has 33.7, 44.23, 0.95 and 7.90% for P_2O_5, CaO, MgO and $CaCO_3$ respectively while SSP has 18 and 27.0% for P_2O_5 and CaO respectively. Soil samples were collected analyzed for both physical and chemical properties according to IITA (1982). ANOVA was performed on all data.

RESULTS AND DISCUSSION

At 6MAT, the cumulative height of coffee seedlings was significantly ($p<0.05$) higher due to SSP application with or without mycorrhizal inoculation compared with coffee seedling height in the control and SRP application with or without mycorrhizal inoculation (Table1). Coffee seedling height was not significantly affected as a result of AMF inoculation in Mambilla soil (Table 1). This is consistent with the observations of Siqueira *et al*. (1998). The stem diameter of coffee seedlings at 6MAT in Mambilla soil was 43.0% and 59.3% higher (significant) in the SSP application (with or without mycorrhizal inoculation) than the stem diameter of coffee seedlings treated with SPP and the control (with or without mycorrhizal inoculation). The effect of the mycorrhizal inoculation was not significant on the number of leaves of coffee seedlings. In Ibadan soil, the influence of P application and mycorrhizal inoculation was not significant on the height, stem diameter and number of leaves of coffee seedlings at 6MAT (Table 1) The plant height, stem diameter and number of leaves of coffee seedlings at 6MAT ranged from 12 to 14 cm, 0.26 to 0.36 cm and 10.00 to 13.00 respectively. Mycorrhizal inoculation without P application significantly ($p<0.05$) increased the pH of Ibadan soil at 6MAT. The significant response of coffee seedlings to phosphate fertilizers in Mambilla soil could be as a result of available P that was critically deficient while in Ibadan soil the available P was at sufficiency level, hence no response to P application (Ayodele and Agboola, 1998). The cumulative effect of SSP application (with or without AMF) on available P was significantly ($p<0.05$) higher compared with SRP and the control with or without mycorrhizal inoculation in Ibadan soil. The root colonization ranged from 45 to 70% in Ibadan soil while it ranged from 45 to 89% in Mambilla soil. Similarly, the root colonization of coffee seedlings at 6 MAT was significantly higher as a result of AMF inoculation without P-fertilizer application compared with root colonization under SSP application and AMF inoculation in Mambilla soil.

CONCLUSION

SRP had a comparable effect with SSP on the growth of coffee seedlings especially under AMF inoculation; hence, SRP is a promising substitute for inorganic phosphate sources for coffee seedling production in Nigeria.

Table 1. Influence of phosphate fertilizers and Arbuscular Mycorrhizal inoculation on the growth of coffee seedlings in Mambilla and Ibadan soils.

	Treatments Plant Height (cm)		Stem Diameter (cm)		Number of Leaves		Leaf Area (cm^2)	
	6 Months After Transplanting							
	Mambilla	Ibadan	Mambilla	Ibadan	Mambilla	Ibadan	Mambilla	Ibadan
P_1M_0	18.67	14.07	0.46	0.30	14.50	25.74	12.00	4.68
P_1M_1	17.25	12.50	0.40	0.30	12.00	12.00	2.89	5.50
P_2M_0	15.24	13.50	0.34	0.36	11.95	12.00	22.11	6.54
P_2M_1	10.70	12.50	0.26	0.31	11.00	10.00	4.05	6.70
P_0M_0	10.70	13.05	0.22	0.26	7.50	13.50	3.73	4.93
P_0M_1	15.71	14.00[a]	0.32	0.35	11.75	13.00[a]	24.42	15.05
SE	3.63	0.67	0.06	0.06	2.47	1.57	5.08	1.88

M_1 = with mycorrhizal inoculation, M_0 = without mycorrhizal inoculation, ns = not significant, P_1=SSP, P_2= SRP, P_0=no P application (control).

Table 2. Soil chemical properties and root infection as influenced by phosphate fertilizer application to AMF inoculated coffee seedlings in Ibadan and Mambilla soils.

	Treatments pH		OC (g/kg)		Avail P (mg/kg)		Root infection (%)	
	Mambilla	Ibadan	Mambilla	Ibadan	Mambilla	Ibadan	Mambilla	Ibadan
P_1M_0	6.40	5.75	2.72	5.62	10.80	6.18	66.50	66.95
P_1M_1	5.80	6.00	4.22	5.50	1.47	8.91	45.00	70.90
P_2M_0	6.50	5.80	3.17	5.54	6.62	4.36	66.35	68.55
P_2M_1	5.70	5.70	4.74	5.50	4.98	5.61	77.50	68.50
P_0M_0	6.40	5.85	3.26	5.15	6.79	5.38	62.55	45.30
P_0M_1	5.90	6.70	4.66	5.35	9.18	4.36	89.50	70.00
SE	0.34	0.28	0.52	0.28	2.76	0.41	12.24	10.08

OC = Organic Carbon, Avail. P = Available P.M_1 = with mycorrhizal inoculation, M_0 = without mycorrhizal inoculation, ns = not significant, P_1=SSP, P_2= SRP, P_0=no P application (control)

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Cup Taste Profile Evaluation on the Hybrid Progeny of Congolese and Guinea Groups of Robusta Coffee (*Coffea canephora*)

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SUMMARY

A study to evaluate cup taste profile variation on the progenies of hybrid between *C. canephora* groups of Congolese (represented by BP 409 clone) and Guinean (represented by Q 121 clone) was conducted. The study was aimed to describe the genetic background of taste profile on Robusta coffee. The progenies along with two parental were grown at Kaliwining Station (± 45 m a.s.l., dry climate) of ICCRI, Jember East Java. Hybrids of Congolese x Guinean and Guinean x Congolese consisted of 48 plants and 49 plants, respectively. Coffee cherries were processed through dry method by fully sun drying. Medium size of coffee beans was taken for roasting observation and conducting cup taste evaluation Cup taste profile was evaluated by sensorial specialists of ICCRI by observing fragrance, aroma, flavor, body, bitterness, astringency as well as after taste. Cluster analysis was applied on the data collected. The results showed that Congolese and Guinean had different cup profile. Guinean performed stronger quality and intensity of fragrance, quality and intensity of aroma and astringency than that of Congolese. In contrast, Congolese performed stronger intensity of flavor, body, bitterness as well as quality and intensity of aftertaste. The two clones performed similar quality of flavor, which Guinean having unique nice dried fruit note and Congolese chocolaty note. In term of cup profile, the progenies of Congolese and Guinean crossing were divided into three groups i.e. one group of Congolese, one group of Guinean and the other one did not belong to the two parental. The last group was considered to have bad cup quality profile.

INTRODUCTION

Robusta production has bean reached up to 85-90% of Indonesian total coffee production. This condition has bean placed Indonesia to be the 4th largest producer of Robusta coffee in the world after Brazil, Vietnam, and Columbia (Anonim., 2009). However, Robusta coffee has bean known for their less quality of cup taste comparing to Arabica coffee which resulted in significantly price difference in world coffee trade, and become economicly disadvantage to the producer countries. Furthermore, market competition of Robusta coffee will be tighten due to intensive development programs in many producere countries such as Vietnam, Thailand, Brazil, dan Uganda (Moschetto et al., 1996). Thus, it should be programmed nationally to develop superiorty of specific characteristic of produced coffee bean, mainly for the quality of cup taste.

Recently, it has bean known that cup taste characteristic of Robusta coffee is divided into two distinct characteristic which caused by their different genetic groups namely Guinean and Congolese (Yahmadi and Mawardi, 2001). The two groups is show different cup taste characteristics in the preference, aroma, acidity, body, and bitterness. Guinean type tend to

have less preference and aroma, that less prefered rather than the Congolese (Charrier and Eskes, 2004). In other side, breeding activities of Robusta coffee should involving parentals that came from those two genetic groups because of their high geneticly distance, in order to increase the chance of obtaining heterosis effect of the agronomic characteristics of progenies, mainly for yield. Fortunately, geneticaly yield and cup taste quality are independent which means increasing cup taste quality will not be gave of negative effect to the yield.

By now, ICCRI with Nestle Research and Development Centre-Tours, French are involving in the genome project of Robusta coffee which are using the parentals coming from those genetic groups of Robusta coffee (Priyono et al., 1999). As part of the research collaboration, this research was aimed to find out variation of cup taste profile on the population derived crossing from those parentals for consideration in the selection programs of superior planting materials.

MATERIALS AND METHODS

Planting material which used in this research were the population derived from artificial crossing between Congolese type (BP 409) and Guinean type (Q 121), and it reciproc, which consisted of 48 and 49 genotypes, respectively. This population is planted in Kaliwining research station, Jember, East Java, Indonesia, at 45 m above sea level (asl) with dry climate condition according to Schmidt & Ferguson (Schmidt and Ferguson, 1951).

Harvest were done only on red berries which then processed using dry process method with fully sun drying. Sample bean was taken 150 g for each genotype, only from medium bean size (sieved at screen 5.5-6.5 mm) at 11-12% moisture content. Roasting were done using Probat roaster at medium roast, and grinded with rough level. Observation of cup taste were done using three replication, each 10 g grinded bean/cup by three ICCRI's panelist using consencus method.

Observation were done before brewing for Intensity of Fragance (IFR) and Quality of fragrance (QFR), while parameter observed after brewing were Quality of Aroma (QAR), Intensity of Aroma (IAR), Quality of Flavour (QFL), Intensity of Flavour (IFL), Body (BOD), Bitterness (BIT), Astrigency (AST), Quality Aftertaste (QAT), Intensity Aftertaste (IAT), Balance (BAL) and Preference (PRE), as well as cup taste defect. It also observed roasting characteristics for enrich the data, involving bulk density, outturn of roasting, and apparent swelling.

Result of observation was perfoming using simple statistik profile, and also analyzed using cluster analysis which complete linkage as the method with euclidean distance.

RESULTS AND DISCUSSION

Roasting Characteristics

Roasting characteristic of the two parental type is only show difference for the apparent swelling. Roasting outturn and bulk density are relatively similar as shown in Table 1. The progenies look likes have similar characteristics to Guinean type which was then confirmed by cluster analysis as shown in Figure 1. Most of progenies were found have similar roasting characteristics to Guinean type rather than to Congolese type.

Table 1. Roasting characteristics of Congolese and Guinean parental and their progenies.

Genotypes	Out-turn of roasting	Bulk density		Apparent swelling
		before roasting	after roasting	
BP 409	0.84	0.71	0:43	1:38
Q 121	0.84	0.75	0:40	1:55
Progenies	0.86	0.76	0:42	1:53

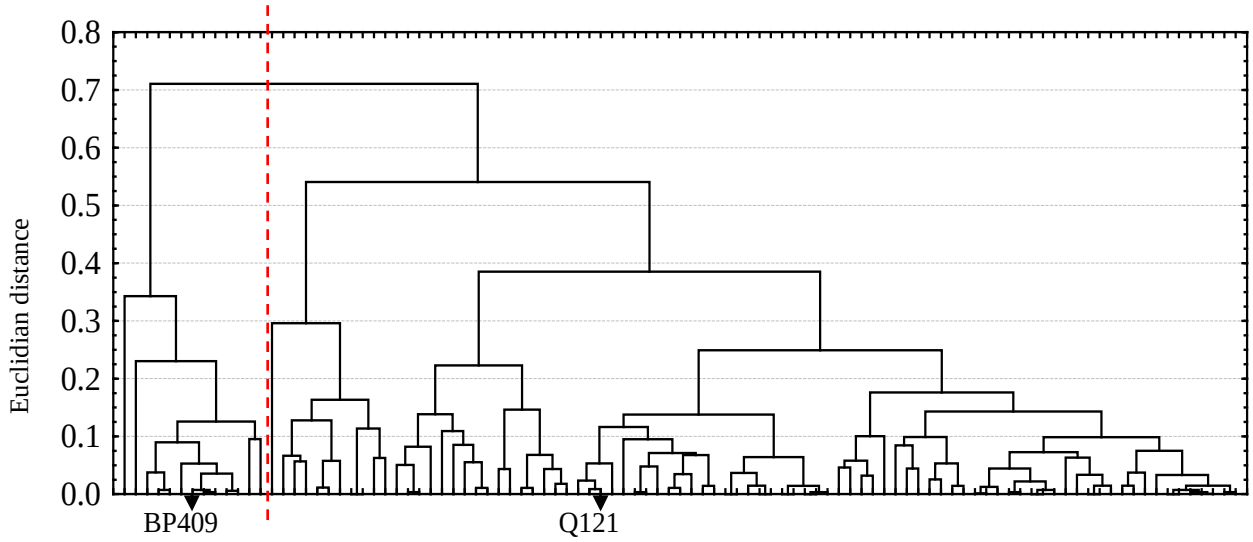

Figure 1. Clustering of roasting characteristics of Congolese x Guinean.

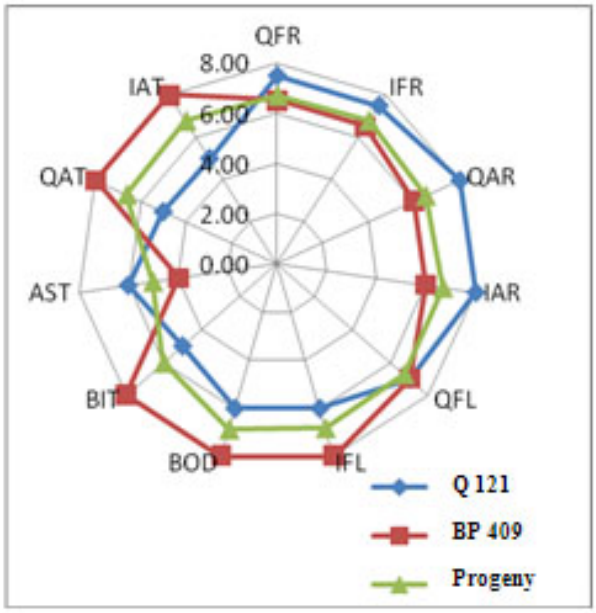

Figure 2. Cup taste profile of Congolese (BP 409) x Guinean (Q 121).

Profile of Cup Quality

Cup taste profile of the parental and their progenies is shown in Figure 2. According to the Figure 2, cup taste profile between the Congolese type (BP 409) and the Guinean type (Q121) are different on QFR, QAR, IAR, IFL, BOD, BIT, AST, QAT, and IAT characteristics. Congolese type have strong IFL, BOD, BIT, QAT, and IAT, but weak in QFR, IFR, QAR, IAR, and AST characteristics. In contrast, Guinean type has strong characteristics before brewing (QFR, IFR, QAR, and IAR) and weak characteristics after brewed (IFL, BOD, BIT, QAT, and IAT). However, those types have similar value on the characteristic of QFL, but in different flavor types. Congolese produce chocolaty flavor, while the Guinean produce dry fruit flavor that associated to green and fermented taste. Those condition then impacted to the cup taste preference which more preferred for the Congolese rather than the Guinean as shown in Table 2. This result is confirming the same found of Moschetto et al. (1996).

Table 2. Panelist preferences of parental.

Genotype	Parameters			
	Clean	Balance	Preference	Comment
Q 121	5	6	6	Floral, Green
BP 409	7	8	8	Chocolaty

In other side, cup taste profile of the progenies in overall shows average values comparing to their parental in all parameters observed. But actually, next analysis using cluster dendogram shows difference in cup taste profile which can be divided into three different groups as showed in Figure 3, namely *Guinean* group (A, marked by the position of Q 121), *Congolese* groups (B, marked by the position of BP 409), and unclear group (C). Cup taste profile of each group which shown in Table 3 confirm the close relation of group A to the Guinean type and group B to the Congolese type. Unfortunately, the last group (C) is identified as the group having bad cup taste profile.

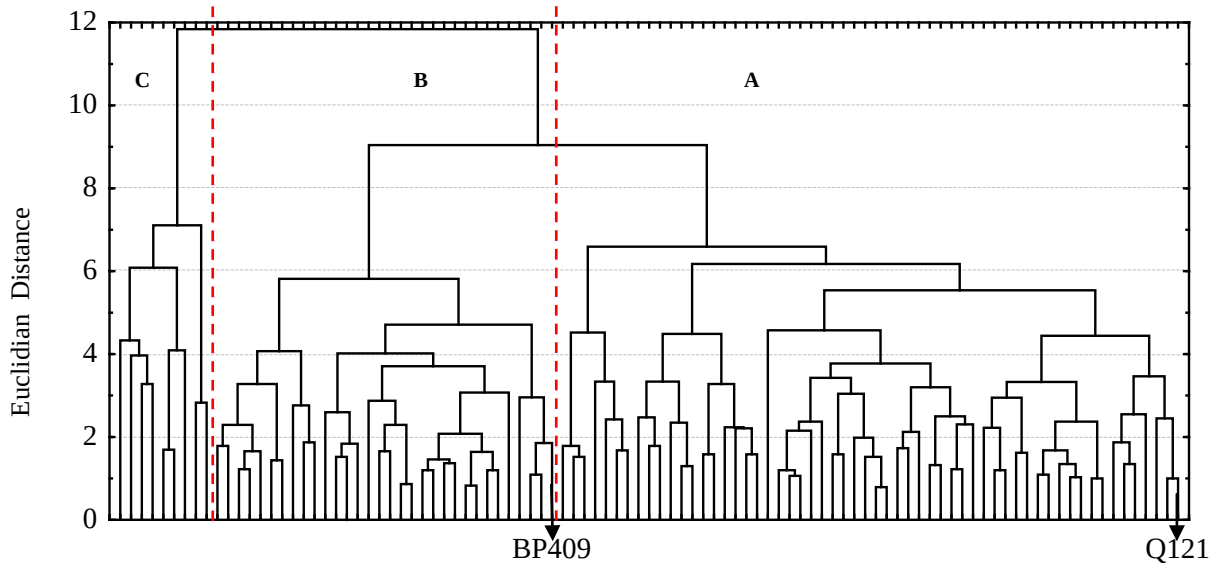

Figure 3. Clustering of cup taste profile of Congolese (BP 409) x Guinean (Q 121).

Table 3. Profile of cup taste quality for each groups compared to the parental.

Parameters	Cup taste score				
	Group A (57 genotypes)	Group B (32 genotypes)	Group C (9 genotypes)	BP 409	Q 121
QAR	5.08	6.95	6.51	6.00	8.00
IAR	6.81	7.02	6.47	6.00	8.00
QFL	4.78	7.52	6.59	7.00	7.00
IFL	6.00	7.55	6.55	8.00	6.00
BOD	6.42	7.71	6.47	8.00	6.00
BIT	7.58	6.23	5.59	8.00	5.00
AST	5.08	4.55	5.28	4.00	6.00
QAT	4.75	7.75	6.32	8.00	5.00
IAT	6.83	7.70	6.27	8.00	5.00
BAL	4.78	7.70	6.29	8.00	6.00
OVE	4.67	7.78	6.32	8.00	6.00

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Evaluation of Alternative Containers for Producing Cloned Seedlings of Coffee Conilon

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SUMMARY

There was an evaluation, during the period from October/2009 to fevereiro/2010, about the use of alternative containers for production of cloned seedlings of coffee conilon compared with traditional polyethylene bags. The experimental design was randomized in blocks with six treatments (T1 (control) - polyethylene bags (control); T2 - used plastic cups; T3 - bio pot (pot of recycled paper); T4 - bags of jute fiber tissue fabric; T5 - bags of TNT (Nonwoven Fabric); T6 - used bottles “PET”) and ten repetitions. The evaluated characteristics were diameter of the stem, plant height, number of leaves, root length, fresh weight of shoot, fresh weight of roots, total fresh weight, dry weight of shoot, dry weight of roots and total dry weight. The seedlings grown in used plastic cups were those that presented the worst results when compared with the seedlings derived from polyethylene bags (control), and they were significantly lower than the witnesses as to the root length, fresh weight of roots and the total fresh weight. The seedlings grown in bio pot and containers of jute showed best results in the evaluated characteristics when compared to control, being higher on the total fresh and dry weight of leaves and did not differ in other traits, demonstrating the feasibility of replacement of polyethylene containers with materials produced by other more ecologically viable.

INTRODUCTION

The coffee is characterized as a crop of major importance for the Brazilian economy. Currently, Brazil is the world's largest producer of coffee with a production of around 38,8 million bags in 2009 (Conab, 2010), moreover, it is the largest exporter and second world's largest consumer of the beverage.

Of all coffee produced in Brazil, about 75% comes from coffee Arabica and 25% from coffee conilon. Espírito Santo in Brazil is characterized as a state that produces two varieties of cultivated coffee, presenting itself as the second largest national producer, with about 9,7 million bags in 2009, and it is also the first largest producer of coffee conilon, about 7,3 million bags (Conab, 2010).

The cultivation of coffee involves several practices that promote increased productivity, reduction of cultivated areas beyond the increase in the producer’s income. The seedling production of coffee is one of the most important steps for the proper development of the crop, because seedlings of good quality show as being the base for a culture, especially in the case of a perennial crop like coffee. Increasingly widens the need to obtain healthy and vigorous plants, and that have a good "fixation" in the field, thus occupying less space of nursery, fewer amounts of seeds, substrate, and manpower.

Within the production of coffee seedlings, the use of polyethylene bags (derived from petroleum) on the packing of cuttings, presents itself as the practice being used by almost all nurseries in the country (Guimarães and Mendes, 1998). However, this material has the disadvantage of being slowly degraded in nature when they are not recycled, demanding the return of this material from the field.

Nowadays, we can observe such a public awareness about the use of materials that are environmentally correct, socially equitable and economically viable. Within this vision, the development of containers for coffee production from non-polluting materials or materials that promote the recycling of existing products, it is shown as an alternative for the small producer who typically has few financial resources to invest in farming, and it also represents a gain for the environment since much of this material being used is forgotten in nature.

Another factor of great importance for the producer is the possibility of developing containers for coffee production that do not need to be removed from the seedling at planting moment, and this may reduce damage to the seedling root system, besides, to facilitate this step and it becomes less costly to the producer.

Therefore, the objective of this project is to develop containers for producing cloned seedling of coffee conilon, from alternative materials obtained from recycled products. Furthermore, it aims to develop containers that facilitate the management of the producer, and which can reduce production costs, as the manpower and the purchase of cheaper materials (sub-products), thus reducing environmental contamination by using products that potentially were being dumped into the environment. Given the importance of coffee to the economy of the estate of Espírito Santo, about 70% of producers may benefit from carried containers, thereby promoting changes in habits and seeking sustainable solutions for Brazilian agricultural sector.

MATERIALS AND METHODS

The experiment was conducted from October 2009 to February 2010 in the greenhouse of IFES-Itapina, located at coordinates 19° 29'52,7"S and 40º 45'36,9"W in the city of Colatina-ES.

We evaluated various alternative containers for growing seedlings of coffee conilon, as the treatments, namely: (T1 (control) - polyethylene bags (control); T2 - used plastic cups; T3 - bio pot (pot of recycled paper); T4 - bags of jute fiber tissue fabric; T5 - bags of TNT(Nonwoven Fabric); T6 - used bottles “PET”).

All containers had the same volume capacity of soil (600 ml) and were provided with holes in the bottom half for drainage (Guimarães and Mendes, 1998). The substrate used in all containers was composed of a mixture of 70% of ravine land and 30% humus manure plus chemical fertilizer.

For the production of clones it was used cuttings of clone number 06 of coffee conilon variety "Vitória". The experiment had six treatments, as described in randomized block design with 10 repetitions, each consisting of six seedlings. The seedlings were growing by 120 days in the nursery until the time of field planting. After this period, the experiment was dismantled and assessed the following characteristics:

- Diameter of stem (DS): measured in the neck region, using a caliper ruler in millimeters;
- Height of plants (HP): measured the neck until the apical meristem of orthotropic branch in centimeters;
- Number of leaves (NL): counted all leaves of each container;
- Root length (RL): measured from the tip of the root emitted more extensive in centimeters;
- Fresh weight of shoot (FWS) and fresh weight of roots (FWR): the seedlings were removed from the container and washed in water. Then the root system was separated from the shoots, cutting the stem at the height of the neck, and placed on absorbent paper to remove excess water and then were weighed on an analytical balance and digital in g / parcel;
- Total fresh weight (TFW): it was made the sum of the fresh weight of roots with the fresh weight of shoot and the result in g / plot;
- Dry weight of shoots (DWS) and dry weight of roots (DWR): the root systems and aerial parts of each plot were wrapped separately in paper bags, properly labeled, and dried in an oven with forced air at 60 °C until constant weight, and weighed in a digital analytical and expressed in g / plot;
- Dry weight total (DWT): it was the sum of the dry weight of root and shoot dry weight and the result in g / parcel.

Data was subjected to analysis of variance and treatment means were compared with the average of control (polyethylene bags) by the Dunnett test at 5%.

RESULTS AND DISCUSSION

The data analysis showed how the diameter of stem only from the jute seedlings differed from the control, with thicker stems than the control (Table 1). The absence of differences in this parameter is mainly due to the fact that cloned seedlings are produced by cuttings, which are standardized at planting, varying little so far to be taken to the field.

The seedlings from the containers jute and TNT showed height of plant and number of leaf significantly higher than the seedlings from polyethylene bags, and all other containers did not differ from the control (Table 1).

The length of seedlings from TNT, the bottles and the plastic cup, was significantly lower than the control (Table 1). In seedlings from the plastic bottles and glasses it was observed that the roots had to be reeled or grown in a spiral shape featuring the "crooked pawn." This characteristic is due to the fact that the containers have rigid surface without grooves, since the containers made of rigid plastic, such as tubes, for example, must submit, within, in the longitudinal grooves to provide better targeting of roots in vertical direction, preventing folding or growth in a spiral shape inside the container (Guimarães et al., 19989).

The fresh weight of seedling from jute and TNT showed significantly higher seedlings of control (Table 1). While the seedlings from the plastic cup showed fresh mass significantly lower than control, directly affecting the total fresh weight which was also significantly lower than the mass of the seedlings produced in polyethylene bags (Table 1). The other treatments did not differ from seedlings in polyethylene bags (control) on the characteristics mentioned above (Table 1).

Table 1. Mean values diameter of the stem (mm), height of plant (cm), number of leaves, root length (cm), fresh weight of shoot (g), fresh weight of roots (g), total fresh weight (g), dry weight of shoot (g), dry weight of roots (g) and total dry weight (g).

TRAT	DS	HP	NL	RL	FWS	FWR	TFW	DWS	DWR	DWT
polyethylene	**6,75**	**11,60**	**10,30**	**21,94**	**8,25**	**12,19**	**20,44**	**1,91**	**1,82**	**3,72**
plastic cups	6,49 ^{ns}	13,35 ^{ns}	9,68 ^{ns}	17,25 $^{-}$	10,41 ^{ns}	10,52 $^{-}$	17,35 $^{-}$	1,63 ^{ns}	1,55 ^{ns}	3,18 ^{ns}
Bio pote	6,73 ^{ns}	15,70 ^{ns}	11,12 ^{ns}	19,93 ^{ns}	11,71 ^{ns}	11,73 ^{ns}	23,44 $^{+}$	2,69 $^{+}$	1,88 ^{ns}	4,57 ^{ns}
Jute	7,36 $^{+}$	16,96 $^{+}$	13,56 $^{+}$	22,43 ^{ns}	15,38 $^{+}$	12,56 ^{ns}	27,94 $^{+}$	3,39 $^{+}$	2,22 ^{ns}	5,61 ^{ns}
TNT	7,02 ^{ns}	17,96 $^{+}$	12,16 $^{+}$	16,04 $^{-}$	13,92 $^{+}$	10,81 ^{ns}	21,31 ^{ns}	2,81 $^{+}$	1,88 ^{ns}	4,60 ^{ns}
bottles	6,33 ^{ns}	11,13 ^{ns}	10,33 ^{ns}	17,02 $^{-}$	7,36 ^{ns}	11,11 ^{ns}	18,47 ^{ns}	1,77 ^{ns}	1,65 ^{ns}	3,42 ^{ns}
DMS	0,45	4,14	1,47	2,38	4,56	1,56	2,74	0,53	0,45	0,90

In each row, means followed by +, - or we are bigger, smaller or not different from control (polyethylene bags), respectively, by Dunnett test at 5% probability.

The negative result in the production of fresh root biomass, interfering directly in the fresh biomass total and presented by seedlings grown in plastic cups, is again because of the hard, flat surface of the container. The root restriction imposed by the walls of containers and the reduced volume, reduces some important parameters in evaluating the quality of seedlings such as biomass production, height and leaf area Townend and Dickinson, 1995).

When it comes to the dry weight to leaves obtained in seedlings grown in bio pot, jute and TNT, they seemed significantly heavier than the dried leaves of the control treatment, since the root and total dry weight of seedlings did not differ from control treatment (Table 1).

According to the results, it is observed that the seedlings produced in containers Bio jute pot did not differ significantly lower than in any of the variables, not differing or being above the seedlings from the polyethylene bags. These data are of great importance, demonstrating the feasibility of production of coffee conilon seedlings in these types of containers. This importance is due to the fact that with this procedure we do not use containers made with petroleum products (polyethylene) anymore which is a non-renewable natural resource, thus reducing possible environmental contamination, since most containers that are thrown to the environment taking years for its total decomposition, besides we can use containers made from recycled materials that would be potential environmental contaminants.

It was observed in this experiment that many roots drilled over from the sides of containers bio pot and jute, highlighting the possibility of direct planting of seedlings with these respective containers in the field without the need to set aside the same, depending on the degree of decomposition of these materials. Such information has such as an importance, because the techniques for transplanting to the field cause profound changes in the structure and architecture of the coffee root system (Rena and Damatta, 2002). The main element of change is the removal of part of the main root of the changes produced in the bag during the planting in the field, which would not happen to the production of seedlings in containers made with degradable materials.

The drawback observed in this study for the use of these containers is that because of the long period for the production of seedlings in the nursery; these containers have deteriorated almost completely, derailing the transport of seedlings by the producer. So, experimental adjustments are necessary to promote the extension of life cycle of these materials still in the nursery.

Regarding the use of cups and plastic bottles for the production of cloned seedling of coffee conilon, they seemed to be unviable because of the main fact of hard and flat surface of the material, which promoted undesirable deformations on roots of the seedlings.

ACKNOWLEDGEMENTS

To IFES/CNPq by scholarship the student of scientific initiation technology, the general direction of the IFES - Campus Itapina by support and project financing and the company to RECICLAL SA. Industry and Commerce (Salvador/BA) for donating the bio pots used the help of all times.

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Employment Generation in Brazilian Coffee Regions

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SUMMARY

Due the specific characteristics of the coffee production on each of the main Brazilian states producers of arabica and robusta coffee, a better understanding of the structural links between production and industrialization of coffee on those states and the national economy can provide subsides for public policies implementation, essential to plan the coffee production and increase the sector competitiveness. Therefore, this study analysed the employment generation in the production and coffee industrialization in the major Brazilian production regions, based on an inter-regional input-output model, with seven regions, which represent the main coffee-producing states - Minas Gerais, Espirito Santo, São Paulo, Paraná, Bahia and Other States - with 44 sectors each, in a system of 308 sectors.

INTRODUCTION

The coffee crop was introduced in Brazil at the beginning of the eighteenth century. Nowadays its production is dispersed on a large part of the national territory. The migratory character/ aspect of the coffee production remains from the colonial era, and resulted in relevant geographical shifts and structural changes in the Brazilian coffee production. While the coffee production is widespread in the country, it is currently concentrated in six states: Minas Gerais, Espírito Santo, São Paulo, Paraná, Bahia and Rondônia. The diversity of social, cultural and, especially, environmental conditions – such as soil, topography, altitude, latitude and rainfall indices - in each of those states, resulted not only in different producing regions and types of coffee, but also in different structures of production, technology and competitiveness. Thus, in the states of Minas Gerais, São Paulo and Bahia the culture of *Coffea arabica*, known as Arabica coffee, is predominant, while in the states of Espirito Santo and Rondonia dominates the culture of *Coffea canephora*, generally known as Robusta coffee (Conillon variety), that is used mainly in the coffee industry or in blends with Arabica coffee. For many decades, coffee was the main product of Brazilian exports, and despite the reduction of its share in those exports, is still very important for the country, especially on the social aspect. It is present in at least 370 thousand rural properties, 70% of them with familiar character, distributed in 2000 municipalities, in 17 States of the Federation. Furthermore, the manual harvesting accounts for great part of agricultural employment, and for up to 50% of the costs of their production. Each one of those producing states presents segments of their respective coffee production chains with distinct structural and technological levels. However, in most producing regions the prevail production systems is based on coffee intensive labour, especially during the harvesting, that can extend from May to September, due the region and climate (Bliska et al., 2009; Bliska and Guerreiro Filho, 2007). Due to technological and structural characteristics of the coffee sector, in each region of Arabica and Robusta coffee, where seasonal and intensive use of labor occurs, this study aims to provide subsidies to improve the understanding of structural relationships between coffee production and industrialization sectors and the national economy. Another goal is to provide subsidies for

implementation of public policies, planning of the coffee park and to increase the competitiveness of the national coffee in general.

MATERIALS AND METHODS

To analyze the behavior and current importance of the sectors of agricultural production and industrialization of coffee for each of the main Brazilian producers, for their respective state economies and other sectors of national economy, regarding to generation and expansion of employments, it was built an inter-regional input-output model, for the year 2002. This system has seven regions and 44 sectors per region, a total of 308 sectors, and it is consistent with the productive structure of the Brazilian economy, which is reflected in the reformulation of the System of National Accounts, occurred in 2007. The survey of structures and technical coefficients of/for production of Arabica and Robusta coffee, in the main producer states - Minas Gerais, Espírito Santo, São Paulo, Paraná, Bahia and Rondônia - was conducted between September 2005 and August 2006. This survey was used as a parameter to build the inter-regional input-output model, with seven regions, representing, respectively, those six major producer states and the region RBR, which include other Brazilian states where coffee production is not significant or there is no coffee production at all. This paper specifically examined the direct, indirect and induced effects of employment generation, and the effects of employment multiplier, Type I and Type II, for the sectors and countries that compose the system (Guilhoto, 2007; Guilhoto and Sesso Filho, 2005; Leontief, 1966; Miller and Blair, 1985).

RESULTS AND DISCUSSION

To facilitate the understanding of the relationship occurring among employment coefficient, coefficient of employment multiplication (or multiplier) and coefficient of employment generation (or generator), the results obtained by applying the proposed methodology are presented in Table 1, and the results are summarized below:

- The total of employments created by an increase of one million reais on the Brazilian coffee production are: (1) Arabica – 121 direct employments, 14 indirect and 71 induced, in a total of 207 employments; (2) Robusta – 192 direct employments, 20 indirect and 74 induced, in a total of 286 employments.
- For multiplier effects, “Robusta” and “Arabica” sectors have the two smaller effects among the 44 sectors of the economy.
- In contrast, the coffee industry presents the 4th largest multiplier effect, which again indicates the importance of coffee agribusiness for the Brazilian economy.
- Minas Gerais State: the generators of total employment of “Arabica” and “Industry coffee” are, respectively, the 2nd and 7th largest coefficients in the period. That is, in the state responsible for 50% of national coffee production, the public policies directed to the coffee sector should be carefully examined before being implemented effectively, because they may cause significant effect on the employment generation and, consequently, on the state economy.
- Espírito Santo State: “Robusta” is the 1st, “Arabica” is the 3rd and “Coffee industry” is the 4th largest generators of total employments. Therefore, policies related to the coffee production and industrialization may cause significant social and economic impacts in this state.

Table 1. Effect of an increase of 1 million R$[1] on the coffee production.

Region		Employment generating					Multiplier effect	
		Direct	Indirect	Induced	Total	Rank[2]	Type I[3]	Type II[4]
Brazil (as a whole)	Arábica	121	14	71	207	4	1,12	1,71
	Robusta	192	20	74	286	1	1,11	1,49
	Industry	6	91	67	164	8	15,91	26,84
Brazilian coffee regions								
Minas Gerais	Arábica	117	14	83	214	2	1,12	1,84
	Industry	7	86	78	171	7	12,66	23,26
Espírito Santo	Arábica	142	13	77	233	3	1,09	1,64
	Robusta	177	17	76	270	1	1,09	1,52
	Industry	7	116	69	192	4	18,44	28,83
São Paulo	Arábica	93	9	62	164	3	1,10	1,77
	Industry	6	69	59	133	8	13,23	23,69
Paraná	Arábica	154	19	66	238	1	1,13	1,55
	Industry	4	90	68	162	6	22,11	37,93
Bahia	Arábica	180	33	109	321	6	1,18	1,79
	Robusta	232	40	131	404	3	1,17	1,74
	Industry	7	90	99	197	20	13,75	27,76
Rondônia	Robusta	193	35	119	346	2	1,18	1,80
	Industry	9	147	107	263	5	17,53	29,58
Other States	Arábica	201	22	71	294	2	1,11	1,46
	Robusta	293	39	63	395	1	1,13	1,35
	Industry	8	89	70	167	8	11,97	20,55

- São Paulo State: coffee sector has the 3rd and industry the 8th position on the employment generation. This is a surprising result, due to the high degree of industrialization, involving very important sectors, such as production and industrialization of sugar, alcohol, livestock, citrus, dairy products and vegetable oils.
- Paraná State: arabica producer, characterized by small family farms, the coffee sector is the most important sector regarding employment generation.
- "Robusta" sector stands out in Bahia (third largest), Rondônia (second) and Rest of Brazil (the first) States.
- "Arabica" sector stands out in Minas Gerais (2nd-largest), Espírito Santo (3rd), São Paulo (3 rd) and Paraná (1st) States.
- "Robusta coffee" is the 1st largest total employment generation per 1 million of reais, while "Arabica" sector is the 4th- largest employment generator, among 44 sectors considered.
- Employment multipliers – Type I and Type II: the coffee industry had the 5th largest multiplier among the 44 sectors.

There are no indications that the production of Arabica and Robusta coffee is significant as employment multiplier, from the creation of a new employment, or from increasing the income of the population due to the creation of a new employment.

These results highlight the importance of farming and the coffee industry for both economies, state and national as a whole, and indicate that the impact of the implementation of public policies that act on sectors of agricultural production of coffee, arabica and robusta, and the sector of industrialization (or solubilization, roasting and grinding) should be significant on the employment generation in both economies, state and national as a whole.

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Influence of Full Sunlight on Trapping of the Coffee Berry Borer *Hypothenemus hampei* Ferrari

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SUMMARY

Trapping is a method currently used to control the coffee berry borer (CBB) *Hypothenemus hampei* Ferr. It was developed then validated in plantations of *Coffea arabica*, of moderate "technical input", cultivated under shade at medium altitude, and representative of many coffee plantations of Central America. Trapping has been little studied in farming systems in full sunlight (which is generally associated with high "technical input"). An experiment was set up in Nicaragua to evaluate the effectiveness of trapping within this specific framework. The experimental design comprised thirteen random sites in the studied plantation. Each one was composed of two paired plots of similar configuration: one with trapping, the other without. In full sunlight conditions there was a linear relation between the initial infestations of CBB and the captured females, but the efficiency was low. Populations of CBB developed better under self-shading and at the same time, trapping helped to reduce infestations where coffee trees formed a closed cover. Possible solutions to improve the performance of the trapping method in full sunlight conditions are laid out, in particular, changing pruning practices of the coffee trees and stripping branches of residual berries after harvest.

INTRODUCTION

Coffee berry borer trapping is a method that involves capturing migrating females emerging from dry berries left on the ground or on branches after harvesting (Dufour, 2005). Trapping was developed in plantations with low intensification: moderate "technical input", traditional tall varieties, low planting density and permanent shade. The quick results obtained encouraged growers to use this control method. The purpose of this work was to assess the effectiveness of the trapping method in estate plantations: high "technical input", dwarf varieties, high planting density and full sunlight.

MATERIALS AND METHODS

The test was set up in an adult *Coffea arabica* plantation (Var. catimor) of 500 ha, located in Matagalpa region, Nicaragua, at an altitude ranging between 700 and 1050 m. This plantation was cultivated in full sunlight with a density of 7140 plants/ha. The general set-up comprised 13 groups of plots of 7000 m² associated per pairs (sites) and distributed randomly in the plantation. The plots of each site (trapping and control) were selected according to their similarity in physical and agronomic characteristics (Figure 1).

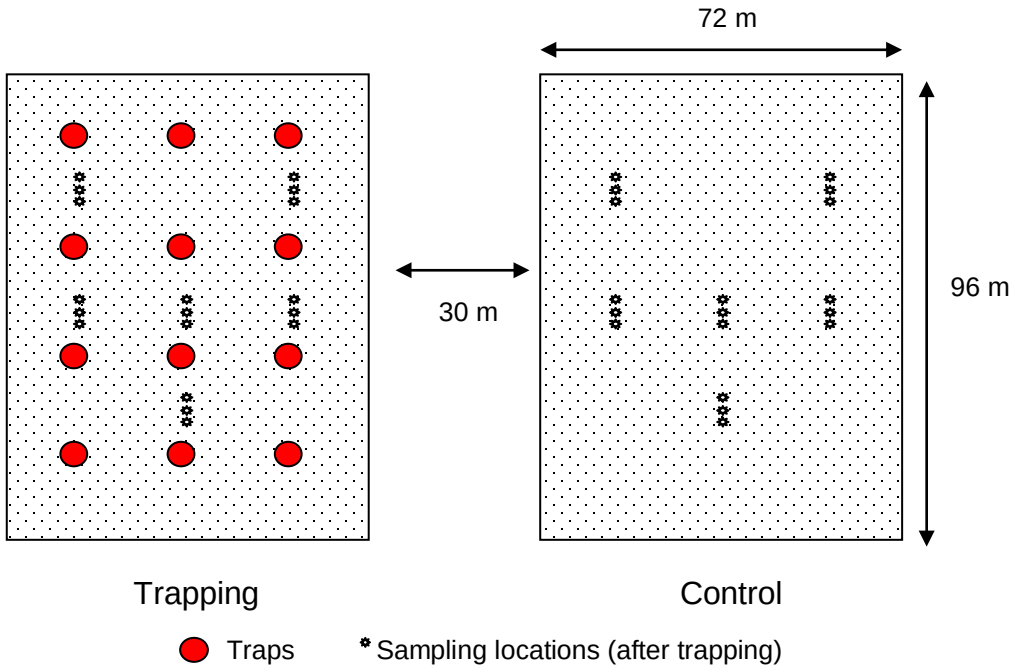

Figure 1. Set-up per site.

Only one agronomic variable was taken into account: the type of cover provided by the actual foliage of the coffee trees which is determined by the size and the structures of the coffee trees (Table 1).

Table 1. Classification of the sites according to the type of cover.

Sites	**Type of cover**
Triangulo, Limón, Zapote, Palo Solo	No cover
Panorama, Guacha, Santa Fé, Naranjo, Casa de zinc	Sparse cover
Piedras negras, Casa de piedra, Los Pérez, Pedrera	Closed cover

The sampling method used has been described by Dufour et al. 2004:

- before trapping the following were estimated: the number of infested residual berries (soil + branches) for 15 coffee trees/plot, the number of females per coffee tree via random sampling and then a dissection of 50 infested berries (soil + branches) by plot.
- after trapping, the number of infested new berries on branches for 18 coffee trees/plot were estimated (Figure 1).

The trapping method (Dufour, 2005) used 12 BROCAP® traps/0.7 ha. Each trap was hung from a coffee tree branch so that the entry of the trap was located around 0.5 m from the ground (Figure 2).

The quantity of captured females was represented by the volume occupied by these females placed in one or more 25 ml graduated cylinders and immersed in 90% ethanol.

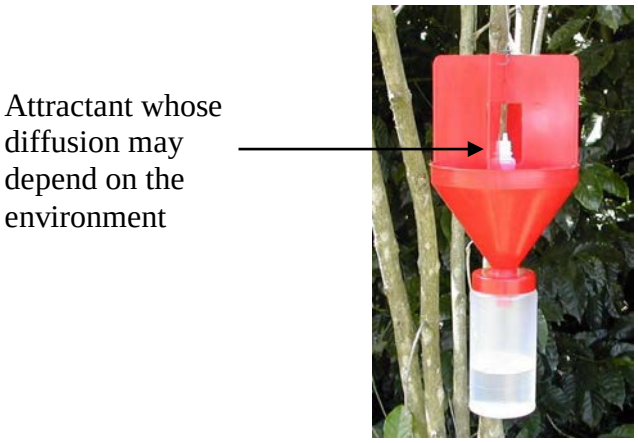

Figure 2. BROCAP® trap.

RESULTS

Trapping in terms of the quantity of females caught

Under full sunlight conditions, there was a linear relation between residual females and captured females. For 100 females/coffee tree, 5.3 ml were caught per trap (Figure 3). A similar relation was found in shaded plantations (Dufour et al., 2007). For 100 females/coffee tree, 25.6 ml were caught per trap, i.e. five times more. Trapping works under full sunlight conditions, but its efficiency is low.

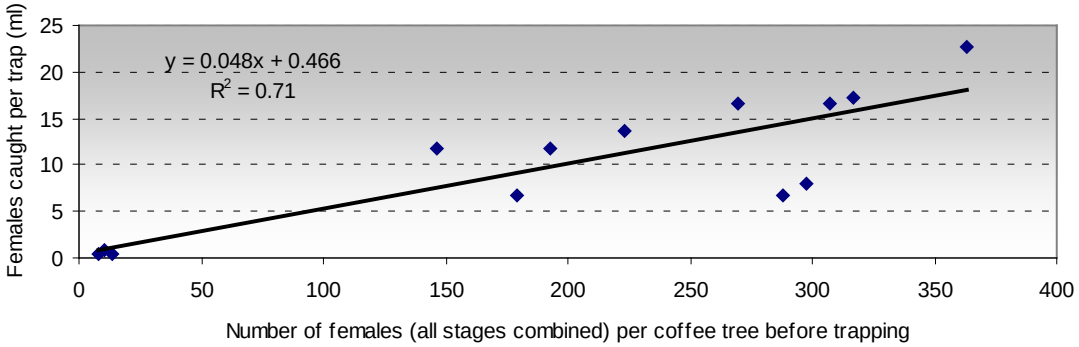

Figure 3. Correlation between the quantities of residual females and captured females.

Trapping efficiency under full sunlight conditions depending on the cover formed by the coffee trees

Trapping helped to reduce infestations in plots where the coffee trees formed a closed cover. This type of cover is conducive to attractant diffusion inside the coffee plantation. There was no effect where the cover was sparse or nonexistent (Table 2).

The CBB developed better in plots where the actual cover of the coffee trees provided substantial self-shading. Self-shading tended to slow down residual berry desiccation, which would be conducive to CBB survival (Table 3).

Table 2. Differences between the number of infested berries from plots with and without trapping, under three types of cover (Friedman test at the 0.05 level).

Type of cover	Trapping plots	Control plots	Friedman test
No cover	1440	1208	NS
Sparse cover	3991	3715	NS
Closed cover	4638	8451	S

Table 3. Differences between attack rates on the new crop in plots with or without trapping (Kruskall and Wallis test and multiple comparison test at the 0.05 level).

Type of cover	Plots	Rank sum	Mean of attack rates	Groups	
No cover	8	46	2.0	A	
Sparse cover	10	137	8.2	A	B
Closed cover	8	168	15.8		B

CONCLUSION

Satisfactory trap operation depends on the existence of shade or self-shading. Under full sunlight conditions, trap use should therefore be reserved for plantations with closed cover where the CBB develops better. In this context, the efficiency of CBB control could be improved by changing some agronomic practices: pruning lower branches to aerate plots and stripping branches of residual berries which provide shelter for colonizing females.

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Yield Differences of "Conilon" Coffee Plants Propagated by Cuttings and Seeds along a Nine Year Period

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SUMMARY

World's coffee production in the last years has being of more than 110 million sacks, produced essentially in developing countries. Brazil is the world's biggest coffee producer and exporter, and has in this culture an important source of incomes, employment and local development in the producing or processing regions. The objective of this work was to evaluate the yield differences of plants of *C. canephora* cv. Conilon implanted from seed or cuttings, along a 9 year period, in Vila Valério, Espírito Santo, Brazil. The experiment was performed in randomized complete block design, with two treatments (seedlings originated from seeds and branches), and 12 replicates with five plants per plot, implanted by 2 x 1 m, in November 1999. The plant production was analyzed along a 9 year period, from 2001 (17 months) until 2009 (113 months). The production from the plants grown from cuttings was higher until the 6th harvest (although not significantly in the 3rd, 5th and 6th) and in the 8th. Plants propagated from seeds presented marginal (not significantly) higher values in the 7th and 9th harvests. Despite the fact that in the last 5 harvests only once a significant higher yield was reported for cutting implanted plants, the accumulated production over a 9 year yields was higher in *ca*. 5.000 kg ha^{-1} when compared with plants implanted from seed over the same period. That shows the strong advantage from implanting this crop from cuttings instead of seeds, with a clear impact on yields, particularly in the early harvests.

INTRODUCTION

The genus *Coffea* comprehends at least 103 species, with commercial relevance for *C. arabica* and *C. canephora* (Davis et al., 2006). World's coffee production in the last years has being of more than 110 million sacks, produced essentially in developing countries (ICO, 2010). Brazil is the world's biggest coffee producer and exporter, and has in this culture an important source of incomes, employment and local development in the producing or processing regions.

C. canephora is a diploid (2n = 22 chromosomes), allogamous species, self-sterile with a gametophytic incompatibility. When propagated through seeds, *C. canephora* cv. Conilon

plants shows large variations concerning the productivity, plant architecture, disease resistance, fruit maturation, size and shape of seeds, fruits and leaves (Conagin and Mendes, 1961; Bragança et al., 2001). On the other hand, vegetative propagation maintain the genetic characteristics of a selected plant, granting a certain crop homogeneity concerning grain maturation, yield potential and other desirable agronomic characteristics (Weigel and Jurgens, 2002), showing as well production precocity (Bragança et al., 2001). In this way, the use of cuttings has become quite advantageous, using orthotropic branches.

Nevertheless, the resistance and durability of such plants are still a matter of debate. It is known that the root system shows differential characteristics according to the species, genotype, plant age, year season, climate, crop density, biotic stresses, soil texture and structure, etc. (Lynch, 1995). However, it was reported that until the 6th year Conilon plants did not show differences concerning the root system (superficial area and length) until a depth of 60 cm, either propagated from cuttings or seeds (Partelli et al., 2006).

The main objective of this work was to evaluate the yield differences of plants of *C. canephora* cv. Conilon implanted from seed or from cuttings.

MATERIAL AND METHODS

The experiment was carried out in the North of Espírito Santo State Brazil (Vila Valério -18º 57` South; 40º 17` West). In this region, the tropical climate is predominant (humid and hot summer, dry winter), with annual precipitation average of 1200 mm and annual temperature average of 23 ^{o}C. The predominant soil is a distrofic Red-Yellow Latosol (typic Hapludox) (EMBRAPA, 1999).

The experiment was performed in randomized complete block design, with two treatments (seedlings originated from seeds and branches), and 12 replicates with five plants per plot, implanted by 2 x 1 m, in November 1999. The plant production was analyzed along a 9 year period, from 2001 (17 months) until 2009 (113 months). From 2002 onwards the conventional fertilization system was substituted by agricultural organic procedures. After 2006, chemical and organic fertilization were implemented, without pest/disease control products application. A one-way ANOVA ($P < 0.05$) was applied to evaluate differences between the yield obtained from plants originated from seeds and cuttings. A further comparison was carried out using an F test (95% confidence level). This work had the collaboration of Valnei Marcos Partelli in the experiment management and data collecting.

RESULTS AND DISCUSSION

The productivity of plants derived from cuttings was higher in the 1st, 2nd, 3rd, 4th, 5th, 7th and 8th year (significantly in the 1st, 2nd, 4th and 8th) (Table 1). The average production of the experimental period (9 years) in the plants originated from cuttings and seeds was 2934 e 2350 kg ha^{-1} year^{-1}, respectively. For the same period, the sums of all yields were 26407 and 21147 kg ha^{-1} year^{-1} for the plants from cuttings and from seed, respectively, what implicated and accumulated difference of 5260 kg ha^{-1}. That clearly points to a strong production advantage in the *C. canephora* cv. Conilon plants originated from cuttings. Such differential was higher than 2 average annual yields from plants originated from seeds, and could be (at least partially) explained by the fact that the plants originated from cuttings are physiologically adults already at the implantation.

Since the development of the root system is similar in the two types of plants, the higher productivity of cutting plants might be related to their ability to emit a higher number of branches and nodes (Partelli et al., 2006), what could have been responsible for the higher initial productions.

Furthermore, the better productivity in plants originated from cuttings might have arise from a good and uniform genetic material, possible due to this cloning process (Weigel and Jurgens, 2002), while the plants originated from seeds would have presented genetic variability not always maximizing the yield capacity (Conagin and Mendes, 1961).

We conclude that *C. canephora* cv. Conilon implantation through cuttings offer higher production advantages as compared to plants originated from seed.

Table 1. Production (kg ha^{-1}) of *C. canephora* cv. Conilon, implanted from cuttings or seeds, from 2001 (17 months) until 2009 (113 months) after implant, in Vila Valério, Espírito Santo State Brazil.

Implantation	Production (kg ha^{-1}) along time of implantation (months)								
	17	**29**	**41**	**53**	**65**	**77**	**89**	**101**	**113**
Cutting	421 [a]	5795 [a]	1629 [a]	3135 [a]	1562 [a]	4325 [a]	2330 [a]	4349 [a]	2861 [a]
Seed	73 [b]	3220 [b]	1269 [a]	2355 [b]	1549 [a]	3745 [a]	2513 [a]	3446 [b]	2977 [a]
CV	50.2	16.9	35.3	26.2	34.9	27.4	22.2	26.1	45.6

In each harvest, means followed by different letters represent significant differences (F test, 95% confidence level).

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Effects of Shade Tree Composition on the Coffee Quality in Agroforestry Systems of the Kodagu District, South-Western India

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SUMMARY

Indian coffee is grown mostly in the Western Ghats, one the world hotspots of biodiversity, under the shade of multi-strata systems that are among the most diverse in terms of native shade tree species in the world. Two studies were undertaken to evaluate 1) the effect of shade tree composition on Arabica and Robusta quality by comparing shade cover made predominantly of a mix of over 20 native tree species to shade cover made with an increasing proportion of the exotic species (*Grevillea robusta*); and 2) the specific effect of individual shade tree species on Robusta and Arabica quality under the canopy of 4 native species and the exotic species.

Results indicate that the exotic species is not a suitable shade tree for Robusta coffee especially in wet conditions (western zone) as proportion of large beans and cup quality significantly decrease. In the drier conditions of the Eastern zone, *Grevillea robusta* does not affect greatly Arabica except for bean size when the proportion of this exotic species is very high (>50%).

INTRODUCTION

India is the fifth largest world coffee producer with Robusta coffee representing more than 70% of its production and grown mainly at altitudes of 800-1000 m. This confers to Indian Robusta an international fame as high-altitude Robusta and a quality premium on the international market. Furthermore, Indian Robusta is mainly grown under the shade of multi-strata systems, mostly in the Western Ghats, one the world hotspots of biodiversity (Myers et al., 2000). Although less dense than for Arabica, shade tree composition in Robusta systems is constituted by highly diverse native species, but the conservation of such biodiversity-rich coffee systems is a challenge with the recent introduction of *Grevillea robusta*, a fast-growing exotic timber species (Garcia et al., 2010). No information is found in the literature regarding the effect of shade level on Robusta quality and even less on the specific effect of individual shade tree species. Although more studies have been published on Arabica quality (Guyot et al., 1996; Muschler, 2001; Vaast et al, 2005), these studies mainly deal with shade level and no publication was found in the literature regarding the specific effect of individual shade tree species on Arabica quality.

In this paper, we present the results of two studies on Arabica and Robusta. The first one reports on the effect of shade tree composition on Arabica and Robusta quality by comparing shade cover made predominantly of a mix of over 20 native tree species to shade cover made

with an increasing proportion of the exotic species (*Grevillea robusta*). The second study concentrates on the specific effect of single shade tree species by comparing quality of Arabica and Robusta grown under the canopy of 4 native species and the exotic species.

MATERIAL AND METHODS

Effect of shade composition on coffee quality

Ten kg of ripe Robusta berries were taken at the peak of harvest in 102 Robusta coffee agroforestry systems along an East-West transect (Deciduous Eastern zone: ~1200-1800 mm/year; Central zone: ~1800-3000 mm/year; Evergreen Western zone: ~3000-5000 mm/year) in the Cauvery watershed of the Kodagu district. Five coffee samples were taken per farm.

Shade level was estimated with a densiometer (Lemmon, 1956) and tree inventories were done species by species. In each zone, farms with shade cover ranging from 40% to 80% were selected and with range of the exotic species (*Grevillea robusta*) ranging from <10%, 10-30%, 30-50% and >50% of the shade tree species composition. The same type of study was undertaken for Arabica, but only in the Eastern zone where it is cultivated. 40 farms with shade cover from 50-75% and over 75% were selected and with the range of the exotic species (*Grevillea robusta*) comprising 0-10%, 10-20%, 20-50% and over 50% of the shade tree species composition.

Effect of single shade tree on coffee quality

The second study concentrates on the specific effect of single tree species on Arabica and Robusta quality under the canopy of 5 tree species, 4 native species (Artocarpus heterophyllus, Dalbergia latifolia, Acrocarpus fraxinifolius, Ficus racemosa or Lagerstroemia microcarpa) and the exotic species (Grevillea robusta). 10 kg of ripe Arabica or Robusta berries were taken at the peak of harvest under the shade of the 5 trees per species at mid-way to the drip-line. Five farms were selected in each of the same 3 zones for Robusta and only in the East for Arabica.

Processing

For Robusta, ripe berries were processed by the dry method. After drying down to 10-12%, dry berries were de-husked. For Arabica, ripe berries were processed by the wet method (wet de-pulping, anaerobic fermentation for 24 hours, sun-drying down to 10-12% and de-husking). For both Arabica and Robusta, out-turn (% of green beans/fresh berries) and % of green beans with larger sizes (AA beans with diameter > 7.0 mm) were recorded. Cup testing was undertaken at the Coffee Lab in Bangalore with cup quality scored on a scale from 0 to 10.

RESULTS AND DISCUSSION

Effect of shade composition on coffee quality

For Robusta (Table 1), increasing the % of *Grevillea robusta* in the shade composition does not have any significant effect on the out-turn (% of green beans / fresh berries) or the % of beans of large sizes mainly due to the fact that results are highly variable. On the other hand,

an increasing % of the exotic species results in a decrease in cup quality (Table 1) as well as aroma and body (data not shown).

Table 1. Effects of % *Grevillea robusta* in the canopy composition on out-turn (% green beans / fresh berries), % of large beans (%AA) and cup quality (scale of 0-10) of Robusta in the Eastern (dry), Central (wet) and Western (very wet) zones of the Kodagu district.

Ratio %	**East**	**Central**	**West**
<10%	21.5 a	21.3 a	21.6 a
10-30%	22.4 a	23.0 a	23.0 a
30-50%	22.0 a	22.9 a	20.0 a
>50%	22.9 a	20.2 a	20.0 a
Zone Mean	22.0 A	21.8 A	21.1 A
% AA			
<10%	27.1 a	31.1 a	25.4 a
10-30%	27.6 a	31.8 a	30.7 a
30-50%	37.9 a	30.2 a	30.3 a
>50%	33.0 a	39.4 a	35.3 a
Zone Mean	30.7 A	33.2 A	30.4 A
Cup quality			
<10%	5.58 a	5.57 a	5.54 a
10-30%	5.73 a	5.56 a	5.42 a
30-50%	5.29 b	5.60 a	5.25 b
>50%	5.28 b	5.33 b	5.25 b
Zone Mean	5.49 A	5.51 A	5.43 A

** Within a zone, small letters indicate statistical significance (P=0.05) according to the Newman and Keuls test.*
*** Between zones, capital letters indicate statistical significance (P=0.05) according to the Newman and Keuls test.*

For Arabica, shade level (50-75% and >75%) has no effect on quality (data not shown). Increasing the % of Grevillea robusta does not affect out-turn or cup quality, but decreases the proportion of large beans (Table 2).

Effect of single shade tree on coffee quality

For Robusta (Table 3), the results show a tendency of the out-turn (% of green beans / fresh berries) to increase from East to West (hence from dry to very wet conditions) whereas bean size (% of AA) tends to decrease from East to West. Shade by the exotic species *Grevillea robusta* results in a higher proportion of beans with large sizes in the East in comparison to the native species, but to a lower one (along with *Artocarpus*) in the West (Table 3). These results also show that shade of the exotic species leads to a lower cup quality in the West. This indicates that *Grevillea robusta* is not a suitable shade tree for Robusta coffee in wet conditions.

Table 2. Effects of % *Grevillea robusta* in the canopy composition on out-turn (% of green beans/ fresh berries), % of large beans (%AA) and cup quality (scale of 0-10) of Arabica in the Eastern zone of the Kodagu district.

% Grevillea	Out-turn (%)	AA (%)	Cup quality (over 10)
0-10	15.3 a	46 a	5.48 a
10-20	16.0 a	41 a	5.64 a
20-50	15.6 a	41 a	5.65 a
>50	15.2 a	34 b*	5.54 a

**Small letters within a column indicate statistical significance (P=0.05) according to the Newman and Keuls test.*

Table 3. Effects of tree species on out-turn (ratio of green beans / fresh berries). % of large beans (AA) and cup quality (scale of 0-10) of Robusta coffee in the Eastern (dry). Central (wet) and Western (very wet) zones of the Kodagu district.

Out-turn (%)	**East**	**Central**	**West**
Artocarpus	20.1 a	20.8 a	21.4 a
Dalbergia	19.9 a	21.7 a	20.4 a
Lagerstroemia	20.1 a	20.6 a	21.6 a
Grevillea	20.6 a	20.9 a	21.8 a
Zone Mean	20.2 B	21.0 A	21.3 A
AA (%)			
Artocarpus	38.1 b*	31.2 a	19.0 b
Dalbergia	32.2 b	29.9 a	23.7 a
Lagerstroemia	36.2 b	31.5 a	24.7 a
Grevillea	47.5 a	27.0 a	17.7 b
Zone Mean	38.6 A**	29.9 B	21.2 C
Cup quality			
Artocarpus	5.68 a	5.78 a	5.75 a
Dalbergia	5.61 a	5.66 a	5.70 a
Lagerstroemia	5.60 a	5.74 a	5.74 a
Grevillea	5.65 a	5.79 a	5.54 b
Zone Mean	5.64 A	5.74 A	5.68 A

**Within a zone, small letters indicate statistical significance (P=0.05) according to the Newman and Keuls test.*
***Between zones. capital letters indicate statistical significance (P=0.05) according to the Newman and Keuls test.*

For Arabica in the Eastern zone, out-turn and proportion of large beans are lower under the shade of the native species *Dalbergia* than under any other species, including the exotic one

(Table 4). On the other hand, coffee quality is lower under shade of *Acrocarpus* and *Artocarpus*, but not under *Grevillea robusta*.

Table 4. Effects of tree species on out-turn (ratio of green beans / fresh berries). % of large beans (AA) and cup quality (scale of 0-10) of Arabica in the Eastern zone of the Kodagu.

Tree species	Out-turn (%)	AA (%)	Cup quality (over 10)
Acrocarpus	0.16 a*	48.9 a	5.39 b
Dalbergia	0.15 a	39.6 b	5.54 a
Ficus	0.16 a	43.3 a	5.61 a
Artocarpus	0.17 a	44.1 a	5.39 b
Grevillea	0.16 a	47.0 a	5.55 a

**Between shade tree species. small letters indicate statistical significance (P=0.05) according to the Newman and Keuls test.*

CONCLUSION

Results indicate that the exotic species, *Grevillea robusta*, is not a particularly suitable shade tree for Robusta coffee especially in wet conditions (western zone) as proportion of large beans and cup quality significantly decrease with increasing % of this species in the shade composition. In the dry conditions of the Eastern zone, *Grevillea robusta* does not affect greatly Arabica quality except for bean size when the proportion of this exotic species is very high (>50%).

Although *Grevillea robusta* does not strongly affect coffee quality, the authors advocate for the maintenance of a high diversity of native tree species in the agroforestry systems of Kodagu in order 1) to maintain the largest range possible of ecosystem services provided by these systems and 2) to improve farmers' livelihoods by reducing their vulnerability to coffee price volatility through access to developing eco-friendly coffee markets and payment schemes for environmental services.

ACKNOWLEDGEMENTS

The authors would like to thank the European Community for financing the CAFNET project under the Programme on Environment in Developing Countries.

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Coffee Agroforestry with Some Timber Shade Trees. Study on Carbon Stock, Mineral Cycle, and Yield

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SUMMARY

Coffee cultivation by using shade trees is an simple of agroforestry, this system could get better ecosystem service and sustainable agricultural. Research of coffee agroforestry with *Tectona grandis, Paraserianthes falcataria, Melia azedarach,* and *Hibiscus macrophyllus* aims to study the possibility of this species of industrial woods as shade trees of *Coffea canephora*. The research was conducted in Jember, Indonesia (45 m asl., D rainfall type according to Schmidt and Ferguson), and arranged in split plot design. The main plot were coffee – *T. grandis*, coffee – *P. falcataria* single row, coffee – *P. falcataria* double rows, coffee – *P. falcataria* var. Solomon, coffee – *M. azedarach*, coffee – *H. macrophyllus*, and coffee – *Leucaena* sp. as control. The sub plots were coffee clones, i.e. BP 534, BP 409, BP 936, dan BP 939. Among those timber trees, *Leucaena* was planted as the alternative shade trees. The result showed that in comparison with coffee – *Leucaena* system, all of coffee agroforestry system improved carbon sequestration. Total C-stock on coffee – *P. falcataria* single row was highest, i.e. 1,007 percent to control while the lowest one was coffee-*T. grandis* 317.44% to control. During one year observation, litter weight of *H. macrophyllus* was heaviest followed by *T. grandis*. The lightest litter was obtained from *M. azedarach*. Based on its mineral contents, litters of *T. grandis* potentially supplied back nutrients that equaled to total Urea, SP-36, KCl, Dolomite, and Kieserite as much as 574.14 g; *P. falcataria* 287.57 g, *P. falcataria* var. *Solomon* 453.59 g, *M. azedarach* 450.84 g, *H. macrophyllus* 877.56 g, and *Leucaena* 445.12 g per tree per year. Because of heavily fallen leaves of *M. azedarach* during dry season and conversely too dense shading of *H. macrophyllus*, cherry yield at 4 and 5 year old by using both spesies were consistently lower than that under *T. grandis, P. falcataria* and control. At those ages, effect of clone on berry yield was still not consistent but there was a tendency that BP 939 was most productive, while BP 534 was the less. Its outturn was not influenced by agroforestry system but by clones. It was concluded that coffee agroforestry improve ecology service, but *M. azedarach* and *H. macrophyllus* were not appropriate to be used as coffee shade trees. *P. falcataria* is recommended as an alternative shade tree beside *Leucaena* sp.

INTRODUCTION

Agroforestry is essentially a cropping pattern that utilizes sunlight and `layered` soil to improve land productivity. In Central America, the shade trees has an important role in conserve natural biodiversity, soil and water quality, and carbon preservation (Vaast and Harmant, 2002). In Indonesia, most of coffee farmers using *Leucaena* and *Gliricidia sepium* as shade because both species can keep well microclimate, legume, and tolerant to be pruned. However, additional use of those species provide less in terms of land productivity (timber yield) and the ability to carbon storage. Environmentally, those species are expected to store carbon faster than traditional shade species, and efficient in pumping nutrients from sub soil

to top soil layer through entirely biomass. To meet the need of wood faced with environmental problems therefore should be found appropriate solutions to produce of wood without damaging the natural preservation (Untung, 1999).

Some industrial timber species that have the potential to developed as shade trees are *Paraserianthes falcataria*, teak (*Tectona grandis)*, *Melia azedarach* and *Hibiscus macrophyllus.* The aim of this study is to study the effect of using of industrial timber species as shade trees and the influence carbon sequestration, mineral cycle, plant growth and coffee productivity.

METHODOLOGY

Research was conducted in the Jember, Indonesia (45 m, D climate type according to Schmidt and Ferguson) in a split plot design. The main plot were nine pattern of agroforestry system i.e.(A) Coffee – teak double row (3 m x 2.5 m x 18 m), (B) Coffee – *Paraserianthes falcataria* in single row (2.5 m x 6 m), (C) Coffee-*P. falcataria* in double row (3 m x 2.5 m x 12.5 m), (D) Coffee – *P. falcataria* var. Solomon in two rows (3 m x 5 m x 12.5 m), (E) Coffee – *P. falcataria* var. Solomon in four rows (3 m x 5 m x 12.5 m), (F) Coffee – *Melia azedarach* in two rows (3 m x 5 m x 12.5 m), (G) Coffee – *M. azedarach* in four rows (3 m x 5 m x 12.5m), (H) Coffee – *Hibiscus macrophyllus* in four rows (3 m x 5 m x 12.5 m), and (I) Coffee-*Leucaena* sp (3 m x 2.5 m) as the control. The subplot was coffee clones, namely BP 534, BP 409, BP 936 and BP 939. The observed parameters were carbon sequestration, litter and biomass weight monthly, litter mineral content, and coffee yield.

RESULTS AND DISCUSSION

Carbon stock

The amount of carbon stored in a coffee plantation depends on the population and type of vegetation (Hairiah and Rahayu, 2007; Yuliasmara and Wibawa, 2007; Yuliasmara et al., 2009). The amount of C stored certainly related to the volume of the plant. In this study, *P. falcataria* is the fastest-growing species while *Leucaena* is lowest. In the meantime, C-stock inside coffee plantation at 5 years old was around 7% in average to total in the area. Carbon stock in the coffee – *Leucaena* pattern (control) is lowest, whereas the highest is on coffee – *P. falcataria* single row (1006.54% to control) followed by coffee – *P. falcataria* var. Solomon four rows (879.9% to control). C stored in the coffee – *T. grandis* pattern cropping was approximately 317.4% to control.

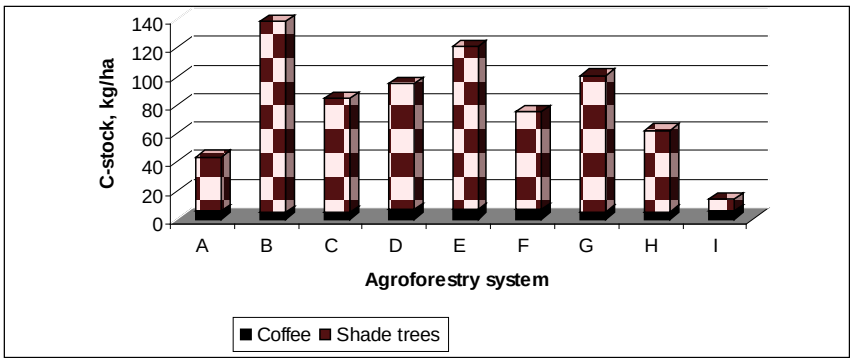

Figure 1. Carbon sequestration between agroforestry system.

Mineral cycle

Litter of *Hibiscus macrophyllus* was heaviest, followed by teak and *P. falcataria* var. Solomon. The lighest litter was obtained from *M. azedarach.* By annually pruning, biomass from *Leucaena* is quiet high. In the equality with total Urea, SP-36, KCl, Dolomite, and Kieserite, litters of *T. grandis* potential to supply back as much 574.14 g; *P. falcataria* 287.57 g, *P. falcataria* var. *Solomon* 453.59 g, *M. azedarach* 450.84 g, *H. macrophyllus* 877.56 g, and *Leucaena* 445.12 g per tree per year. The tendency of micro element (Fe, Mn, Cu, Zn) cycling is quite similar with those macro elements. During dry season, *T. grandis* falling down all of their leaves, and on rainy eason a part of the branches were pruned to give more illumination for the coffee. This result is similar with reported in India that 64-76% of mineral in teak biomass is supplied back to soil (Salleh, 2001). But, if those mineral cycling is correlated with coffee yield, there is still not positive correlation, the impact of others growing factors are more dominant. Nevertheless, in comparison with *Gliricidia sepium*, litter of *P. falcataria* is 81% more, total C organic content 17% higher, N-total 40% higher, and available P 112% higher (Purwanto et al., 2007).

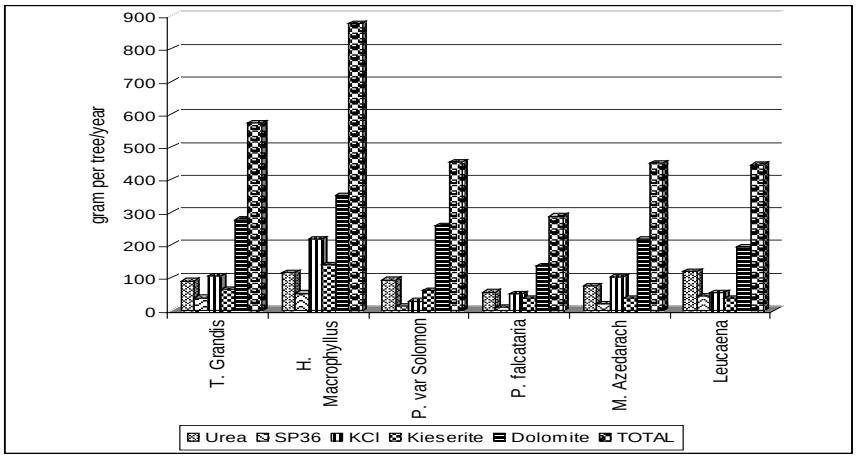

Figure 2. Potency of litter and biomass to supply nutrient (in equality to urea, SP 36, KCl, Kieserite, Dolomite) back to soil.

Coffee yield

There is a tendency that *M. azedarach* and *H. macrophyllus* by using those planting pattern are not suitable for coffee shade trees. *M. azedarach* falling down their leaves during dry season, and on the other side *H. macrophyllus* gave heavy shading. Observation of shading level by using densitometer showed that shading intensity of *H. macrophyllus* was 73%, while *Leucaena* was 49%. Coffee without or less shade trees has less chlorophyll and low electron transport (Chaves et al., 2008). In Indonesia, *Leucaena* is the most ideal for coffee shade trees, while in Costa Rica was reported *Eucalyptus deglupta (*(Rudi and Philippe, 2006).

Table 1. Effect of planting patterns and clones on the average of cherries yield 4 and 5 year old.

Treatment	**Cherries per tree**	
Main plot	4 year	5 year
A. Coffee-Teak	440.1 bc	1,219.0 a
B. Coffee-P. falcataria 1 rows	479.8 ab	902.2 bc
C. Coffee-P. falcataria 2 rows	470.4 b	723.8 cd
D. Coffee-Solomon 2 rows	469.6 b	1,024.0 ab
E. Coffee-Solomon 4 rows	291.4 de	688.0 cd
F. Coffee-M. azedarach 2 rows	338.7 cd	364.4 ef
G. Coffee-M. azedarach 4 rows	99.5 f	316.8 ef
H. Coffee-H. macrophyllus	211.3 ef	259.1 f
I. Coffee-Leucaena sp. (Control)	603.5 a	869.7 bcd
Sub plot		
BP 534	432.3 a	618.1 bc
BP 939	365.9 ab	1,040.0 a
BP 409	377.0 ab	753.4 b
BP 936	337.8 b	551.4 c

Notes: The data in the same column followed the same letter are not significantly different at 5% level according to HSD test.

CONCLUSION

1. Cropping pattern agroforestry enhance the ability to store carbon and improve mineral cycling and soil organic matter content.
2. Cherry yield by using *Paraserianthes falcataria* as the shade trees is similar with under *Leucaena*, but under *Melia azedarach* and *Hibiscus macrophyllus* are consistently lower.
3. The cherry yield is also influenced by clone, BP 936 clone tend the less.
4. Coffee agroforestry improve ecology service, *P. falcataria* in special planting pattern is recommended as an alternative shade tree beside *Leucaena* sp.

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Multiple Income Generation in Coffee Farms with *Paraserianthes falcataria* as Shade Trees

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SUMMARY

Due to increasing demand and decreasing supplies of indusrial wood, there is a need to cultivate timber crops. *Paraserianthes falcataria* is a growing fast tree, and its timber is highly valuable. A research to determine the effect of *P. falcataria* harvesting on coffee damage, coffee production, and land productivity has been conducted in Indonesia (45 m asl., D rainfall type according to Schmidt and Ferguson). Three model of planting systems applied were coffee – *P. falcataria* single row (2.5 m x 6 m), coffee – *P. falcataria* double rows (3 m x 2.5 m x 12 m), and coffee – *Leucaena* sp. (2.5 m x 3 m) as control. *P. falcataria* timber was harvested after 7 years old, and its shading function was replaced by *Leucaena* which had been prepared before. An economic cost-benefit analysis was carried out considering densities of coffee planting and the damage trees, yields, timber volumes and both coffee and timber prices. Damage to coffee crops was quantified in terms of severity levels and then translated into yield losses and their corresponding economic values. The result showed that harvesting of timber trees for double row caused more severe damage of coffee plants than single row system. One year after timber harvesting yield from undamaged trees of double rows was higher than control. Yield from the damages trees the yield was higher in double rows than single row and control. This yield was positively correlate with leaf chlorophyll content and stomatal conductance during rainy season. The financial analysis showed that on average, the net gain derived from timber harvesting was around US$37.43 per tree, and contribute Benefit/Cost 43.75 and 37.87 for double rows and single row system, respectively. Total B/C including coffee yield calculation at that age were 14.17 and 18.38 for the double rows and single row systems, respectively, while B/C coffee monoculture 2.34. This study showed that revenue obtained from timber sales was easily offset the costs of damage to the coffee crop, so this planting models is economically appropriate.

INTRODUCTION

In Indonesia, forest exploitation for timber resources take place very fast because of increasing in consumption for industry and building. The deforestation must be stopped, timber commodity must be cultivated, and in this research two species of timber were cultivated in coffee plantation. Its effect on coffee yield and land productivity, were examined. Attention in shade-grown coffee is now increasing because of an increasing trend toward sustainability and green consumerism. Industrial timber species is very rare to be used as coffee shade trees, although timber yield is potential to improve of farmer's income. Increasingly, farms and ranches in a region also provide industrial wood for sawmills and other wood processing plants.

Paraserianthes falcataria L. (I. Nielsen) or *Peacock-plume* is Leguminousae family, growing fast, tolerant to marginal soils, the canopy directing difuse minor and the timber lately costly

(Heyne, 1987). In Indonesia, the timber is very valuable because is used for plywood industry. Because of its characteristic, this plant has opportunity to be used as shadings of coffee and environmental conservation in the plantation, although it needs to notice its characteristic of being inresistant to wind of high velocity, the risk of damage of the coffee crop when harvested and possibility of strong competition of water and mineral nutrients. Coffee plantations which are managed intensively, has complied with the rules of natural resource conservation. The existence of shading tree a continuing 70-80% of light is very important to ensure a long productive life and productivity levels of coffee and cocoa (Alvim, 1977; Maestri and Barros, 1977). The purpose of this research is to observe impact of *Paraserianthes* harvesting on yield and land productivity.

METHODOLOGY

The research was done in Kaliwining Experimental Station (45 m dpl. tipe iklim D (Schmidt and Ferguson). Industrial timber was planted in 2003. Research methodology using split plot design. The treatments of this research are coffee – *Paraserianthes falcataria* (single row 2,5 m x 6 m and double row 3 m x 2.5 m x 12 m and coffee monoculture (*Leucaena* shading trees). As a sub plot is coffee clones i.e. BP 409, BP 534, BP 936 and BP 939. Between *Paraserianthes falcataria* trees was planted *Leucana* sp. as shading trees when the *Paraserianthes falcataria* harvest. Harvest time of *Paraserianthes falcataria* is after 7 years old. The cutting of *Paraserianthes falcataria* was done very carefully for the coffee plant damage will be less. Observation parameter are coffee yield before and after *Paraserianthes falcataria* harvested; coffee plant damage because of the *Paraserianthes falcataria* harvesting; and timber income benefit/cost ratio.

RESULT AND DISCUSSION

At three years old the highest production was on coffee-*Paraserianthes falcataria* single row treatment. At six years old coffee plant (2009), the *Paraserianthes falcataria* was harvested and the shading function is replaced by *Leucaena* sp. trees. Coffee yield increased, but not the effects of *Paraserianthes falcataria* timber harvesting because the coffee beans was formed before *Paraseranthes falcataria* harvested. The changes of coffee yields is seen in seventh years (2010) because after *Paraseranthes falcataria* harvested and the shading function is replaced by *Leucaena* sp. that was prepared before. Coffee yield increased significantly before *Paraserianthes falcataria* harvested, which is about 127% and 32% respectively for the ex single row and double rows.

One year after timber harvesting (2010), coffee yield from double rows undamaged trees was higher than control. The improvement of coffee yield of the ex double row was 127% and 32% of the ex single row to the control. The coffee yield after 7 years of control decreased compared to 6 years old and this phenomenon is a natural in the coffee. The results in Brazil showed the similar tendency, that coffee is cultivated in agroforestry, particularly species that are not allowed to do pruning, the production is lower than if the shading trees allows for pruning. Coffee yield with shading trees reached 515 kg/ha, while the cultivated monocultures reached 2443 kg / ha (Campanha et al., 2004).

After *Paraserianthes* was harvested, the shading function was replaced by *Leucaena* sp. which have been prepared before. From harvesting of 441 timber trees for double row and 483 trees for single row system caused severe, moderate and minor damage of coffee plants by 6.58%, 3.63% and 4.31% respectively for double row system, and 5.38%, 3.11%, and 2.90% respectively for single row system. The serious damage caused more than 75% coffee

yield loss, moderate 30-50% loss, and minor less than10% yield loss. Nevertheless, the revenue obtained from timber sales was easily offset the costs of damage to the coffee plant. This impact is normal, in Costa Rica, using of timber shade trees (*Cordia alliodora*), harvest of timber shade trees damage cocoa plants. From the 49 harvested timber trees observed, 196 cocoa plants were affected, of which 4% required replanting and 38% coppicing (Ryan et al., 2009).

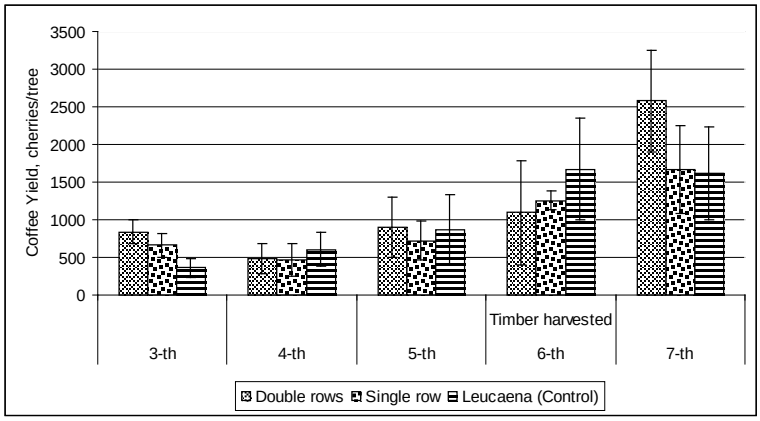

Figure 1. Progress of coffee yield. Histogram ± deviation standard.

Table 1. Cherry yield per tree at one year before and after harvesting of timber shade trees.

	Before	After	Changing, %
Double rows	1095.16	2481.22	126.56
Single row	1254.62	1659.4	32.26
Control	1671.17	1620.55	-3.03

The influence of coffee clones, have not shown consistent results. At the 6 years old (2009) of coffee crop, BP 939 have a highest coffee yields and then BP 409 clone, the lowest is BP 534 clone. One year after *Paraserianthes falcataria* harvested, coffee yield is increase except BP 409 clone. BP 939 clone is the highest. But, the coffee yields is not optimum yet because the coffee crops are still young.

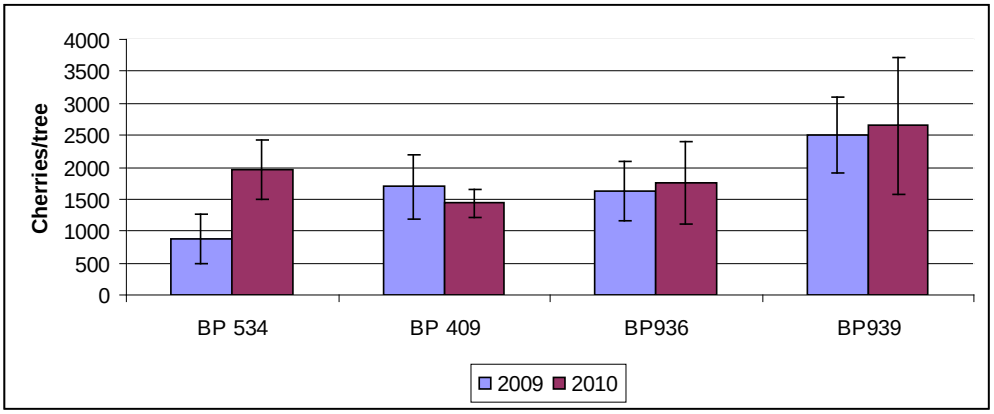

Figure 2. Effect of coffee clone on berry yield at before (2009) and after (2010) the timber shade trees harvested.

PARASERIANTHES FALCATARIA FARMING ANALYSIS

Paraserianthes falcataria was harvested in the beginning of 2009 (7 years old), the prices was US$ 37.43 per tree. The result of coffee farming analysis showed that benefit cost ratio is very high. The prices level in 2008, *Paraserianthes falcataria* as a shading trees with single row and double row pattern increase the land productivity drastically. B/C of single row is 18.4 and 14.2 for double row.

Table 2. *Paraserianthes falcataria* farming analysis (7 years old).

Coffee- *Paraserianthes falcataria* (single row)	**Value**	**Coffee- *Paraserianthes falcataria* (double row)**	**Value**
Coffee:		Coffee:	
R/C	1.73	R/C	1.5
B/C	0.73	B/C	0.5
Paraserianthes falcataria:		*Paraserianthes falcataria:*	
R/C	38.87	R/C	44.75
B/C	37.87	B/C	43.75
Coffee farming:		Coffee farming:	
B/C	18.38	B/C	14.17

CONCLUSION

1. The result showed that harvesting of timber trees for double row caused more severe damage of coffee plants than single row system.
2. One year after timber harvesting yield from undamaged trees of double rows was higher than control.
3. Yield from the damages trees the yield was higher in double rows than single row and control.
4. The financial analysis showed that on average, the net gain derived from timber harvesting was around US$37.43 per tree, and contribute Benefit/Cost 43.75 and 37.87 for double rows and single row system, respectively. Total B/C including coffee yield calculation at that age were 14.17 and 18.38 for the double rows and single row systems, respectively, while B/C coffee monoculture 2.34.
5. This study showed that revenue obtained from timber sales was easily offset the costs of damage to the coffee crop, so this planting models is economically appropriate.

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Characteristics of Anatomy, Morphology and Physiology as Indicators for Yield of Robusta Coffee

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SUMMARY

Yield of coffee is affected by some anatomical- as well as morphological properties and physiological activities. Coffee is a C3 plant that has a photosynthetic saturation at a certain light intensity. To know some anatomical-, morphological- and physiological characteristics those affect growth and yield of Robusta coffee, a study has been done at the Indonesian Coffee and Cocoa Research Institute, Jember, East Java, Indonesia. Fully expanded leaves of eight clones have been measured on their area, stomata density, stomata pores area, palisade index, chlorophyll index, and photosynthetic rate. Clones measured were BP 42, BP 358, BP 409, BP 534, BP 936, BP 939, BP 961 and SA 237. Measurement was done at Kaliwining Experimental Station, Jember, Indonesia, at the altitude of 45 m above sea level. Measurement was stressed to describe the dynamic response curve model and to correlate yield to other parameters. The most dominant parameter that affect yield was determined.

INTRODUCTION

Coffee is one of estate commodities that have a high economic value and as an important income source for Indonesia. Many of coffee plantations in Indonesia are Robusta coffee, but there a little part of those plantations is Arabica coffee. Clones of Robusta coffee cultivated in Indonesia are variable and relatively have different anatomical-, morphological- and physiological characteristics. Their difference in those characteristic might cause a different yield. Coffee is a C3 photosynthetic pathway crop, it means that for optimum growth need only 70% of sunlight and their saturated photosynthesis active radiation (PAR) is around 1000 $\mu mol.m^{-2}.s^{-1}$.

To explore anatomical-, morphological- and physiological characteristics and then to relate to their yield, an observation has been conducted at Kaliwining Experimental Station, Indonesian Coffee and Cocoa Research Institute (ICCRI). The specific objective of the experiment were to describe (1) a dynamic model for photosynthesis rate among varies coffee clones in varying photosynthetic active radiation, (2) to investigate the relationships between photosynthesis rate (P) and other parameters such as chlorophyll index, stomata and palisade towards the yield of coffee clones. Results of those experiments were presented below.

MATERIALS AND METHODS

Fully expanded leaves of eight clones have been measured on their photosynthetic rate using portable gas exchange system Li-6400 *(*LI-COR Inc., Lincoln, NE). Besides, chlorophyll index, stomata density and palisade index also observed. Clones measured were BP 42, BP

358, BP 409, BP 534, BP 936, BP 939, BP 961 and SA 237; respectively. Leaves were taken from collection garden of the Indonesian Coffee and Cocoa Research Institute. Chlorophyll index was measured by using chlorophyll meter, stomata density was observed by using light microscope, and palisade index was observed by using binocular microscope. The data collected analyzed with variance analysis. The difference among parameters was tested by LSD at the 0.05 probability level. The relationship between yield and those observed parameters was done to illustrate a role of those parameters to yield.

RESULTS AND DISCUSSION

Potency of productivity of eight clones observed was varied between 1200 kg/ha to 2800 kg/ha, BP 42 has the lowest of productivity and BP 939 has the highest one.

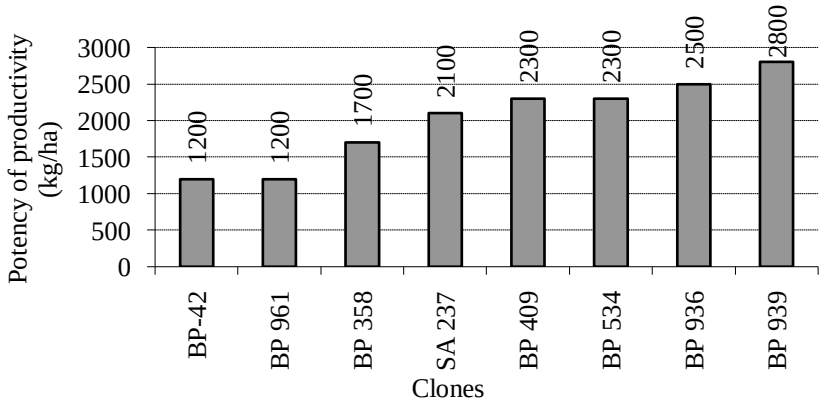

Figure 1. Potency of productivity of eight clones observed.

Leaf area of eight robusta coffee clones those observed was varied between 113-215 cm^2/leaf, whereas BP 534 was the lowest and BP 409 was the highest one. Range values of other parameters as follows: chlorophyll index was 51.23-61.37 (BP 42 was the lowest and BP 939 was the highest), stomata density was 245-466 stomata/mm^2 (BP 961 was the lowest and BP 936 was the highest), stomata pore area was 3.48-7.31 $mm^2.10^{-4}$ (BP 358 was the lowest and BP 534 was the highest), and palisade index was 24.82-36.97 palisade/epidermal cell (BP 42 was the lowest and BP 936 was the highest); respectively.

Table 1. Morphology and anatomy properties of eight coffee clones observed.

Clones	Leaf area (cm^2/leaf)	Chlorophyll index	Stomata density (stomata/mm^2)	Stomata pore area ($mm^2.10^{-4}$)	Palisade index (palissade/ epidermal cell)
BP 42	167.20	51.23	342.20	5.75	24.82
BP 961	172.26	53.70	245.68	3.69	26.66
BP 358	116.22	53.99	263.23	3.48	33.39
SA 237	130.46	56.92	315.87	5.53	24.98
BP 409	214.99	56.59	377.29	5.31	35.09
BP 534	113.01	57.32	421.16	7.31	32.98
BP 936	159.89	59.48	465.03	6.42	36.97
BP 939	136.44	61.37	412.39	5.91	35.54
LSD 5%	41.29	5.52	52.87	3.42	7.019

There were positive exponential relationship between photosynthetic active radiation *(PAR*) and photosynthetic rate (*P*) of eight coffee clones. It means that the higher the PAR, the higher the photosynthetic rate (*P*) until they reach a maximum point and then constant or gradually decrease. This phenomenon is usual for living objects.

Table 2. Exponential relationship between photosynthetic active radiation *(PAR*) and photosynthetic rate (*P.*)

Clones	**Equation**	**R^2**
BP-42	Y = 7 (1-exp(-0.02 x X/7))	0.825
BP 961	Y = 5.8 (1-exp(-0.0123 x X/5.8))	0.829
BP 358	Y = 8 (1-exp(-0.0212 x X/8))	0.964
SA 237	Y = 11.5(1-exp(-0.0297 x X/11.5))	0.967
BP 409	Y = 7 (1-exp(-0.0164 x X/7))	0.903
BP 534	Y = 7.5 (1-exp(-0.0177 x X/7.5))	0.928
BP 936	Y = 11 (1-exp(-0.0239 x X/11))	0.979
BP 939	Y = 13 (1-exp(-0.0203 x X/13))	0.979

Relationships between morphological-, anatomical- as well as physiological characteristics to potential productivity follow a positive linier regression equation.

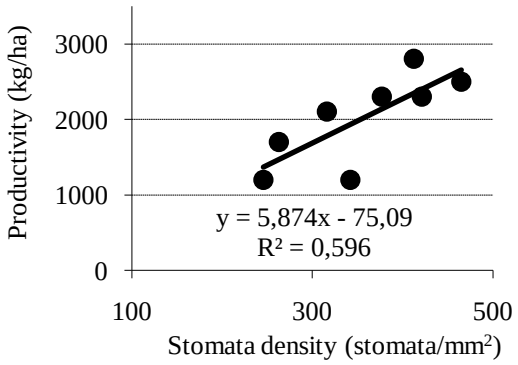

Figure 2. Relationship between stomata density and productivity.

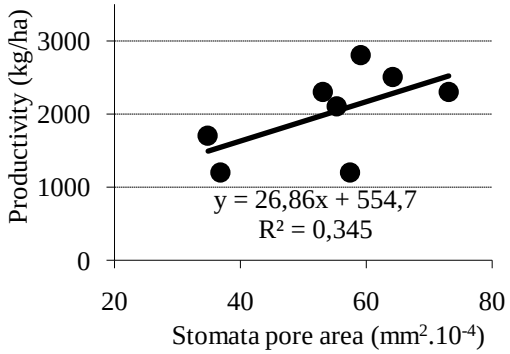

Figure 3. Relationship between stomata pore area and productivity.

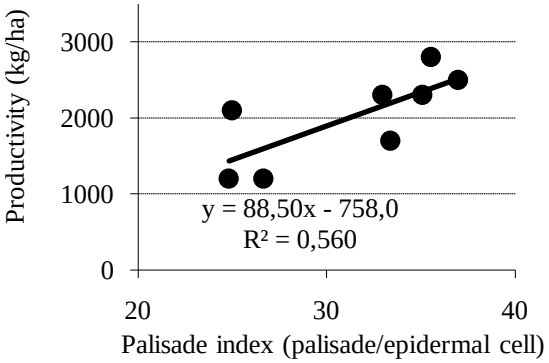

Figure 4. Relationship between palisade index and productivity.

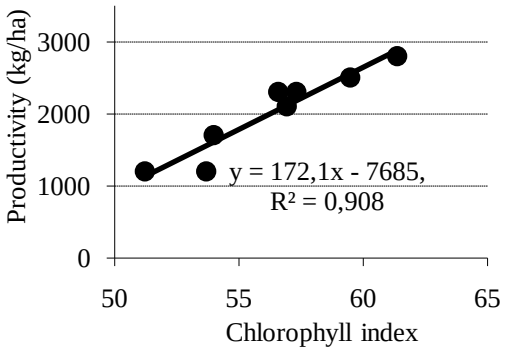

Figure 5. Relationship between chlorophyll index and productivity.

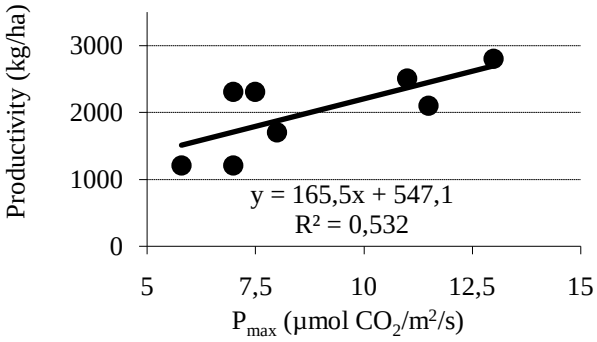

Figure 6. Relationship between maximum photosynthesis and productivity.

PAR is the intensity of radiation that stimulates photosynthesis reaction actively. It is very logic that until an optimum level, the higher the intensity of radiation, the higher the photosynthetic rate. Radiation is very close related to photosynthesis. Photosynthesis only takes place when sunlight is presence.

Stomata density, palisade index, chlorophyll index and photosynthesis were significantly affected productivity of coffee clones. It is very logic because those characters were governed assimilate production. Based on determination coefficient (R^2) value, chlorophyll index was the character that has closest relationship to productivity of robusta coffee clone.

CONCLUSIONS

- Potency of productivity of eight clones observed was varied between 1200 kg/ha to 2800 kg/ha, BP 42 has the lowest and BP 939 has the highest one.
- There were positive exponential relationship between photosynthetic active radiation *(PAR*) and photosynthetic rate (*P*) of eight coffee clones.
- Stomata density, palisade index, chlorophyll index and photosynthesis were significantly affected productivity of coffee clones with positive linier regression equation.
- Chlorophyll index was the character that has closest relationship to productivity of robusta coffee clone.

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Investigation on Main Factors Influencing the Arabica Green Coffee Quality

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SUMMARY

Green coffee samples were produced in controlled conditions to study the influence of different factors on green coffee quality. Factors under study are the variety of Arabica coffee, altitude, geographical areas and intensity of shade for the environment, quality of harvest and post-harvest processing applied to coffee cherries. Green coffee samples were produced at farmer level in Central and South American countries (Costa Rica, Guatemala, Colombia and Brazil) but also in Experimental farms located in China and Thailand. Around 1500 green coffee samples were evaluated for their sensory profile, physical parameters and biochemical composition. Collected data were analysed statistically using analysis of variance (ANOVA) to identify the factors having significant effect on the quality or the biochemical composition of green coffee. Coffee varieties and altitude of production have significant influences on the quality potential of Arabica coffee. The increasing altitude has a positive effect on the perceived acidity and fruity attributes of Arabica coffee, although bitterness and malty attributes are reduced in the same conditions. It was demonstrated that the lipids content is increasing with the altitude. Traditional varieties like Typica, Bourbon and Mundo Novo have a higher quality potential compared to more recent varieties like the one belonging to the group of Catimors.

Modalities of wet processing should be adapted to the potential quality of cherries (altitude and varieties). When the potential is high, sophisticated wet process could optimize the quality of green coffee. In the opposite, if the quality potential is low a simple process (semi-wet) is well adapted.

The presence of green cherries in the harvest is affecting the final quality by the significant increase of defective cups. Sun-drying of parchment coffee in appropriate conditions can significantly reduce the percentage of defective cups, as observed with air circulation in greenhouses. In case of dry process, it appeared that optimal drying conditions are important to avoid the fermentation of the pulp. Mature Arabica cherries are highly susceptible to fermentation and could be easily deteriorated if they are not dried in adapted conditions. Considering all these results, a strategy of actions and decision is proposed for the wet process of Arabica coffee according the quality of cherries.

Results obtained in the study will fuel the port-folio of best practices to be communicated to coffee growers through technical assistance program. It will help to build conditions for a sustainable quality.

INTRODUCTION

Green coffee quality is the consequence of many factors including genetic, environmental, harvesting and processing aspects. The study aimed to identify scientific proof of latent knowledge of agricultural and post harvest practices for achieving highest green coffee quality.

MATERIALS AND METHODS

Around 1,500 green coffee samples were prepared during 3 years in controlled conditions in 4 Central and South American countries (Costa Rica, Guatemala, Colombia and Brazil) and in two Asian countries (China and Thailand). The study focused on the most important factors preliminary determined as the varieties, the environment, the mode and quality of harvest and the post harvesting treatments. Each sample was prepared from 12 kg of cherries and the number of replications was fluctuating between 3 and 5. Green coffee samples were analysed by Near InfraRed (NIR) spectroscopy for their composition, sensory analyses were done by a trained panel of 12 panellists.

RESULTS

Main results obtained in the study can be classified into 4 categories according the factor under study.

Environment

Site, season and altitude of production have significant influence on the cup quality. The effect was observed in Colombia, Costa Rica and Guatemala. Local conditions influence the cup quality, they could be related to the soil fertility or microclimatic conditions and can be assimilated to the "terroir" effect. Almost a systematic influence of the altitude is detected on the sensory profile. The coffee quality is strongly influenced by the seasonal effect, most probably related to annual climatic conditions.

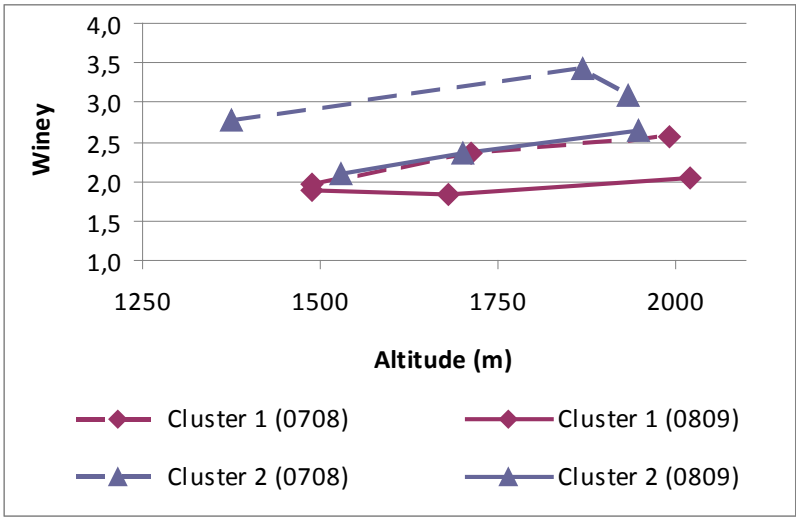

Figure 1. Winey attribute in relation with the altitude (2 seasons & sites in Colombia-Caturra wet process).

Genetic

Varieties have a strong significant effect on cup quality. The influence was observed in Colombia, Costa Rica, Guatemala and Brazil. The sensory intensity of the varieties is influenced by the environmental conditions (altitude, site, year).A negative relation was identified in Guatemala between caffeine content and the fruity attribute. Varieties induce strong sensory differences. They need to be properly selected in relation with the desired sensory profile of the finished product. “Traditional “varieties like Typica, Bourbon are leading to more winey/fruity and acidity.

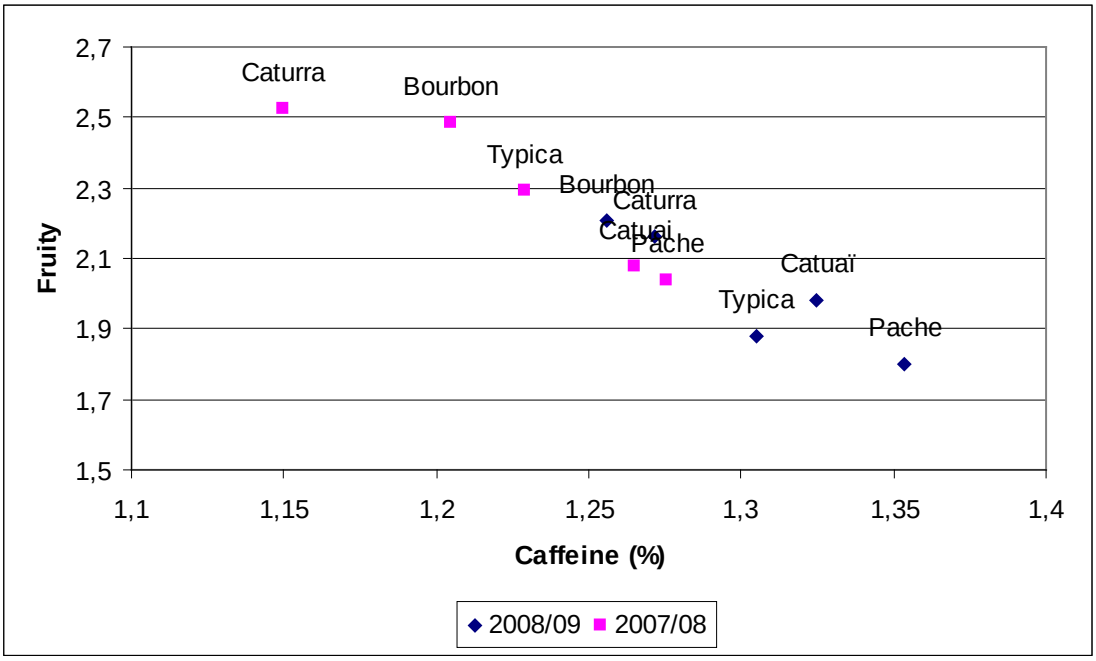

Figure 2. Fruity attribute of varieties in Guatemala in relation with the caffeine content – two seasons.

Quality of harvest

The quality of cherries (green/mature/momified) has a significant influence on the percentage of defective cups mature cherries are very sensitive to fermentation and need appropriate treatment to avoid deterioration. These effects were observed in all investigated countries. The absence or a low percentage of green cherries in the harvest is necessary to avoid defective cups in the finished product. Mature cherries require appropriate post harvesting treatment (wet process or rapid dry process) to avoid fermentation.

Post harvesting treatment

The post harvesting process has a significant effect on the sensory profile. A soaking phase applied during the wet process increases the cup acidity and decreases the bitterness. This effect was observed in all countries. The percentage of defective cups is significantly increased when sun drying of parchment coffee is not applied in good conditions, this factor was only investigated in Colombia. A wet process with a soaking phase can improve the sensory profile. The green coffee composition is modified, especially the chlorogenic acids profile. Sun drying conditions of parchment coffee need to be optimized

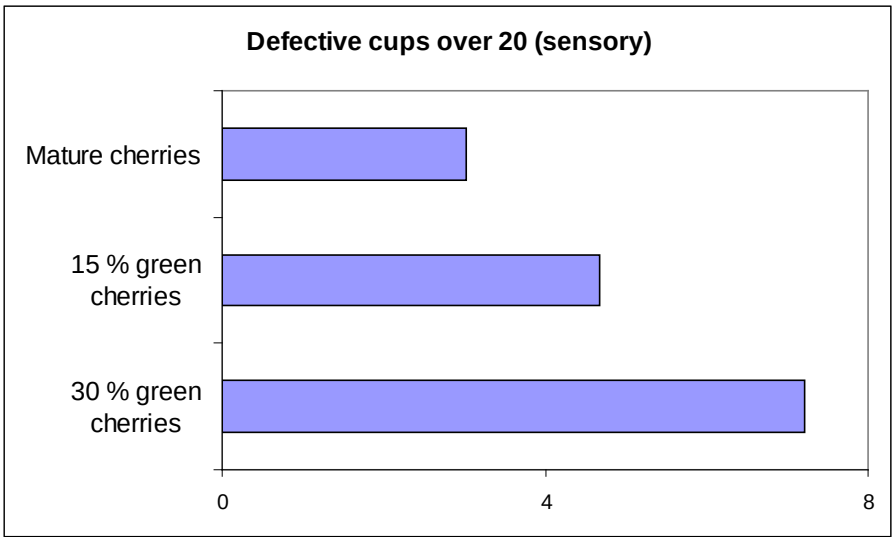

Figure 3. Percentage of defective cups in relation with the quality of cherries.

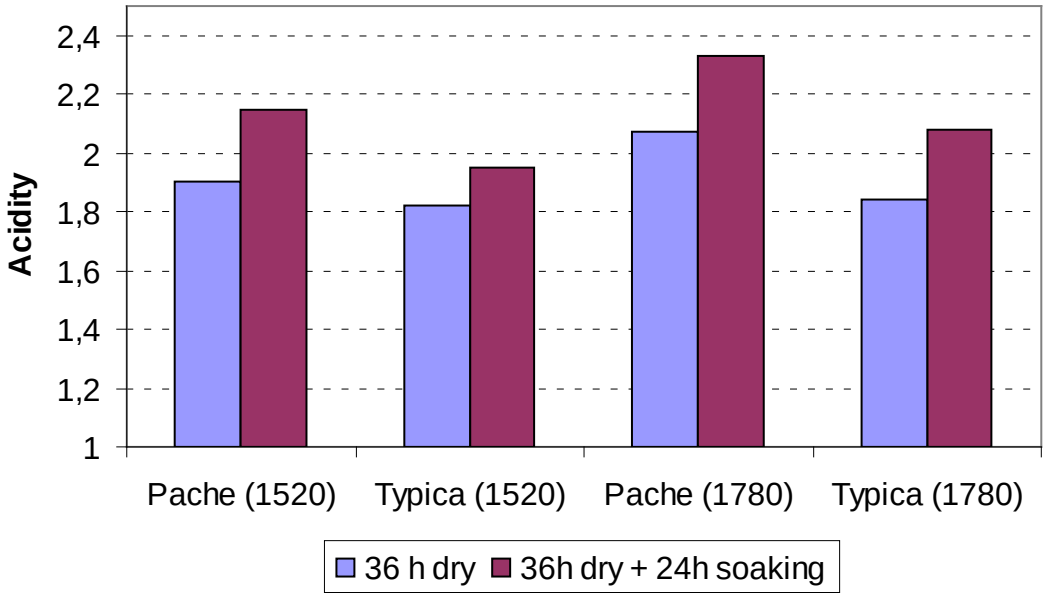

Figure 4. Cup acidity of 2 varieties at 2 altitudes with and without soaking (Guatemala).

CONCLUSIONS

The study allowed to identify factors having significant effect on the sensory profile, like varieties, environmental conditions, quality of harvest and post harvesting process. The understanding of the action of these factors is giving the opportunity to build the quality by applying the conditions leading to the required sensory profile in the finished product.

Micro-Landscape Context Effects on the Dispersal of Coffee Berry Borer (*Hypothenemus hampei*) in Costa Rica

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SUMMARY

This work was addressed to study the effects of landscape on the short distance dispersal (≤140 m) of coffee berry borer (CBB). We conducted a six-month study during the flight period of the CBB (January to July 2009), in six locations of the Turrialba region, in Costa Rica. We measured CBB movement in transects spanning isolated coffee plantations into three adjacent land uses: forest, sugar cane and pasture. At each location, we placed six transects starting 30 m within the coffee plantation, continuing 140 m into each of the adjacent land uses. Within these transects we placed one CBB trap (Brocap®) each 10 m. Despite a low frequency of dispersal events outside of coffee, we found significant differences in the permeability of the three adjacent land uses. The forest generated the greatest friction to CBB movement: the number of CBB captured in forests was only 12 % and 19 % of the number of CBB captured in sugar cane and pasture respectively. This finding suggests that breaking connectivity between coffee plantations may help to reduce CBB dispersal particularly when low permeability land uses such as forests are placed between coffee plots.

INTRODUCTION

The coffee berry borer (CBB) *Hypothenemus hampei* (Ferr.) has been detected in the Costa Rican landscape since 2000 from whence it has rapidly expanded its territory, colonizing new coffee farms. Although the males of the species have atrophied wings and are flightless, females have the ability to fly, and particularly do so when seeking new coffee berries to colonize after harvest. Distances covered by CBB are known to be large enough to reach proximate pest free areas (Baker, 1984); however, the number of individuals able to fly great distances across non-coffee land uses is probably low. As a consequence, CBB dispersal is believed to be facilitated by the connectivity between coffee plantations, but may be hampered by fragmented landscapes when alternate land uses are found between coffee patches.

The aim of this study was to understand the effect of landscape context on the short distance dispersal (≤140 m) of CBB in the Turrialba canton. Turrialba is a low altitude coffee region of Costa Rica under Caribbean influence, favourable to CBB development.

MATERIALS AND METHODS

We conducted a six-month study during the flight period of the CBB (January to July 2009), in six locations of the Turrialba region. Each location consisted of one isolated coffee plantation bordered by two of the following three possible land uses: (1) forest, (2) sugar cane and (3) pasture. Each of the land uses combinations (forest-pasture, pasture-sugar cane, forest-sugar cane, Figure 1) was repeated twice.

Due to the isolation of the studied coffee plantations, we assumed that these were the only sources of CBB in each location. CBB movements from the coffee plantations to the other land uses were studied by using Brocap® traps (Figure 1) baited with a 3:1 mixture of methanol and ethanol (Dufour et al., 2005, 2008).

Figure 1. Simplified representation of the distribution of the BROCAP® traps along six transects in a coffee-forest-sugar cane combination. Other studied land uses combinations were coffee-forest-pasture and coffee-sugar cane-pasture.

At each location, we established six transects, with traps placed each 10 m, starting 30 m within the coffee plantation and continuing 140 m into each of the two adjacent land uses (three transects per land use separated by 30-40 m each) (Figure 1). The captured CBB were collected and counted every ±12 days. We analized data from a total of 120 days of trapping.

Our primary response variable was the number of females captured per day. We used the median data from the three transects in order to normalize the data's distribution. We used a generalized linear mixed model to analyze the data, where the adjacent land use and the distance of the Brocap trap® to the coffee plantation were considered as fixed factors and the collecting date as random factor. We also included altitude as a covariable.

RESULTS

We captured 96.5% of the individuals within the coffee plots and only 3.5% in the adjacent land uses. The majority of the individuals captured outside the coffee plots (30.2%) was found directly on the edge between the two land uses. However, some individuals (2.9%) were found up to 140 m from the coffee edge beyond which we had no traps (Figure 2). Despite this low frequency of dispersal events outside of coffee, we found significant differences in the permeability of the three adjacent land uses. The forest generated the greatest friction to

CBB movement: the number of CBB captured in forests was only 12 % and 19 % of the number of CBB captured in sugar cane and pasture respectively (Figure 3).

DISCUSSION AND CONCLUSION

Although occasional CBB individuals were captured 140 m from coffee edges, our results show that CBB does not regularly disperse outside of coffee. This finding suggests that breaking connectivity between coffee plantations may help to reduce CBB dispersal particularly when low permeability land uses such as forests are placed between coffee plots (compared to sugar cane and pasture which have greater permeability). These results are especially important in the Turrialba region where CBB flights are not so abundant due to the year-round presence of coffee fruits for CBB infestation. Because of this, we recommend that the study be repeated in other regions with synchronized flowering.

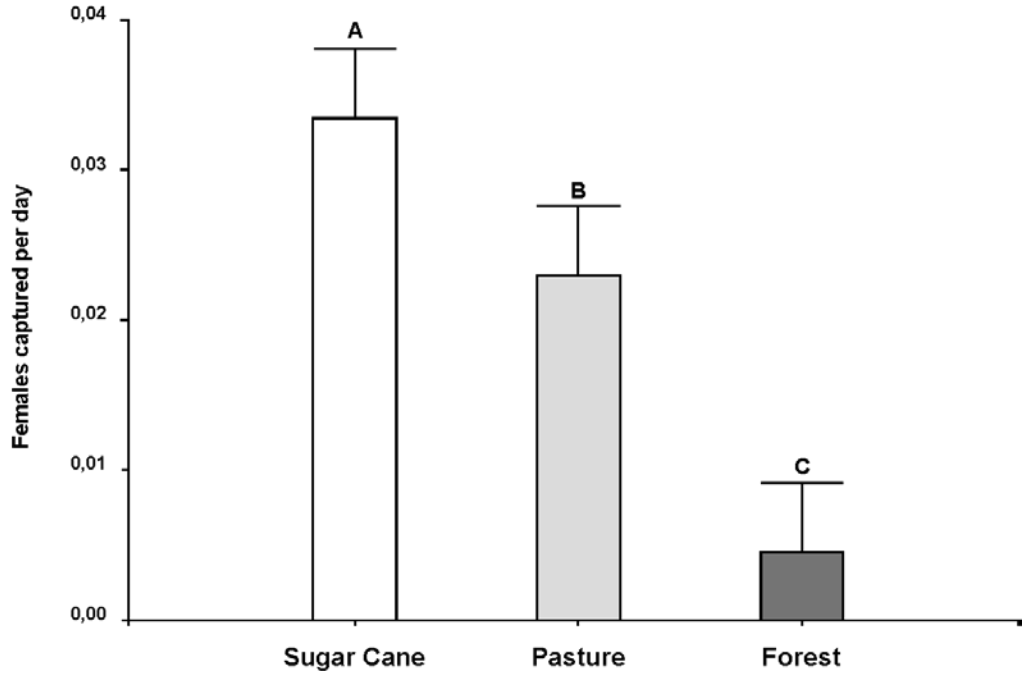

Figure 3. Number of coffee berry borers captured per day with Brocap® traps during a 120-day period in different land uses up to 140 m from a coffee plot (mean of the medians data from three transects).

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Landscape Context and Plot Incidence of Coffee Rust (*Hemileia vastatrix*), Coffee Berry Borer (*Hypothenemus hampei*) and the Root-Knot Nematodes *Meloidogyne* spp. in Costa Rica

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SUMMARY

This work was addressed to study the relationships between landscape context and the incidence of three noxious organisms with different abilities to spread: coffee rust (*Hemileia vastarix*), coffee berry borer (*Hypothenemus hampei*) and the root-knot nematodes (*Meloidogyne* spp.). We analized these relationships at different spatial scales (radii 50-1500 m) in 50 agricultural landscapes of the Turrialba coffee region in Costa Rica, differing in structural complexity. We found positive correlations between coffee berry borer abundance and the coffee area percentage in the landscape. The correlation was strongest at a scale of 150 m. We also found positive correlations between coffee rust incidence and the pasture area in the landscape. The significance of this relationship peaked at the 300 m radius. We didn't find any significant correlation between coffee root-knot nematodes and landscape. These relationships can be interpreted according to the dispersal ability of the studied organisms. Coffee berry borer is specific to coffee and can fly only at short distances. Coffee rust is an airborne pathogen which is probably favoured by open spaces. Nematodes are almost immobile. We hypothesize that fragmenting coffee regions may help to reduce coffee berry borer dispersal. In contrast, fragmentation of coffee landscape by pasture may increase coffee rust dispersal.

INTRODUCTION

Disease and pest attack intensities are mainly determined at the plot level through interactions between the host, noxious organism, environment and agricultural management (Zadoks and Schein, 1979). However, the immigration of noxious and beneficial organisms from outside may also affect pest and disease incidences at the plot scale. Successful immigration is facilitated in landscapes with greater connectivity between resources patches (Zadoks, 1999). Functional connectivity of landscapes depends on the distribution and density of specific land uses, on how these are perceived (hostile or not) by specific organisms, and on organisms' dispersal ability to move across non-habitat areas. In a given landscape context, higher connectivity is therefore expected for generalist noxious organisms with high dispersal abilities. Here, we study the relationship between coffee pest and disease incidence in coffee farms and landscape context. We hypothesize that greater coffee cover within the local context (<1500 radius) will increase pest and disease incidence whereas greater forest cover will decrease it. We use three focal organisms to test these hypotheses: (1) coffee rust (*Hemileia vastatrix),* (2) coffee berry borer (*Hypothenemus hampei*) and (3) the root-knot nematodes (*Meloidogyne* spp.). These noxious organisms differ by their host specificity and dispersal ability. Coffee rust is coffee specific. Its uredospores are spread by wind over great

distances and can even cross oceans (Bowden, 1971). The coffee berry borer is very specific to coffee, however it has been found to reproduce in several plant species (Damon, 2000). The females are able to fly, and probably can be transported by convection winds, over a few hundred meters (Baker, 1984). Root-knot nematodes are able to infect different plant species and, when not dispersed through human activities, can be considered nearly immobile.

MATERIALS AND METHODS

We conducted a one-year survey starting in November 2008 through November 2009 on 50 coffee plots in the Turrialba region of Costa Rica (Figure 1a). The plots were distributed within a wide range of landscape contexts, from highly fragmented (Figure 1b) to intact coffee plots (Figure 1c). Coffee plots were comprised of eight rows of 15 coffee plants (120 coffee trees per plot). We quantified noxious organism incidence on five systematically distributed coffee trees in each plot, in 2-5 evaluation periods depending on the organism: February-March, May, June-July, September, October-November. We measured coffee rust incidence in all five periods by counting the number of diseased and healthy coffee leaves on three branches per coffee plant (a total of 15 branches per plot). We estimated coffee berry borer abundance four times only (excluding February-March) by counting the number of bored coffee fruits on four branches per coffee tree (20 branches per plot) and the total number of fruiting branches. We assessed root-knot nematodes population densities twice only (May and September) by sampling coffee roots from the four neighboring coffee plants of each of the five selected trees (20 subsamples per plot).

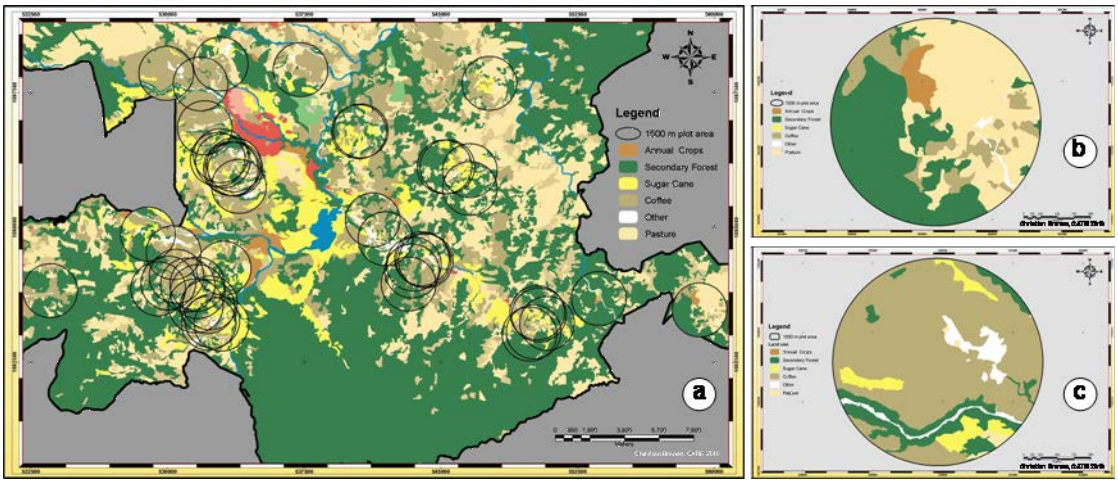

Figure 1. Landscape description within circular sectors of 1.5 km radius centered on the 50 surveyed plots (a), and two selected landscape contexts from highly fragmented (b) to almost intact coffee plots (c), Turrialba, Costa Rica, 2009.

In our analyses we used the maximum annual percentage of diseased coffee leaves as our descriptor of coffee rust infection. In the case of the coffee berry borer we used the maximum annual number of bored coffee fruits estimated per coffee plant. Finally, for the root-knot nematodes, we used the average population density per 100 g of coffee roots.

To describe the landscape context, we first classified a 2005, 1 m^2 resolution aerial image of the landscape by assigning the land uses within a 1500 m radius around each sample point to one of four land uses: coffee, sugar cane, pasture, and forest. We further verified this classification through ground-truthing. Then, we subdivided the 1500 m radius plot into 12

nested circular plots with the following radii: 50, 100, 150, 250, 300, 350, 400, 450, 500, 1000, 1500 m, and calculated the proportion of each land use at each scale.

Finally, we examined the correlations between plot level pest and disease descriptors and landscape context at each scale mentioned above to determine whether landscape context impacts pest and disease incidence, and at what scale.

RESULTS

We found diverse responses to landscape context for each of the study organisms. There were no correlations between landscape context and population densities of root-knot nematodes as expected. We found multiple significant positive correlations between coffee berry borer infestation and proportion of the landscape in coffee (Figure 2a). The significance of this relationship peaked at the 150 m radius (r=0.28, $P<0.05$; Figures 2a and 3a). Similarly, we found multiple significant positive correlations between coffee rust incidence and proportion of the landscape in pasture (Figure 2b). In contrast to the coffee berry borer, the significance of this relationship peaked at the 300 m radius (r=0.35, $P<0.05$; Figures 2b and 3b). In addition, multiple negative correlations were obtained for coffee berry borer and the proportion of pasture in the landscape (Figure 2b) and for the coffee rust with the proportion of coffee in the landscape (Figure 2a).

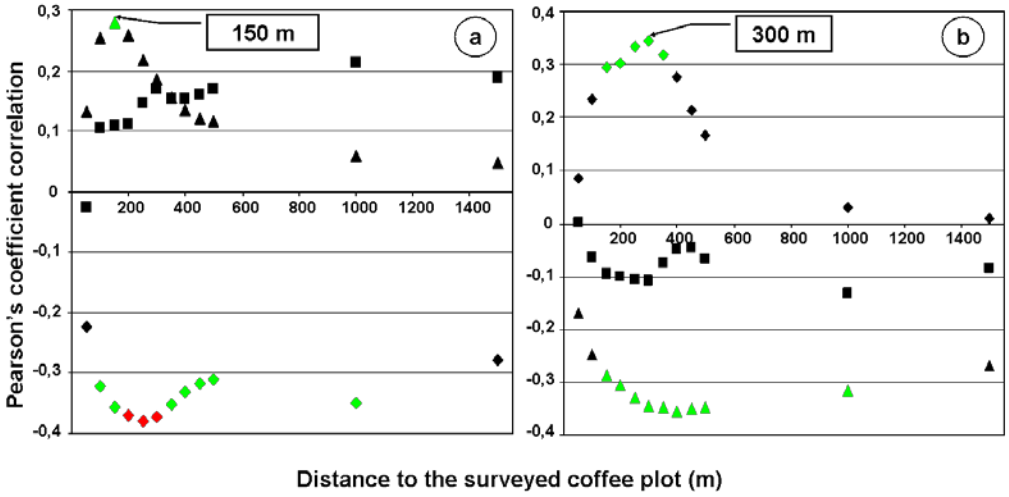

Figure 2. correlation of coffee berry borer abundance (▲), coffee rust incidende (♦), Meloidogyne spp. population density (■) versus the percentage of coffee (a) and pasture (b) areas at 12 spatial scales, $P>0.05$ (black) $P<0.05$ (red).

DISCUSSION AND CONCLUSION

Our results can be interpreted according to the dispersal ability of the studied organisms. Coffee berry borer has low dispersal ability and perceived other land uses as hostile. As a consequence, fragmenting coffee farms at small scales (i.e. interspersing alternate land uses or linear barriers such as riparian corridors) may help to significantly reduce coffee berry borer movement between plots. In contrast, fragmentation of coffee landscape, particularly by pasture, may increase coffee rust dispersal. This is probably because coffee rust is an airborne pathogen whose dispersal is favored by open spaces. Finally, nematodes, which are nearly immobile, were not influenced by landscape context.

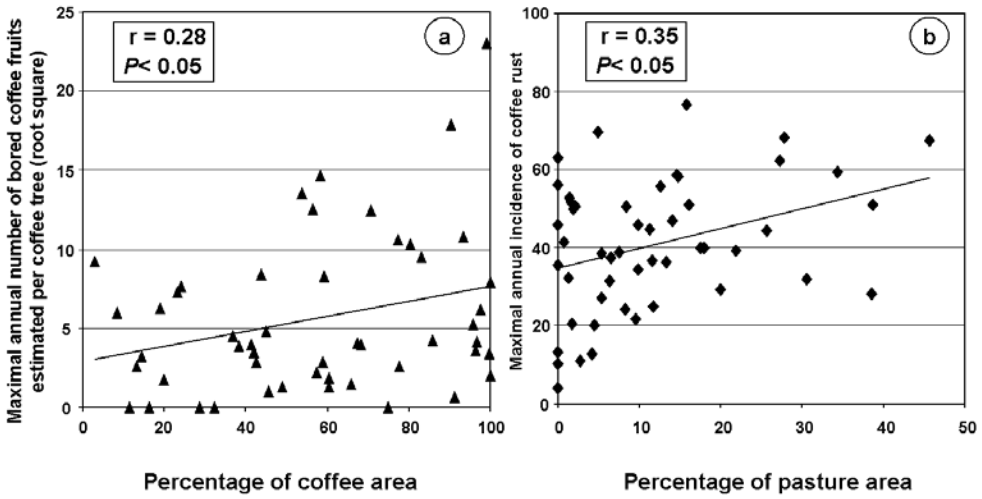

Figure 3. Dependence of coffee berry borer abundance on the percentage of coffee area at the spatial scale of 150 m (a) and of coffee rust incidence on the percentage of pasture area at the spatial scale of 300 m (b).

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Indonesian Green Arabica Coffee Growing Zones Characterization Using Near Infrared Spectroscopy

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SUMMARY

The Specialty Coffee Association of Indonesia (SCAI) is a trade association formed in February 2008, whose goal is to improve the quality of Indonesian Arabica coffee. SCAI represents all steps in the coffee supply chain, from the Indonesian Coffee and Cocoa Research Institute (ICCRI) to coffee retailers and importers. The 93 SCAI members export more than 60% of Indonesia's Arabica coffee. A key concern of SCAI is to increase the traceability of Indonesia's Arabica coffee. In the traditional system, coffee is bought and sold through several layers of village and town collectors, before being exported. This system obscures the origin of the coffee, mixing high and low quality coffee. Importers and roasters are demanding more information about the origins of Indonesian coffee, and they are willing to pay higher prices for traceable coffee. Since 2008, SCAI has implemented different inter-related projects, designed to increase coffee traceability 1) Working with industry stakeholders to develop digital maps of each Arabica coffee origin 2) Supporting efforts by members to develop Geographical Indications (G.I.), following the lead of Bali Kintamani and 3) conducting researches on the characteristics of coffee type samples from each origin, to create a quantitative method for determining origin. For the present study, three sets of samples were prepared for 3 origins: Sidikilang, Lintong and Aceh Gayo. To produce the type samples, ten-kilogram lots of parchment coffee were purchased from village collectors distributed evenly across each origin. Village collectors typically aggregate and hull coffee from up 40 to 80 surrounding farmers. The coffee samples were processed following the traditional wet hulling or "giling basah" method. A total of 32 samples were processed and sun dried. Near infrared fingerprint of ground (<0.5 mm) green coffees were scanned using monochromator instrument NIRS 6500 (Foss NlRSystems, USA). Caffeine, chlorogenic acids, sucrose, fat; moisture and trigonelline content were predicted using CIRAD green Arabica coffee calibration equation. A one-way ANOVA was done to investigate the effect of growing zone on the content of the 6 predicted constituents. The estimated average values per zone were compared using a Student Newman-Keuls (SNK) multiple pair comparison test, at 5% level. Significant differences were found for sucrose and caffeine content: sucrose content was different for the 3 zones while caffeine content was different for samples from Aceh Gayo. A Linear Discriminant Analysis based on NIR fingerprints allowed a 100% correct classification of Gayo samples; the overall classification according to origins was done with a rate of 93,8%. These results will be presented and discussed in relation with coffee organoleptic profiles and coffee growing zone ecosystem

INTRODUCTION

Buyers will pay more for specialty coffee, if they can be sure where it was grown. In the industry, this is called "traceability". Geographical Indications (G.I.) create a legal framework

for traceability. Once a G.I. is established by the Government of Indonesia, only coffee from that origin can be sold under the name of the G.I.

The first G.I. in Indonesia was for Bali Kintamani coffee and Aceh Gayo coffee has been approved this year. However, it can be difficult to enforce a G.I., because the physical appearance of green coffee from different origins can be similar. Some expert cuppers can identify a coffee's origin by flavor and aroma, but this is a subjective method.

The Specialty Coffee Association of Indonesia (SCAI) and CIRAD, the French Agricultural Research Center for Development, are working to develop scientific methods of identifying coffee from different origins: Near-Infrared Spectroscopy (NIRS). NIRS is a very efficient and non destructive method for high-throughput screening of plant materials for their chemical characteristics. This indirect method is based on vibrational properties of organic molecule chemical bonds and their interactions with infrared radiation. The resulting absorption spectrum can be seen as a fingerprint of the product linked to its properties and its history. Therefore comparing singular fingerprint to a reference spectra database allows samples authentication.

Developing a NIR spectral database using well known (origins, varieties, post harvest treatment...) coffees samples will be a useful tool for G.I. certification.

MATERIAL AND METHOD

Plant material

During the 2008-2009 season, 32 samples of Arabica coffee were collected in the origins of Sidikilang (13), Lintong (9) and Gayo (10) on Sumatra Indonesian island. Figure 1 shows the village locations in Gayo, Sidikilang and Lintong zones where the samples were purchased and de-hulled. The yellow lines are the boundaries of the significant Arabica coffee production areas. To produce the type samples, ten-kilogram lots of parchment coffee were purchased from village collectors distributed evenly across each origin. Village collectors typically aggregate and hull coffee from up 50 to 100 surrounding farmers. The coffee samples were processed following the traditional wet hulling or "giling basah" method, which is normal for Arabica coffee Sumatra, Sulawesi and Flores. The process steps were:

- Pulping, done by farmers in traditional wooden pulping machines
- Fermentation, typically done by storing the wet parchment overnight in a plastic bag
- Hand washing, to remove the mucilage
- Drying for 2 to 3 hours, down to a moisture content of 40 to 50 percent
- Storage for up to one day, before marketing to village collectors
- The type samples were purchased at this stage, and then hulled
- The samples were then separately dried and sorted to specialty grade (no primary defects and a maximum five secondary defects in a 350 gram sample).

At reception the beans were cooled with liquid nitrogen and ground (< 0.5 mm) using a Rescht ZM200 grinder.

Near infrared spectroscopy

About 3 grams of homogenized powder were analysed in NIR using a FOSS 5000 spectrometer equipped with a transport module and small ring cups. Spectra were recorded as log (1/R) in diffuse reflectance from 1100 nm to 2500 nm, in 2 nm steps.

The spectra were mathematically transformed using WINISI 1.5 software (Infrasoft International, Port Matilda, USA): a second derivative of the standard normal variate and a detrend corrected spectrum (SNVD) calculated on five data points and smoothed (Savitzky and Golay smoothing) on five data points.

Figure 1. Google Earth map locations of Gayo, Sidikilang and Lintong growing zones.

The spectral population was structured using a Principal Components Analysis (PCA) and Mahalanobis distances (H) calculated on extracted PCs. The matrix expression of H calculation was: $H = X (X' X)^{-1} X'$, where H was the matrix of H distances, X the matrix of centred spectra data and $(X'X)^{-1}$ the reverse matrix of variance covariance. The Mahalanobis distance is the distance of each sample from the average sample and takes into account the total variability of the population. Generalized H distances (each individual distance divided by the average distance) were expressed as standard deviations, making it possible to define population limits and associate a probability with the H distance. A sample with an H distance over 3 had a probability of less than 1% of belonging to the population.

The statistics, multivariate analyses and discriminant model were performed using WINISI 1.5 software, STATGRAPHIC Centurion XV(StatPoint, Inc., Usa) and XLSTAT version 2008 6.02 (Addinsoft, Paris, France).

Caffeine, chlorogenic acids, sucrose, fat; moisture and trigonelline content were predicted using CIRAD green Arabica coffee NIR calibration equation. A one-way ANOVA was done to investigate the effect of growing zone on the content of the 6 predicted constituents. The estimated average values per zone were compared using a Student Newman-Keuls (SNK) multiple pair comparison test, at 5% level.

RESULTS AND DISCUSSION

Near infrared spectra

The whole set of samples presented similar spectra: principal absorption bands were identical; the only variation between spectra was observed for absorbance intensities (Figure 2). The spectral shape conformed to that of classical ground green coffee spectra, with typical absorption bands for water (1350 nm and 1908 nm) and for fat (1720 nm and 2308 nm). This weight of water and oil in the spectral fingerprint was illustrated by the representation of absorbance standard deviation at each wavelength as illustrated on Figure 3. The highest standard deviations were observed for water and fat absorption bands.

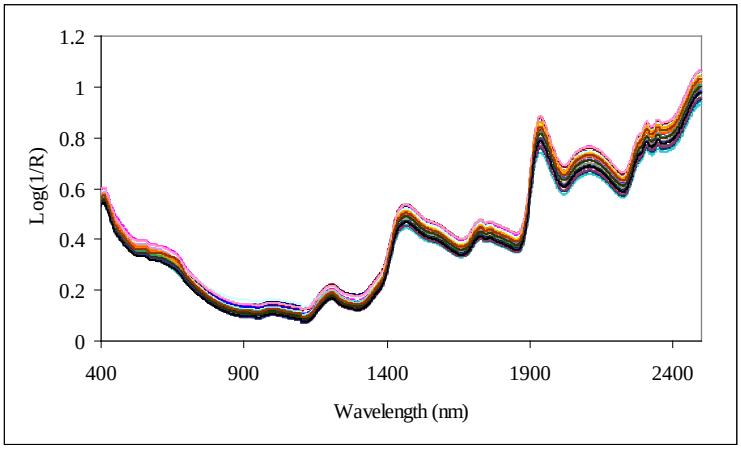

Figure 2. Near infrared reflectance spectra of the 32 Indonesian green coffee samples.

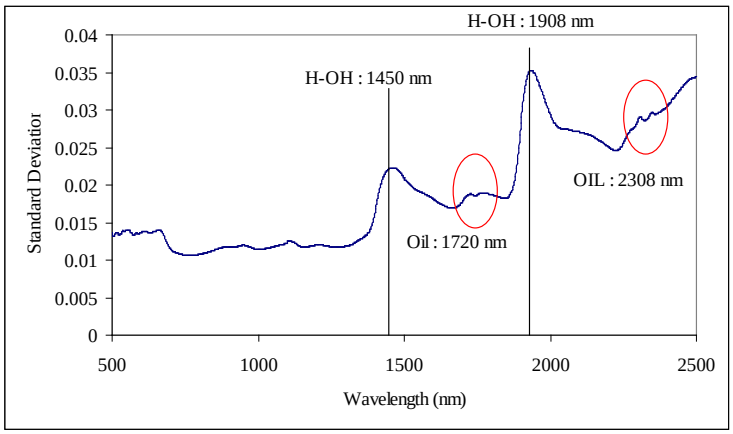

Figure 3. Absorbance standard deviation at each wavelength.

These observations confirmed that spectra can be used for analysis: there were no atypical spectra and no special deformation or pollution within absorption bands. Differences observed for water are normal for green coffee and corresponded to differences in sample drying. No group of spectra was observed according to growing zone origin or due to the intensity of water absorption bands; differences in water content resulting in a random spread of absorbance intensity. That is to say that sample moisture content is not responsible for any clustering of the spectral fingerprints.

A principal component analysis was done on the spectral matrix, using centred data and variance/covariance matrix as metric. 32 PCs were retained to describe the whole set of spectra, these 32 PCs explained 99.7% of the total inertia. The first three PCs explained respectively 51.07%, 26.41% and 10.60% of the spectral variability. For the 32 samples, the maximum Mahalanobis distance from the mean was 2.38. No sample was an outlier. This result confirms the visual approach, no sample was atypical. That is to say 1) the NIR fingerprint is representative of the sample, 2) no sample was atypical due to an NIR specific fingerprint. So, we can conclude that no specific biochemical change due to origins occurred during production or post harvest treatment. Thus, if differences due to origins exist between coffees, these differences are subtle and result from constituents in lower concentration than moisture or fat.

Prediction of chemical composition

CIRAD NIR predictive models were applied to these coffees in order to predict: moisture, caffeine, sucrose, trigonelline, fat and chlorogenic acids (CGA). The overall (32 samples) descriptive statistics are reported in Table 1.

Moisture content ranged between 11.8% and 13.2%, with an average content of 12.6%; these values corresponded to relatively high moisture content for commercial coffees. The average moisture contents per growing zone were quite similar and the moisture content followed a normal distribution. There was no separation of growing zones according to moisture content.

Table 1. Descriptive statistics for the 6 predicted constituents.

Statistique	DM	Caffeine	Trigonelline	Fat	Sucrose	CGA*
N	32	32	32	32	32	32
Minimum	86.84	1.11	0.71	12.61	7.94	8.63
Maximum	88.16	1.51	0.87	14.80	9.60	9.85
Mean	87.43	1.26	0.78	13.63	8.98	9.09
SD**	0.31	0.09	0.04	0.61	0.47	0.24

** CGA : total chlorogenic acids **SD : Standard deviation*

Caffeine content ranged between 1.11% and 1.51% with an average value of 1.26%. The caffeine contents observed were typical of Arabica coffees. Samples from Lintong presented the lowest average content (1.20%) close to samples from Sidikilang (1.22%), while samples from Gayo had the highest average content (1.36%); 40% of Gayo samples were distinguished by a caffeine content higher than 1.4%.

Fat content ranged between 12.61% and 14.80% with an average content of 13.63%. These values are relatively low for Arabica coffees. The average fat content was similar for the 3 growing zones, and fat content distribution was rectangular between the extreme values (12.61% and 14.80%).

Trigonelline content ranged between 0.71% and 0.87% with a uniform distribution over growing zones. Average content was similar for the 3 zones.

The same observation was found for CGA. CGA content ranged from 8.63% to 9.85% with a uniform distribution over growing zones. Only one sample from Gayo (sample1, 794/09) was distinct with a CGA content equal to 9.85%.

Sucrose content ranged from 7.94% to 9.60%, samples from Gayo having an average value lower (8.47 %) than those for Lintong and Sidikilang samples (9.04% and 9.33%).

The sucrose distribution showed that 50% of the samples from Gayo had lower sucrose contents (< 0.85%) than other coffees.

Analysis of variance

In order to confirm the previous observations a one-way factorial ANOVA was done to investigate the effect of growing zone on the content of the 6 predicted constituents. According to the ANOVA results, there was no effect of growing zone on fat, trigonelline or CGA content. Growing zone had a slightly significant effect (Fisher value = 3.83) on moisture content. According to the SNK test, samples from Sidikalang had significantly different moisture content than Gayo and Lintong samples, which were not significantly different. Sucrose content was significantly different for the 3 zones. Estimated average contents for each zone were: Sidikilang 9.3%, Lintong 9.0% and Gayo 8.5%. Caffeine content was significantly different for Gayo coffees while coffees from Sidikilang and Lintong did not have significantly different caffeine content. This result is illustrated in the graph of estimated mean caffeine content per growing zone (Figure 4).

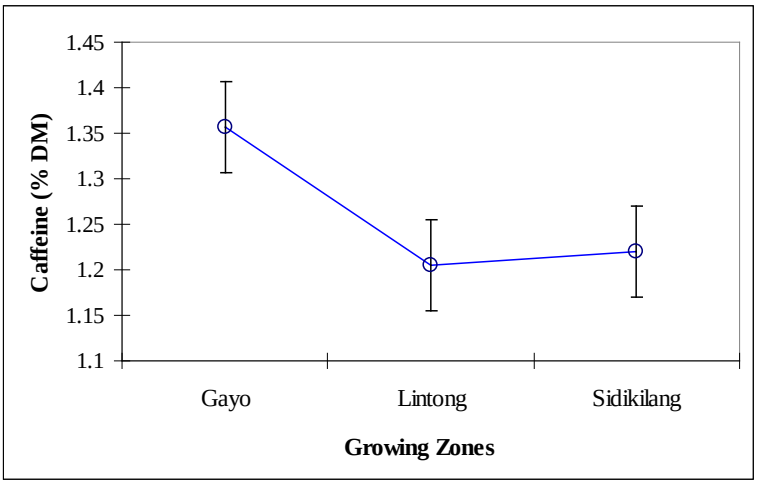

Figure 4. Mean caffeine content (% DM) per region.

Growing zones classification: Linear Discriminant Analyses

Linear discriminant analysis (LDA) is a supervised statistical method used to find a linear combination of features which characterize or separate two or more classes of objects or events. LDA allows a description of samples according to classes and a class affectation prediction for unknown samples. In the present study classes are defined by the growing zones, thus 3 classes were retained, whilst the quantitative variables (explicatory variables) were the first 8 principal components extracted from the PCA analysis done on the spectral data. The two first discriminant functions were retained and explained 100% of the variability. The Mahalanobis distances between the groups barycentre (Table 2) calculated for the 2

functions indicated the proximity of Lintong and Sidikilang samples and the dissimilarity of Gayo samples.

Table 2. Mahalanobis distances between groups barycentre.

	Gayo	Lintong	Sidikalang
Gayo	0	34.955	30.360
Lintong	34.955	0	3.731
Sidikilang	30.360	3.731	0

The scatter plot (Figure 5) of the 32 sample scores for the two discriminant functions, with 95% confidence ellipses highlights the separation between Gayo samples and the two other growing zones. The classification rate was 100% for Gayo samples while one sample from Lintong was recognised as Sidikilang and one sample from Sidikilang was recognised as Lintong.

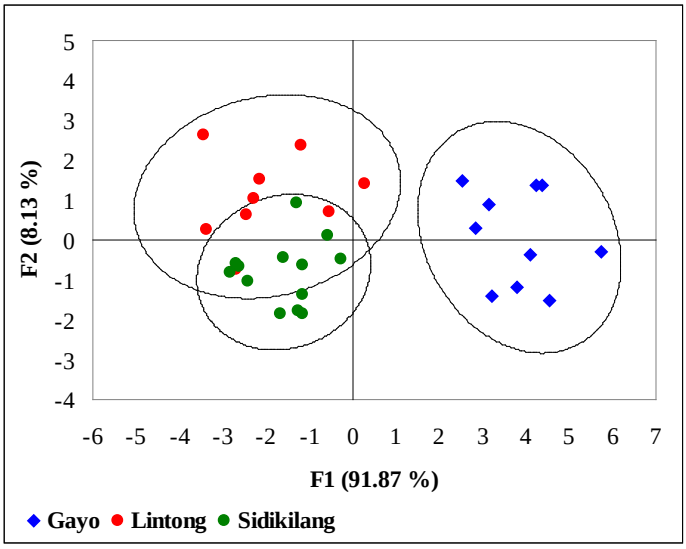

Figure 5. Scatter plot of samples scores for the 2 discriminant functions (95% confidence ellipses).

CONCLUSION

These Indonesian green coffees were similar to other Arabica coffees, their near infrared fingerprints being close to the CIRAD green Arabica NIR database. The predicted values for six major constituents showed high moisture content (12.6% on average) for commercial coffees. Differences were found for caffeine and sucrose contents between the 3 growing zones. Caffeine content was higher for Gayo samples (1.36%) than the two other regions (around 1.20% caffeine) and sucrose content was different for the 3 zones (highest content Sidikilang 9.3%; lowest content Gayo 8.5%).

Gayo sample NIR fingerprints were sufficiently different from other samples, to allow a 100% of separation of Gayo green coffee samples. The separation, based on spectral data, of Lintong and Sidikilang samples had a classification rate close to 90%. The discrimination observed was not due to moisture or fat, two constituents with a high response in NIR, but

certainly due to minor constituents in the coffee. This result is more reliable for future development as moisture is dependent on drying and fat is dependent on maturity.

This preliminary study shows that NIRS can be applied as an efficient tool for G.I support and definition.
To confirm these initial results, an experimental design should be developed in order to identify variability sources such as year effect and/or processing effect.

More investigations need to be conducted for coffee biochemical characterization in order to understand the origin of the spectral discrimination. Constituents such as proteins, amino acids, fatty acids and poly-phenols should be analysed.

Coffee Varieties Behavior (*Coffea canephora* Pierre) in Growing Area of High Altitude in the Northwest Region of Rio De Janeiro State - Brazil

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SUMMARY

In Brazil, Conilon coffee production stands out in Espírito Santo, Rondônia, Bahia and Pará States, the largest producers. It is also grown in Vale do Rio Doce - Minas Gerais and in the north and northwest of Rio de Janeiro, being grown in areas with altitude up to 400 meters and below latitude 21° South. The Conilon coffee has a higher growth rate when the temperature is between 18 to 27 °C, however, shows sharp fall in growth in winter, associated with low temperatures. The objective of this study was to evaluate the performance of four coffee Conilon varieties in areas of high altitude in Rio de Janeiro State. The experiment was installed on Candelária site, in February 2004, in Bom Jesus do Itabapoana – RJ, at an altitude of 680 meters. Three clonal varieties were planted (EMCAPA 8111 – early ripening; EMCAPA 8121 – maturation intermediate, EMCAPA 8131 – late maturing) and a seed variety (EMCAPA 8151 – Robusta Tropical). The experimental design was in a completely randomized blocks in a spacing of 2.5 x 1.2 m with four treatments and 6 repetitions, with experimental plots of 12 plants. On average four crops (2006 to 2009), the yields Robusta Tropical and early varieties (14.5 and 22.2 bags/ha, respectively) were lowers than clonal varieties of middle and late (26.8 and 34.7 bags/ha, respectively). This may be related to the stress caused by cold (early) and/or type of propagation (robusta tropical) as conilon coffee plants from cuttings produce more in the first harvest compared with those from seeds. The varieties of *C. canephora* have different tolerance when exposed to areas of high altitude, low temperatures, which should be related to defense mechanisms and acclimation between the different varieties.

INTRODUCTION

Brazil has a coffee crop of 6.41 billion pits in an area of 2.33 million ha, with the main producing states, Minas Gerais, Espirito Santo, São Paulo, Bahia and Parana (CONAB, 2010). Coffee production Conilon stands out in the states of Espirito Santo and Rondonia. It is also cultivated in Pará Vale do Rio Doce in Minas Gerais, in southern Bahia and in the north and northwest of Rio de Janeiro, is cultivated in areas with altitude up to 400 meters and below latitude 21° South.

The current forecast for domestic production of processed coffee indicates 47.04 million bags of 60 kilos of green coffee, an increase of 19.2%, or 7.57 million bags, compared with production of 39.47 million bags obtained from the 2009 harvest. The largest increase will occur in the production of Arabica coffee, estimated at 35.31 million bags, representing a gain on the previous harvest of 22.3%. For the production of Robusta (conilon) forecast points to production of 11.73 million bags, or 10.7% (CONAB, 2010).

The seasonal growth in the coffee plant fluctuations have been studied in some producing regions. It has been observed that for latitudes higher than 15° S, the greatest vegetative growth with longer and warmer occurs days, as well as with higher precipitation, where the lower vegetative growth rates were observed in the colder months with shorter days (Barros et al., 1997; Libardiet al., 1998; Silva et al., 2004). Such decay in the growth rate is not related to photoperiod (Amaral et al., 2006) or with the presence of fruits in the branches, Although the branches without fruits may present higher rates (Libardiet al., 1998). It has been reported that the C. arabica low growth phase "seems to occur at temperatures lower field than 14 °C (Amaral et al., 2006), which could be related to minimum air temperatures. However, *C. canephora* is more sensitive to low temperatures, what it's evolution probably related with ecological conditions in the low lands of the African continent (Davis et al., 2006).

In many plants low positive temperatures affects several components of the photosynthetic machinery, leading to a reduction in stomatal conductance, net photosynthesis, photochemical efficiency of the photosystem II, thylakoid electron transport, the activity of carbon metabolism enzymes, as well as changes in the Promoting structure and composition of photosynthetic pigment complexes, the observed in C. arabica and C. canephora plants (Ramalho et al., 2003; Silva et al., 2004; Partelliet al., 2009 Partelli et al., 2010).
In the State of Rio de Janeiro there Conilon coffee plantings on a small scale in the north and northwest, although there are good growing conditions in much of the state. Thus, knowledge of the behavior of different varieties of Conilon coffee in areas of high altitude, can assist in the management and the process of selection of cold tolerant varieties.

Thus, the purpose of this study was to evaluate the performance of four varieties of Conilon coffee in high-altitude areas in the State of Rio de Janeiro.

MATERIAL AND METHODS

The experiment was installed on site Candelaria in February 2004, in Bom Jesus do Itabapoana - RJ at an altitude of 680 meters. Four varieties were planted, three propagated by cuttings, EMCAPA 8111 (early ripening), EMCAPA 8121 (intermediate maturation) and EMCAPA 8131 (late maturing) and a variety of seed, EMCAPA 8151 (Robusta Tropical). The seedlings of clonal varieties were sold by the producer Ozílio Partelli Vila Valério – Espírito Santo state. The experimental design was completely randomized, a spacing of 2.5 x 1.2 m with four treatments and six replications, with plot of 12 plants.

The crop was harvested in June and July due to the difference in time of maturity among varieties. From the amount produced per plant was estimated productivity benefit in sacks of 60 kg ha^{-1}. The production data obtained from each cultivar were subjected to analysis of variance and means compared by Tukey test at 5% probability.

RESULTS AND DISCUSSION

On the average of four crops (2006 to 2009), Emcapa 8111and Emcaper 8151 had the lowest average yield (14.5 and 22.2 bags per hectare, respectively), being surpassed by clonal varieties of middle and late maturity (26,8 and 34,7 bags per hectare, respectively) (Table 1).

Table 1. Yield (bags / ha) of different varieties of conilon coffee grown in high altitude area in the Northwest Fluminense.

Variety	Productivity				
	2006	2007	2008	2009	Mean
Emcapa 8111	5,54 c	11,59 c	22,4 b	18,5 b	14,5
Emcapa 8121	12,93 b	21,36 b	53,4 a	19,3 b	26,8
Emcapa 8131	24,08 a	30,69 a	56,2 a	27,8 a	34,7
Emcaper 8151	9,91 bc	10,89 c	52,8 a	15,1 b	22,2

Means followed by same letter in a column do not differ significantly by Tukey test at 5% probability.

The lower yields of varieties Robusta Tropical and EMCAPA 8111 may be related to stress caused by cold (early ripening cultivar) and / or type of propagation (Robusta Tropical), since the coffee plants from cuttings produce more crops in the first compared with those from seeds (Partelli et al., 2006).

The performance of Conilon coffee altitudinal on two floors in the Vale do Rio Doce in Minas Gerais was evaluated Matiello et al., 2004), noting that Conilon coffee produced well in areas of high altitude and it adjusts to the cold conditions.

The varieties of C. canephora have different tolerance when subjected to high-altitude areas with low temperatures, which should be related to defense mechanisms and acclimation among different varieties, a fact described by (Partelli et al., 2009; 2010) to study different genotypes under low-temperature conilon coffee in a controlled environment. So the next harvests will provide important results on the performance of each variety and viability of commercial plantations of conilon coffee in areas of high altitude in the northwestern state of Rio de Janeiro, as well as help in choosing varieties adapted to these climatic conditions.

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Shade Level and Species Composition Affect Carbon Sequestration in Coffee Agroforestry Systems of the Kodagu District, South-Western India

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SUMMARY

Carbon (C) sequestration of Arabica and Robusta agroforestry systems (AFS) was assessed with respect to adjacent reference forest patches in the Kodagu district, the most important coffee region of the Western Ghats, one the world hotspots of biodiversity. Results indicate that coffee AFS with native species sequester large amount of C (190-200 t/ha, above and belowground) on par with forest. Robusta AFS shaded with the fast growing exotic species, *Grevillea robusta*, sequester less (only 2/3) than native AFS or forest. Carbon sequestration decreases from East to West following a contrasted rainfall gradient (from dry to very humid conditions) for all systems including forest. Tree biomass and soil are the 2 major carbon pools as they represent over 90% of the total carbon whereas the contribution of coffee plants and litter are minimal. These preliminary results confirm the strong potential of coffee AFS, particularly shaded with native tree species, to act as C sink in the major coffee producing region of India.

INTRODUCTION

India is the fifth largest world coffee producer with both Robusta (70%) and Arabica (30%) grown under the shade of multi-strata agroforestry systems (AFS), mostly in the Western Ghats, one of the world hotspots of biodiversity (Myers et al., 2000). For both Robusta and Arabica AFS, tree composition ranges from highly diverse in terms of native tree species to mostly constituted by one recently introduced exotic species (*Grevillea robusta*).

In the Kodagu district, the most important region for coffee production in India, ecological conditions vary from very humid (> 5000 mm of annual rainfall) in the evergreen Western belt to drier ones (1200 mm) in the deciduous Eastern belt and this results in a decreasing tree cover and shade level from East to West.

Over the last 25 years, the landscape has strongly evolved. Coffee plantations have expanded toward the Western part of the Kodagu district, reducing forest areas to fragments, similarly to the Eastern zone. Due to the stem borer (*Xylotrechus quadripes*), many farmers have converted Arabica to Robusta, more resistant, easier to manage and requiring less shade. This shift, along with irrigation to stimulate coffee flowering at the onset of the rainy season, has led to a reduction in shade density and diversity (Garcia et al., 2010). Furthermore, native trees are being replaced by fast growing tree species, particularly the exotic species *Grevillea robusta*, due to tree rights and land tenure issues that prevent farmers to fell and freely market wood from native species, and to the willingness of farmers to diversify their revenues through production of wood and pepper.

In this study, we present the impact of shade tree composition and coffee management (Arabica or Robusta) on carbon sequestration (above and belowground) by comparing shade cover made predominantly of a mix of native tree species to shade cover mainly constituted by the exotic species *Grevillea robusta* in reference to forest patches along a West-East rainfall gradient in the Cauvery watershed of the Kodagu district.

MATERIAL AND METHODS

Three contrasted zones were selected in terms of rainfall regime along the West-East transect (Deciduous Eastern zone: ~1200-1800 mm/year; Central zone: ~1800-3000 mm/year; Evergreen Western zone: ~3000-5000 mm/year) in the Cauvery watershed, the central watershed of the Kodagu district. A minimum of 5 sites comprising a forest reference and various coffee systems (Arabica or Robusta shaded by either predominantly native or exotic tree species) were studied in each zone with a total of 22 sites and 67 plots surveyed.

To compare accurately the different systems in the highly variable ecological conditions of the Kodagu district, plots close to each other (<300 m) were selected within a site to have similar rainfall regime, topographical and soil characteristics (notably soil texture). General information was collected through interviews with farmers on plantation age, farm size and agricultural management including year of conversion of forest to coffee, replacement of native trees by exotic ones, and conversion of Arabica to Robusta. This allows us to select plots that were under the same system for more than 10 years.

Plots were selected as "Native" when the shade composition comprises more than 90% of native tree species whereas plots were selected as "Exotic" when shade was made over 60% of *Grevillea robusta*.

In each selected plot, aerial canopy biomass was assessed via the allometric relationship developed by Chave et al. (2005) after measuring the height and girth at breast height of all the trees in a circular sampling subplot with a 15m radius (700m²) and using species specific wood density data from ICRAF (2009).

The equation specific to "Wet forest stand" of tropical areas is:

$$\text{Aerial biomass (in kg)} = 0.0776 \times (\rho D^2 H)^{0.94}$$

with ρ the wood specific density (in g/cm^3), D the tree trunk diameter at breast height (in cm) and H the total tree height (in m).

These data were also used to calculate plot tree density and basal area.

Shade level was assessed with a densiometer (Lemmon, 1966).

Root biomass was assessed via the shoot root ratio equation developed by (Cairns et al., 1997): RBD = exp (- 1.085 + 0.9256 * ln(2*ABD)) with RBD and ABD respectively the root and above ground biomass density of the plot.

Aerial biomass of Robusta and Arabica plants were estimated from allometric relationships based on destructive samplings of 25 plants.

In this study, aerial and root biomasses (shade trees and coffee plants) were converted into amount of carbon by assuming a ratio of 0.5 (IPCC, 2003).

Soil samples were also collected in the central part of each plot at two depths (10 and 30 cm) with a cylindrical auger to measure soil bulk density and soil fraction larger than 2 mm. Only the superficial soil horizons were collected as a preliminary study on various soils representative of the watershed (data not presented) show that 70% of the soil carbon is concentrated in the top 30 cm.

Litter was also collected avoiding contamination by soil particles via washing in the laboratory. After oven drying, soil and litter carbon content were measured on aliquots by a non dispersive infrared sensor associated with a furnace (Total Organic Carbon V CSH – CSN and Solid Sample Module SSM 5000A, Shimadzu Scientific Instruments).

Table 1. Mean shade level (%), basal area (m^2/ha) and carbon sequestered (t/ha) in reference forest (Forest) coffee (Arabica or Robusta) agroforestry systems shaded predominantly with native tree species (Native) or exotic species (Exotic) in 3 ecological zones (East~1200-1800, Central ~1800-3500 and West ~3500-5000 mm/year) in the Cauvery watershed of the Kodagu district.

	ZONE			Overall
System	**EAST**	**MIDDLE**	**WEST**	**Watershed**
Shade level (%)				
Forest	61	87	91	76
Arabica Native	69	-	-	69
Arabica Exotic	66	-	-	66
Robusta Native	53	47	32	43
Robusta Exotic	55	41	36	45
Basal Area (m^2/ha)				
Forest	37	42	22	34
Arabica Native	32	-	-	32
Arabica Exotic	27	-	-	27
Robusta Native	28	28	26	27
Robusta Exotic	20	16	13	17
Carbon (t/ha)				
Forest	222	189	163	196
Arabica Native	206	-	-	206
Arabica Exotic	183	-	-	183
Robusta Native	192	172	179	182
Robusta Exotic	163	131	115	138

RESULTS AND DISCUSSION

In Table 1, these preliminary results show that coffee AFS composed of Arabica shaded by either native or exotic tree species sequester C at the same rate as reference forest. To a lesser

extent, this also appears to be the case for Robusta AFS shaded with native species. The results also confirm that the conversion of Arabica to Robusta reduces shade level. A similar trend was observed for basal area, but not for tree density (data not shown). It must be noticed that C sequestration of Robusta shaded by exotic species is much lower than reference forest and other coffee AFS. An important aspect to consider is the fact that C sequestration decreases from East to West (hence from drier to wetter conditions) in reference forest and Robusta shaded with the exotic species.

With values in the range of 140-220 t/ha, total carbon sequestered in the present coffee systems are well above the median C sequestration potential of AFS estimated at 95 t/ha for tropical AFS by (Albrecht and Kandji, 2003). They are in the same order of magnitude with that of a Robusta AFS shaded by *Albizia* spp. in West Africa (Dossa et al., 2008) and comparatively higher than Arabica AFS studied in Latin America (Siles et al., 2010; Harmand et al., 2007).

In Table 2, it can be observed that tree biomass and soil are the 2 major carbon pools as they represent over 90% of the total carbon whereas the contribution of coffee plants and litter is minimal. Arabica C stocks appear low comparatively to Latin American studies (Siles et al., 2010; Harmand et al., 2007) whereas and Robusta C stocks are similar to those of the African study (Dossa et al., 2008).

Table 2. Mean carbon sequestered (t/ha) in the various components of reference forest (Forest) and coffee (Arabica or Robusta) agroforestry systems shaded predominantly with native tree species (Native) or exotic species (Exotic) in the Cauvery watershed of the Kodagu district.

Carbon (t/ha)					
System	**Tree**	**Coffee**	**Soil**	**Litter**	**Total**
Forest	97	-	97	2,4	196
Arabica Native	88	4,8	112	1,6	206
Arabica Exotic	73	3,3	105	2,2	183
Robusta Native	78	13,0	90	1,8	182
Robusta Exotic	47	10,1	78	1,9	138

CONCLUSION

It can be emphasized that Arabica AFS, particularly with native species, sequesters comparable amount of C as reference forest. On the other hand, a decline in C sequestered in Robusta AFS shaded with the exotic species *Grevillea robusta* is quite noticeable, especially in the wetter zones (Western and Central) where basal area declines strongly in comparison to Robusta associated with native tree species.

This study confirms the strong potential of coffee AFS shaded by native species to act as C sink. Consequently, incentives and policies should be put in place to reward farmers maintaining a high density and diversity of native tree species and to avoid a rapid conversion to coffee AFS predominantly shaded with exotic species and hence to preserve the largest range possible of ecosystem services, including carbon sequestration, provided by these coffee systems. This could be achieved by a combination of eco-friendly coffee labels and

payment for environmental services by local and/or international schemes such as REDD (Reduction of Emissions from Deforestation and forest Degradation).

ACKNOWLEDGEMENTS

The authors would like to thank the European Community for financing the CAFNET project under the Programme on Environment in Developing Countries.

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The Influence of Primary Processing Methods on the Cup Taste of Arabica Coffee from the Indonesian Island of Flores

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SUMMARY

The cup taste profile of specialty coffee origins is commonly attributed to a broad range of climatic, environmental and geographic factors unique to each area of production. The method of processing fresh coffee cherry to green bean is less commonly considered as a major factor in a coffee's unique cup taste. This research investigated how coffee processing method can influence coffee cup taste by evaluating 3 commercial coffee processing methods in use in the Indonesian specialty coffee origin of "*Bajawa*" on the island of Flores. The 3 processing methods assessed were: *Full-Washed* (*FW*), *Wet-hulled (WH)* and *Pulped-Natural* (*PN)*.

Fresh coffee cherry of 3 separate varieties (*Typica, S795* and *Hibrido de Timor (HdT*)) was harvested and processed to dry coffee by three different processing methods. Three replications of each of the 3 processes x 3 varieties were conducted, giving 27 samples. Each of the 3 replications within each process x variety group was combined to produce 9 composite samples. 67 coffee cuppers in Indonesian, USA and Australia evaluated the 9 samples by using a blind cupping, comparative preference testing methodology.

Cupper's preferences revealed that processing method clearly influenced coffee cup taste. *PN* processing was ranked the most preferred by cuppers across all 3 varieties. In Indonesia *PN* is often viewed as an inferior form of processing. However, the research demonstrated that applying consistent quality control to *PN* processing can produce high quality coffee in Flores. *PN* processing has a number of advantages in Flores's relatively dry environment, highlighting opportunities for the investigation of this processing method in this origin in order to develop unique flavor profiles which are preferred by the international specialty coffee industry. This research also highlights the importance of considering traditional practices and local conditions along with market requirements when making recommendations for coffee value adding and quality improvement.

BACKGROUND

Flores lies within the Lesser Sunda island chain of Eastern Indonesia and is characterised by a relatively dry agro-ecological climate where surface water is scarce and the coffee season coincides with a dry season. Flores produces an estimated 3000 tons of Arabica coffee of 3 main varieties, *Typica, S795* and *Hibrido de Timor*, all by smallholder growers at altitudes from 1000 m to 1400 m. A typical coffee farm in Flores is characterized by minimal inputs of both capital and labour, with an almost total absence of synthetic fertilisers and farm chemicals, but with a high level of staple food production of both maize and rice. Food

security concerns appear to be an important factor inhibiting greater investment in improved coffee farm management (Neilson et al., 2010).

The Flores coffee industry is undergoing rapid change, with commercial traders introducing a diversity of coffee processing and trade systems. (Marsh and Neilson, 2007). These new entrants to the Flores coffee industry have largely chosen to bypass *PN* processing and increasing numbers of farmers are encouraged to sell fresh cherries to cooperatively-owned processing units to be processed as *FW* or to prepare wet-parchment coffee for sale to be processed as *WH* by commercial buyers. However, the majority of farmers still use traditional *PN* processing and sell ungraded green beans. Both *FW* and *WH* require relatively large amounts of water (up to 5 liters per kg of cherry) for the washing stage of the process with equivalent amounts of waste water being produced. *WH* is an Indonesian variation of the FW process where fully washed parchment is hulled while still at 30% to 40% mc to produce a green bean ready for drying. *PN* is the drying of coffee with the mucilage intact on the parchment after the skin of the fresh cherry is removed by a pulper, with no washing. This process is also described as *"Descascado"*.

Traditional farmer-processed *PN* coffee from Flores is often viewed as inferior, as it can have flavour defects, due to poor quality control at farmer level such as non-selective harvesting, delayed pulping and haphazard drying. This research compares the merits of applying quality controls to the traditional method of *PN* processing with the two newly introduced processing systems. This research attempts to determine if *PN* processing creates inherently lower quality coffee, while at the same time considering the environmental, resource and financial constraints of the Flores farm system.

METHODS

Fresh coffee cherry (300 kg) of a single variety was harvested and sorted to a high standard and then divided into three identical 100 kg lots of cherry. Each lot of cherry was processed by one of three different process methods to yield dry coffee. This process was twice repeated during the coffee season, giving 3 replication samples for each process method for a single variety. The entire procedure was replicated using 2 further coffee varieties giving 27 samples representing 3 repetitions of 3 process methods applied to 3 varieties of coffee, all processed on the same site during a 6 week period in 2009 coffee season.

Each process x variety group of 3 replication samples was cupped by the ICCRI cupping panel and found to have good consistency. Each of the 3 replications within each group were then combined to produce 9 composite samples, representing 3 process methods applied to 3 varieties of coffee.

Sets of 9 x 300g samples were sent with randomly chosen blind sample codes (A to I) for cupping by 67 of speciality cuppers and coffee industry professionals in Indonesia, USA and Australia. Cuppers were presented with 3 sets of 3 blind samples and informed that each of the 3 sets of coffee were of a single undisclosed variety which had been processed 3 ways. Using the blind cupping, comparative preference testing methodology cuppers were asked to rank each coffee in the set of 3 according to their personal or commercial preference from "Prefer Most" "Mid Preference" to "Prefer Least".

The "Melbourne Preference" data was obtained from a cupping workshop held in November 2009 where 43 cuppers assessed the 9 samples, blind cupping from coffee samples prepared in air pots. The "International Preference" data was obtained from 24 cuppers who

participated in 5 separate evaluations in: USA (2 commercial coffee companies with 2 and 3 cuppers) Australia (5 cuppers), ISCA (Indonesian Speciality Coffee Association, 11 cuppers) and ICCRI (3 cuppers). 30 cuppers also evaluated the samples using the SCAA (American Speciality Coffee Association) cupping evaluation methodology.

RESULTS

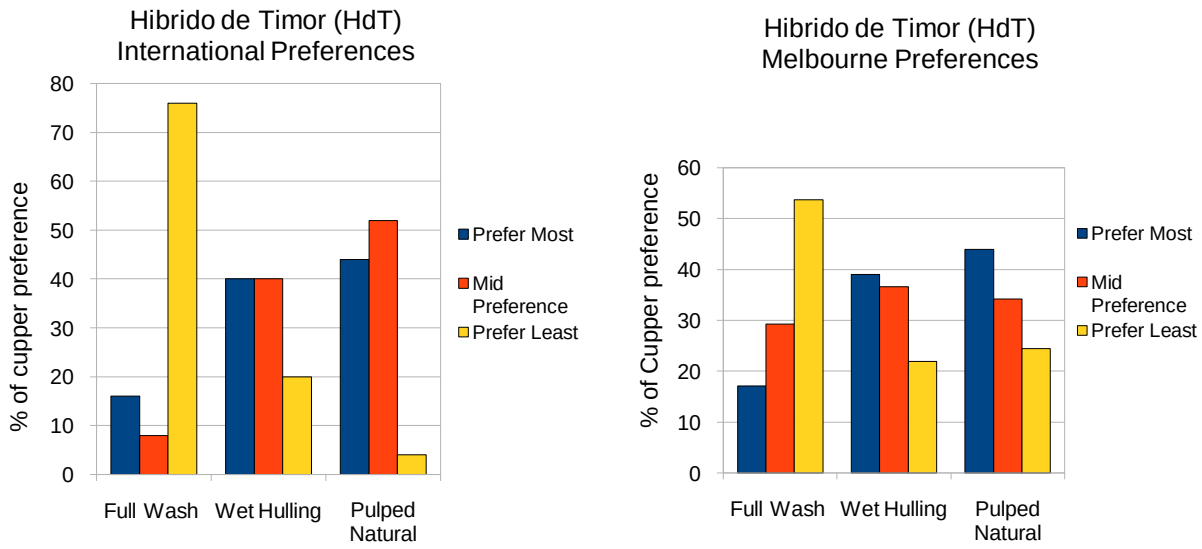

Figure 1. Preference cupping results for *Hibrido de Timor* (*HdT*).

The *HdT PN* was the ranked the Most Preferred by the larger proportion of cuppers in both International (44%) and Melbourne (43%) cuppings and also received a high proportion of the Mid Preference ranking (52% and 35%). *WH* was ranked Most Preferred by the second largest proportion of cuppers in both cupping groups (39% and 40%) and also received a high proportion of the Mid Preference ranking (37% and 40%). FW in contrast, was ranked Least Preferred with a high proportion of both groups giving it the lowest ranking; Melbourne (76%) and International (54%).

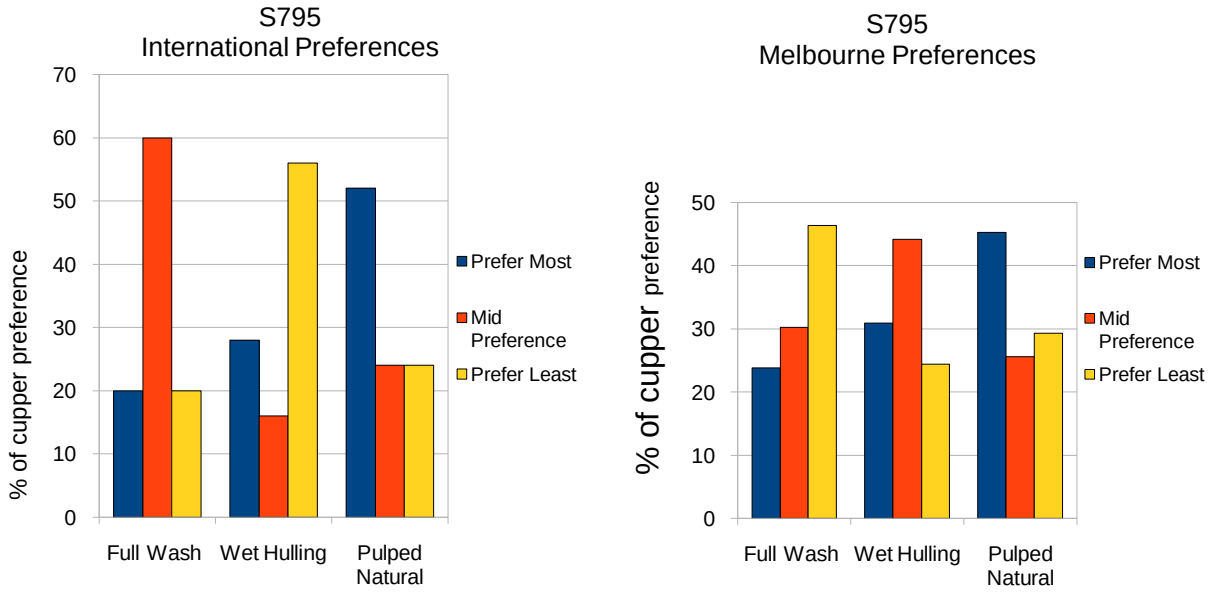

Figure 2. Preference cupping results for *S795*.

The *S795 PN* was ranked the Most Preferred by the larger proportion of cuppers in both International (53%) and Melbourne (46%) cuppings. *WH* was ranked Most Preferred by the second largest proportion of cuppers in both cupping groups (28% and 31%). However Mid and least Preferences for PN, WH and FW were not consistent between the two cupping groups indicating the preferences due to processing method may not be pronounced for this variety.

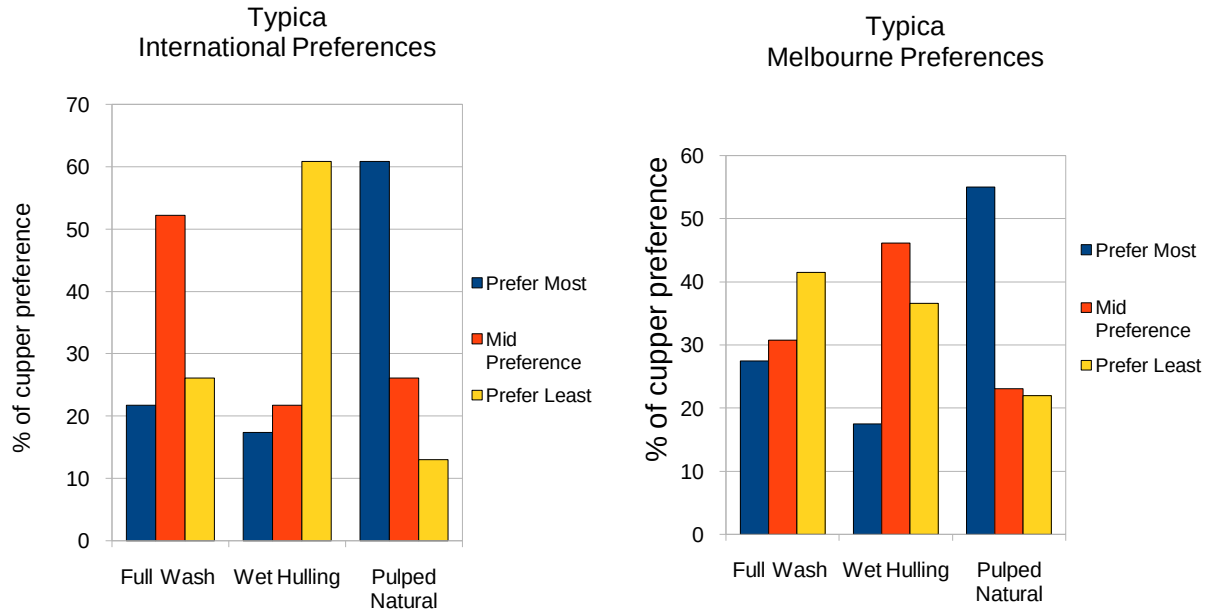

Figure 3. Preference cupping results for *Typica*.

Table 1. SCAA Cupping Data for 9 samples.

Blind Code	Variety	Process	CQI (5)	ICCRI (9)	SCAI (8)	Commercial (8)	Average 30 of Cuppers
H	*Hibrido de Timor*	Full Wash	79.75	79.63	81.16	74.31	78.64
C	*Hibrido de Timor*	Pulped Natural	76.95	78.44	82.38	76.47	78.71
F	*Hibrido de Timor*	Wet Hulling	77.95	83.09	82.13	78.13	80.65
A	*S 795*	Full Wash	80.20	83.92	83.25	80.25	82.14
D	*S 795*	Pulped Natural	80.75	81.93	83.25	81.78	82.04
G	*S 795*	Wet Hulling	79.15	83.52	82.03	82.72	82.18
E	*Typica*	Full Wash	78.40	79.09	82.19	75.84	78.94
I	*Typica*	Pulped Natural	77.45	78.26	82.00	79.38	79.28
B	*Typica*	Wet Hulling	75.30	79.39	79.98	75.66	77.87

The *Typica PN* was ranked the Most Preferred by the larger proportion of cuppers in both the International (61%) and Melbourne (55%) cuppings. *FW* was ranked Most Preferred by the second largest proportion of cuppers in both groups (22% and 28%). Mid and Least Preferred for *PN, WH* and *FW* were not consistent between the two cupping groups, however *WH* was clearly Preferred least by International cuppers (61%) and the second highest Preferred Least in Melbourne (36%).

The average SCAA scores (maximum 100 points) is presented for 4 groups of cuppings, CQI (Coffee Quality Institute): 5 cuppers, ICCRI: 9 cuppers, ISCA: 8 cuppers, Commercial speciality coffee companies in the USA: 8 cuppers. The average score for each sample for all 30 Cuppers is also presented. SCAA cupping methodology is an internationally recognised system of sensory evaluation of a coffee's flavor. It assesses and scores 10 specific flavour and quality attributes and gives a numerical indicator of a coffee's commercial value on a scale out of 100.

DISCUSSION OF RESULTS

Results demonstrate a clear preference for *PN* processing for all 3 varieties across 2 cupping groups with *Typica* variety receiving the highest preference for PN processing.

The *HdT PN* was ranked the Most Preferred sample by the larger proportion of cuppers in both groups. The *WH* was also a highly preferred method of processing for HdT. *FW* was ranked Least Preferred by a high proportion of cuppers in both groups. These results indicate that the *HdT* variety performs best when processed as *PN* and *WH* and is far less preferred when processed as *FW*. The average SCAA score for the *HdT FW* was the lowest of the 3 process methods for this variety. In earlier ICCRI coffee variety research, *HdT* was noted to have improved quality when processed by *WH* rather than by *FW*.

The *S795 PN* was ranked the Most Preferred by the larger proportion of cuppers in both groups, while *WH* was ranked Most Preferred by the second largest proportion of cuppers in both cupping groups. However Mid and Least Preferred for *PN, WH* and *FW* varied between the two cupping groups indicating the preferences due to processing method may not be pronounced for this variety. SCAA scoring gave S795 *PN, WH* and *FW* the highest scores for all samples indicating that the S795 variety creates consistently good coffee under all 3 processing systems.

The *Typica PN* was ranked Most Preferred by 61% of International cuppers and 55% of Melbourne cuppers. *FW* was ranked Most Preferred by the second largest proportion of cuppers in both groups. *WH* was Preferred Least by the largest proportion of International cuppers (61%) and received the second highest Preferred Least in Melbourne (36%). SCAA scores for Typica *WH* were the lowest of the 3 process methods for this variety. This reflects previous ICCRI coffee variety research where Typica was noted to have improved quality when processed by *FW* rather than by *WH*.

SCAA cupping results confirm that all 9 coffees samples were good quality coffees and that all varieties and processes scored in relatively tight range of 77.87 to 82.18 points out of maximum of 100. The results for SCAA cuppers were less conclusive in determining differences due to processing, as this quality evaluation system gives a commercial rating to coffees based on the intensity of specific coffee cup characteristics such fragrance, aroma, flavour, acidity, body, and the presence of essential components such as sweetness and balance rather than taster preferences. Only 10% of points are allocated for the cupper's

personal preference in the SCAA scoring methodology and coffees with different flavor characteristics can achieve similar overall SCAA scores. Thus, SCAA scores serve to demonstrate that the samples are of a good quality, but do not identify the presence or difference of particular flavours created by different processing methods.

CONCLUSIONS

This research demonstrates that under Flores conditions and for the 3 varieties evaluated, *PN* processing creates a highly preferred coffee compared to *FW* and *WH*, indicating that processing does have an identifiable influence on cup taste.

PN processing is often viewed as an inferior form of processing, perhaps because of its relative low technology and is often inconsistent due to poor quality control by smallholders. If consistent quality control is applied to *PN* processing this research shows that the resulting coffee is highly preferred by the specialty coffee industry. The demonstrated quality results for *PN*, coupled with the low water use, low waste output and minimal processing equipment requirement indicates that *PN* processing in the Flores coffee industry warrants further investigation.

The similar SCAA scores for each of the 3 processes for each variety demonstrates that all coffees samples were of good quality and of a similar commercial quality. The results also indicate that SCAA system effectively reduces the effect of cupper's preferences in its scoring system as there was a clear preference for *PN* in all 3 varieties which was not evident in the SCAA results.

SCAA scores also demonstrated that there are clear differences between varieties with *S795* scoring 2 to 3 points higher than *Typica* and *HdT* for all forms of processing. The influence of varieties on cup taste is the subject of a related paper from this research.

The preference testing results also indicated that, under Flores conditions, individual varieties respond to specific processing. Results indicate that *HdT* is more preferred if processed by *WH* or *PN* while *Typica* is more preferred if processed by *PN* or *FW*. The influence of cup taste by specific processing methods on specific Arabica varieties is the subject of a related paper from this research.

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Coffee Bourbon Pointu of Reunion Island: The Post-Harvest Process Is One of the Keys to Achieve the Best Sensorial Quality

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SUMMARY

Producing coffee in a European country is a challenge according to the cost of labour. Faced to the need to substitute non profitable crops like perfume plants (*Geranium rosa*), farmers of the Reunion Island, the French island in the Indian Ocean (Figure 1), decided to grow coffee helped by a development project ("Café Bourbon pointu de la Réunion").

This project has been set up to study the feasibility of producing a high value coffee, a "gourmet coffee". The coffee Bourbon pointu, caffeine low, is well known for its fruity taste since the 19th century. It received 39 gold medals at the 1869 Colonial Fair and the gold medal at the 1897 Anvers World Fair. The coffee Bourbon pointu cultivation is abandoned in 1940 because of the production costs that makes this cultivation non profitable. It is now boosted for a high value niche market.

Four years of agronomic trials (screening of the lines, development of good cultural methods) and experiments on the post-harvest process give a lot of results. Crossing these results with sensorial evaluations led to produce a high value coffee. Studies about the behaviour of the coffee trees in various environmental conditions permitted to define the favourable "terroir" to produce a high value coffee.

This paper focuses on the post-harvest processes and the method used to define the process giving the best sensorial results.

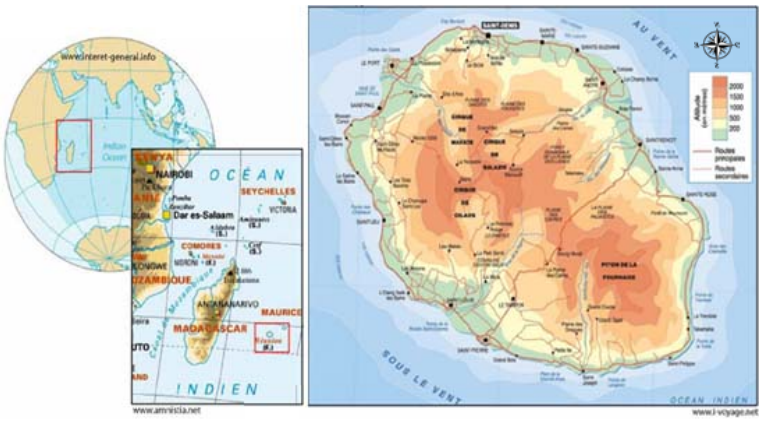

Figure 1. Geographic location of Reunion Island.

MATERIALS AND METHODS

In 2002, 27 stocks of Bourbon pointu coffee are selected among the coffee trees found in the "creole" gardens. In 2003, 113 plots are planted all around the island to put across experiments in different ecological conditions. These plots are followed up by the agronomists of the project. From the first significant harvest (2006), the cherries are collected by the project and are processed in the project workshop.

All cherries are wet processed; for the first harvest, 7 processes are tested. The pattern of the process (sequence of the phases) is the same but the length of the phases swings and a stepping can be done or not.

The Table 1 gives the details of the seven processes to be tested.

Table 1. Seven post-harvest processes are tested.

Process	Fermentation (hours)	Maceration (hours)	Steeping (hours)	Process length (hours)
A	24	24	0	48
B	24	12	12	48
C	24	24	12	60
D	24	12	0	36
E	12	12	12	36
F	12	12	0	24
G	24	0	0	24

At the end of the process, pulped coffee is washed and spread on trays to dry in the sun (Figure 2) for 9 to 11 days. Once dry (water content between 8 and 9%), coffee is bagged and stored in an air-conditioned room.

Figure 2. Coffee is dried in the sun.

A sensorial evaluation is done on each lot. A sample is drowned in each lot, the coffee husk is mechanically removed and the green coffee is roasted in a laboratory roaster Probat®. The coffee beverage is prepared according to the ISO 6668 standard, with 7 grams of grinded coffee for 100 ml of spring water heated to 94 °C. The beverage is prepared with the method of the pot brewing for 5 minutes in a French press coffee maker (Bodum® type).

The panel for sensorial evaluation is composed of 12 judges trained by the CIRAD experts on sensory evaluation.

Each sensorial attribute is appraised from 0 to 5 (0: lack of the characteristic, 5: strong). A full sensorial profile is described with 14 descriptive variables (Aroma strength, Body, Acidity, Bitterness, Astringency, Sour, Metallic, Harshness, Grassy, Dusty, Woody, Fermented, Persistence, Fruity) and with 2 hedonic variables (Aroma quality, Preference). Then the mean scores are logged in the data base.

The statistical analyses are carried out with XLSTAT® software. The Principal Component Analysis (PCA) and the ANalysis Of VAriance (ANOVA) with Fisher comparison tests are used to cross the data of sensorial evaluation and of the post-harvest processes.

To avoid a systematic error because of environmental differences according to the zones, the database has been reduced to the favourable "terroir" defined in 2008 (about 30 coffee plots situated in the highlands in the West and in the South).

The Table 2 gives the number of samples evaluated and used in the statistical analyses.

Table 2. Number of samples used for the tests and the analyses.

Process	2006	2007	2008
A	70	-	-
B	69	-	-
C	36	-	-
D	35	222	503
E	44	206	-
F	21	156	-
G	24	-	-

RESULTS AND DISCUSSION

An Principal Component Analysis (PCA) is carried out on data of the harvest 2006 (Figure 3).

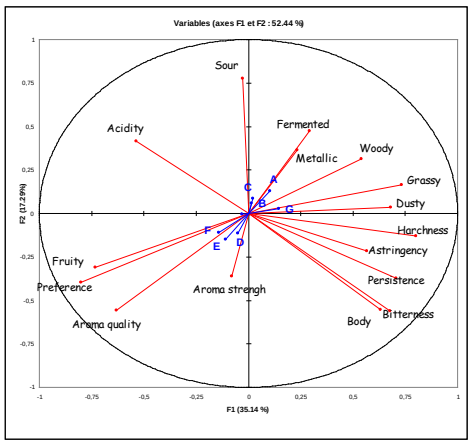

Figure 3. CPA on the 2006 data.

The two first axes give 52.44 % of the variability. The sensory variables are well situated close to the correlations circle. The process variables are not well plotted, far the correlations circle, especially the processes A, B, C and G. The PCA shows that post-harvest processes D, E and F tend to the quality sensory variables (Fruity, Aroma quality and Preference). The other processes take the opposite direction and are closed to Sour, Fermented, Woody and Grassy.

The ANOVA with Fisher comparison tests shows that processes D, E and F give the best results for the characteristics Fruity and Aroma quality (Table 3).

Table 3. Results of ANOVA (Fisher tests) for Aroma quality and Fruity.

Process	Aroma quality (means)	Groups		Process	Fruyt (means)	Groups	
F	3.473	A		F	1.56	A	
E	3.415	A		D	1.33	A	
D	3.373	A		E	1.31	A	
B	3.2		B	G	0.96		B
A	3.174		B	B	0.96		B
C	3.154		B	A	0.88		B
G	3.098		B	C	0.82		B

The processes D, E and F have been used to process the coffee of the harvest 2007.

Table 4. Results of ANOVA (Fisher tests) on 2007 data.

Variables	D	E	F	Significativity
Aroma strebgth	3.43	3.40	3.43	No
Aroma quality	3.38 A	3.32	3.28 B	Yes
Body	2.41	2.42	2.44	No
Acidity	2.63 A	2.58	2.51 B	Yes
Bitterness	2.01	2.04	2.06	No
Astringency	0.98	0.99	0.98	No
Fruity	1.78	1.71	1.64	No
Jarshness	0.75	0.78	0.77	No
Grassy	0.20	0.20	0.17	No
Woody	0.15	0.17	0.17	No
Persistence	2.77	2.78	2.79	No
Preference	3.20 A	3.11	3.06 B	Yes

On 2007 harvest data, an ANOVA is carried out. Results are very close (Table 4). For the most important variables (Aroma quality, Acidity and Preference), the post-harvest process D gives the best results. With this process, the Fruity characteristic tends to be better. This process, (24 hours for dry fermentation, 12 hours for maceration, no steeping) is chosen.

CONCLUSION

The histogram of the evolution of the attributes (Figure 4) shows the progress made in only 3 years, especially for the Fruity taste.

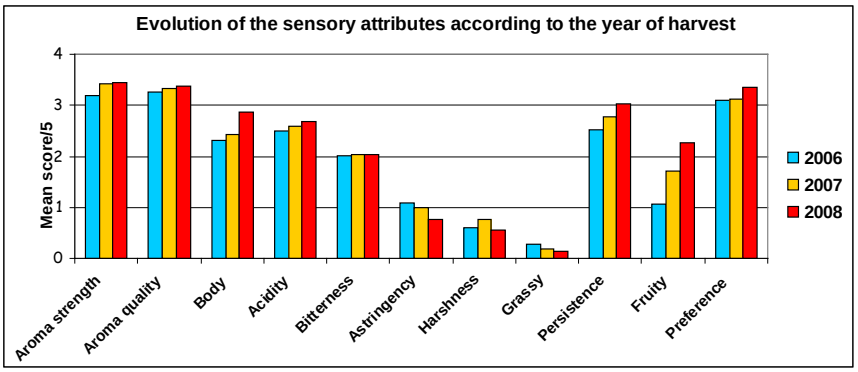

Figure 4. Evolution of the sensory attributes according to the year of harvest.

In 2006, seven post-harvest processes are compared and three of them are chosen according to their good results.

In 2007, the three processes are compared and one (process D) gives the best results.

In 2008, the post-harvest process D is the only process to be used.

To get the best coffee « Bourbon pointu de la Réunion », besides good soil and good environmental conditions, the more efficient post-harvest process is a two phases process with a 24 hours dry fermentation followed by a 12 hours maceration. Steeping is banned. Sun drying is imperative.

Support to Small Coffee Farmer in Thailand Through Large Scale Propagation of *Coffea canephora*

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SUMMARY

A project started in 2005 in Thailand with the objective to afford support to small coffee producers through the distribution of coffee plantlets and technical assistance. Robusta clones are selected in comparative trials organised in different places and is based on criteria of field yield, cup quality and bean size. Clones are propagated through somatic embryogenesis organised in France, nevertheless the technology was transferred to a national laboratory where embryos are produced at a smaller scale.

Plants are sold to farmer at production cost. Annual numbers of plants distributed to farmers are increasing gradually, thanks to the effort of demonstration organised in the producing area and the adoption of farmer for better planting material. Results obtained at farmer level indicate that yield of new planting material is commonly double compared to coffee trees locally cultivated. Strong efforts are invested in the project to rapidly develop and make available new clones able to triple the yield.

In the future, it is envisaged to efficiently combine the technology of somatic embryogenesis and the production of rooted cuttings to leverage the project. Somatic embryogenesis will be reserved for the production of new clones, although confirmed clones will be produced by rooted cuttings.

Large scale production of coffee plantlets of improved clones is a key element to make coffee competitive to other industrial crops and recuperate the farmer commitment for coffee production.

INTRODUCTION

Green coffee consumption in Thailand is continuously increasing by 10% a year although green coffee production is decreasing. The main reasons to explain the national decrease of the production are the competition with other industrial crops like rubber and oil palm. Coffee trees are also old and need to be replaced. The average yield for Robusta in 2009/10 was 0.90 tons of green coffee/ha. Traditionally, Robusta coffee is propagated by seeds in Thailand. Seeds are mostly selected by the producer and are not leading to performing genetic material. Considering the highly heterozygous status of the species and its system of pollination (auto-incompatibility), the usage of vegetative methods of propagation is required to produce performing planting material. A selection and breeding program was initiated in Thailand in 1996 and allowed to select clones adapted to the local conditions and having appropriate characteristics.

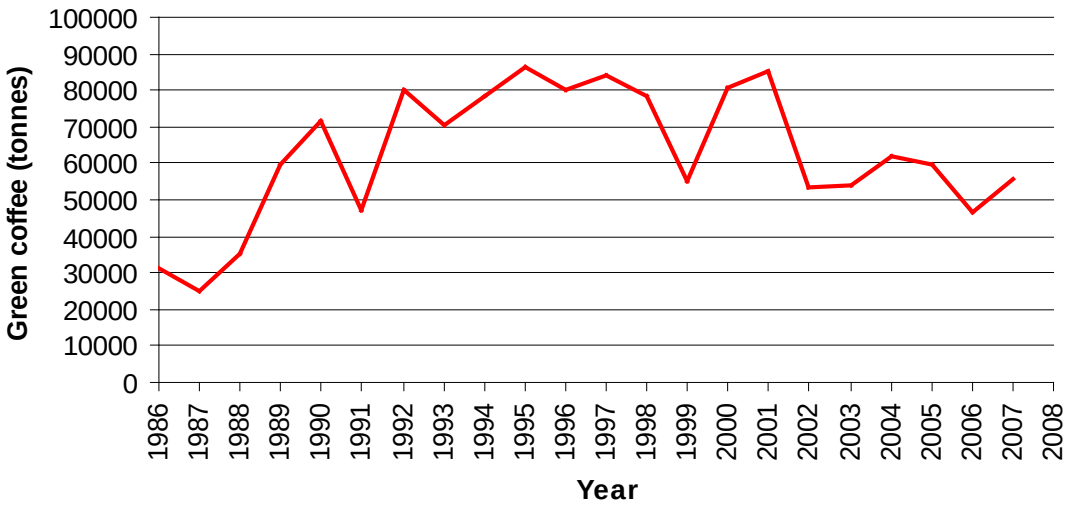

Figure 1. Coffee production in Thailand.

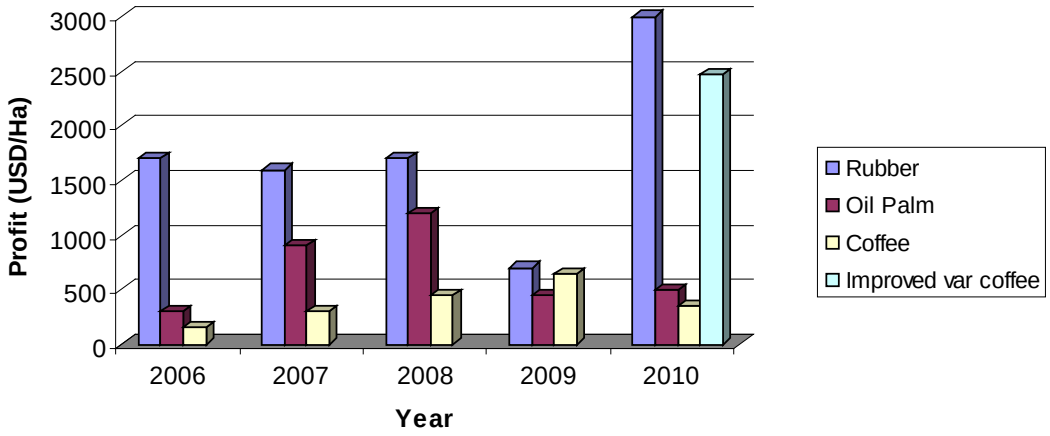

Figure 2. Profitability comparison among para rubber, oil palm, coffee and improved varieties of coffee in Thailand.

MATERIALS AND METHODS

Propagation method

Considering the urgency to produce planting material, it was decided to initiate the propagation program with the system of somatic embryogenesis. This modern technique of In Vitro propagation allows rapidly producing high number of plants and giving also the flexibility to change easily the clones under propagation. The somatic embryos are mainly produced in France (Nestlé R&D Tours) but also in a national laboratory (CHRC) where two scientists were trained on the technique.

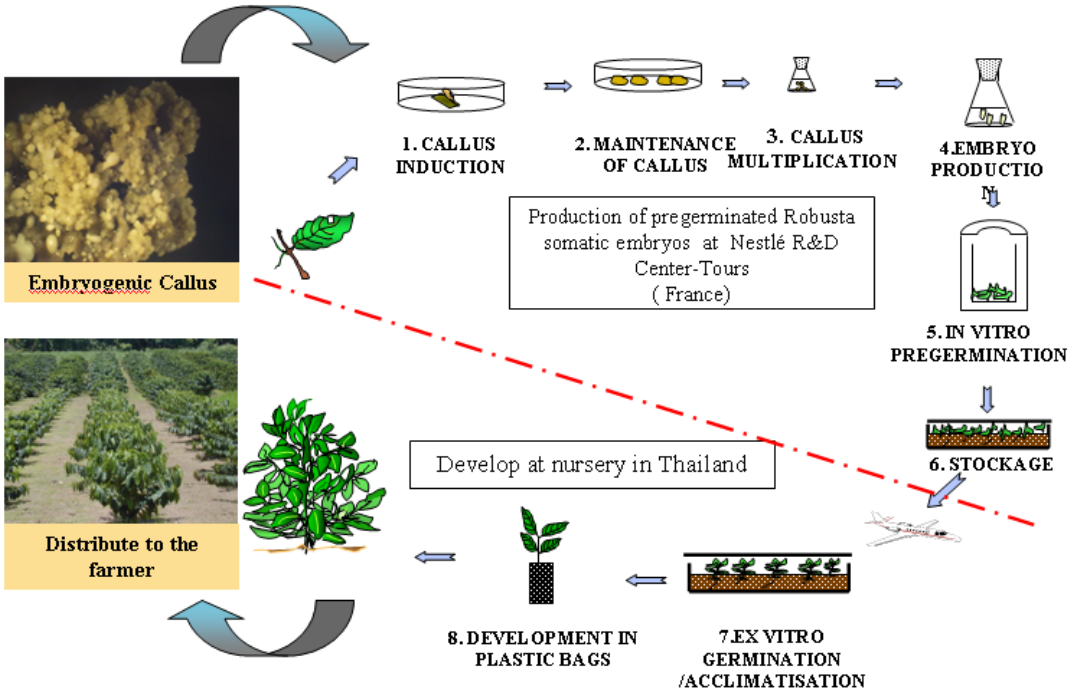

Figure 3. Protocol of Robusta coffee somatic embryogenesis.

Planting materials

The group of clones under propagation is improving thanks to the selection and breeding program. Selection criteria are mainly focusing on field performance combined with green coffee quality

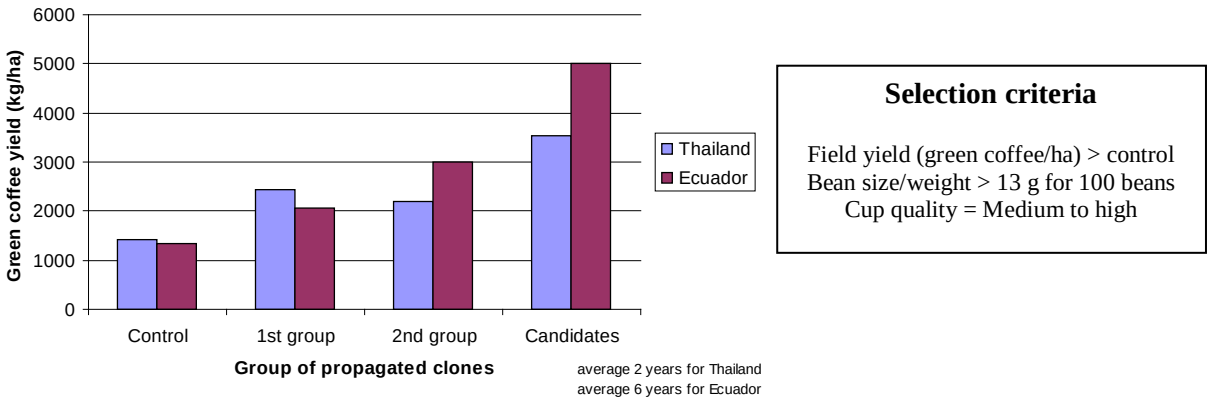

Figure 4. Annual average yeld.

RESULTS

Up to June 2010, 1.81 million coffee plantlets were sold to 4,337 coffee farmers. Farmer plots are under observation to monitor the benefit of the new clones compared to national performance.

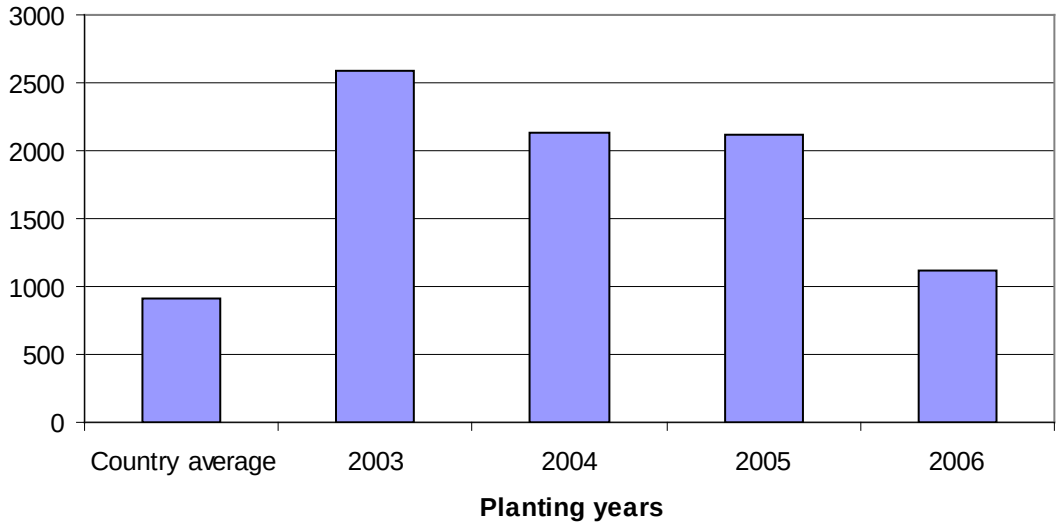

Figure 5. Average yeld 2009/10 (kg green coffee/ha).

PERSPECTIVES

An ambitious project of propagation started in Thailand in 2005 with the objective to replace 10 % of the actual acreage of coffee. The strong competition between industrial crops for the land profitability is putting pressure on Robusta coffee. One way to increase efficiently the coffee profitability is to distribute new clones with much better field performances. The project is using the technology of somatic embryogenesis for a rapid and massive propagation of selected clones and its feasibility is now technically demonstrated. The technique is particularly interesting to face urgent situations, like the one encountered now in Thailand, when large quantities of improved trees are required in a short delay. The method is also very useful to quickly propagate new clones coming out of a breeding program without passing through the phase of wood gardens. It is now envisaged to combine the two systems of propagation, keeping somatic embryogenesis for the new clones and using rooted cuttings for the clones already confirmed. The combination of the two techniques will allow to dramatically increase the size of the project for the benefit of the coffee sector. Strong research efforts allocated in Thailand for the selection and development of better genetic material will be valorised through the propagation and distribution of millions trees.

Coffee and the Environment. A Review

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SUMMARY

Under ideal and stable environmental conditions, the coffee plant is programmed to complete a set of processes like growth and differentiation. However, under a different set of environmental conditions the life cycle may be shortened or lengthened. In this review, the impact of environmental factors with special emphasis on climate change on the physiology and production of *Coffea arabica*. The first part of the review deals with climatic requirements of coffee in different coffee growing zones. Impact of rainfall and moisture stress on the flowering pattern and ultimately the yield of coffee will be discussed in the next section. Temperature effects on the physiology of coffee and its effects on the crop productivity balance will also be addressed. The third section will focus on mitigating and adaptation strategies used to reduce the negative impact of climate change.

INTRODUCTION

The native habitat of all *Coffea* species are the under storey of African tropical forests (DaMatta et al., 2007) with Arabica coffee *Coffea arabica* L originating as an under storey plant in the Ethiopian forest and early plantations were shaded by planting overstorey trees to simulate the natural habitat (DaMatta, 2004). Coffee exhibits typical features of such species such as acclimation of leaves in order to photosynthesize in low light, high leaf area: woody structure ratio and absence of a self thinning mechanism that regulates fruit load which are important features because they affect plant carbon balance: the basis of coffee yields and quality (Franck et al., 2007). Despite being shade adapted, commercial cultivars are increasingly grown in full sun and are able to out yield shade grown plants (Kumar and Tieszen, 1980; Beer et al., 1987). Out of 103 species in the genus *Coffea*, *C. arabica* L. and *C. canephora* Pierre economically dominate world coffee trade with these species being responsible for about 99% of world bean production (DaMatta et al., 2007; Camargo, 2010).

CLIMATIC REQUIREMENT

Temperature is the most relevant climatic factor for coffee production since production responds significantly to seasonal temperature patterns (Gay et al., 2006). The strongest influences on temperature are latitude and elevation. Coffee is grown around the world at latitudes from 24°N to 25°S and elevations ranging from sea level to as high as 7000 ft (Bittenbender and Smith, 2008). In general, high-elevation coffee regions are found in countries at or near the equator, such as Kenya, the New Guinea highlands, and Colombia, while low-elevation coffee regions, such as Hawaii and Sao Paulo, Brazil, are usually at subtropical latitudes (22-25°) . In Ethiopia, coffee is cultivated within the elevation range of 1,000 to 2,100 meters above sea level (Kufa et al., 2001). The optimum mean annual temperature range for Arabica coffee is 18-2 °C (Alègre, 1959). Above or below these temperatures the yield and quality of *C. arabica* is greatly reduced (DaMatta and Cochicho-

Ramalho, 2006). The optimum mean annual rainfall range is 1200-1800 mm for Arabica coffee (Alègre, 1959). The average yearly precipitation in coffee's native habitat is 1,500 to 2,000 mm (Kufa et al., 2001). Arabica coffee requires a less humid atmosphere comparable to that of the Ethiopian highlands (Coste, 1992). Hot winds increase evapotranspiration and therefore rainfall or irrigation requirements to the trees increase (DaMatta et al., 2007). In addition, strong winds may severely damage leaves and buds exacerbating shedding of developing flower and fruits.

WATER RELATIONS IN COFFEE

Synchronization of a group of plants within vegetative and a reproductive pattern seems to depend on the rainfall distribution in equatorial regions (Wormer and Gituanja, 1970). Rainfall requirements depend on the retention properties of the soil, atmospheric humidity and cloud cover, as well as cultivation practices (DaMatta et al., 2007). Reduction in water stress by experimental wetting, rainfall or irrigation may break dormancy and lead to the flowering of coffee (Cannell, 1985). Seasonal showers during the normally dry period were most easily recognized as having had a direct role in flowering onset. These showers are important for the synchronous mass flowering of coffee (Opler et al., 1976). The occurrence of sporadic and sometimes low intensity rains during the latter phases of flower bud development is believed to be one of the uncontrolled factors responsible for several blossom periods in Arabica coffee. In addition, abundant rainfall throughout the year is often responsible for scattered harvest and low yield (DaMatta, 2004). Crisosto et al. (1992) reported that ripe fruits, large and small green fruits, flowers and buds at different stage of development may all be found on the same tree. Annual rainfall in coffee's native habitat is 1500-2000 mm and exhibits a pronounced seasonal distribution in which several months are nearly rainless. This probably contributed to the substantial drought resistance shown by coffee (Meinzer et al., 1990) and to its requirement for a period of reduced water availability to trigger phenological events such as floral bud release (Crisosto et al., 1992). Water shortage during the rapid fruit expansion stage often reduces the growth of berries as ovules do not reach their full size under limiting water. In fact, fruits that expand during the wet weather become larger with larger locules which are subsequently filled with larger beans than fruit which expanded in the hot dry weather (Cannell, 1985).

EFFECTS OF TEMPERATURE ON COFFEE PRODUCTIVITY

The optimum growth temperature often corresponds to optimum temperature for photosynthesis. Photosynthesis in C_3 plants is limited by photorespiration and most of this activity is closely related to temperature. Temperature increases are associated with a rise in atmospheric vapour pressure deficit and therefore decreases in photosynthesis could be due to increase in temperature per se or increase in vapour pressure deficit leading to stomatal closure or both (DaMatta, 2007). Coffee shoot growth is not continuous during the active growing season. Temporary depression in growth of shoots has been attributed to high temperatures (Barros et al., 1997). A relatively high air temperature during blossoming, especially if associated with a prolonged dry season, may cause abortion of flowers (Camargo, 2010). Camargo (1985) reported that at temperatures above 23 °C, the development and the ripening of the fruits are accelerated often leading to loss of quality. However, selected cultivars under intense management have allowed arabica coffee to be spread in marginal areas with average temperatures of 24-25 °C while on the other hand, in regions with a mean annual air temperature below 18 °C, growth is largely depressed (Camargo, 2010). In contrast, DaMatta (2004) reported that photosynthetic rates in coffee may be maximized at higher values even at temperatures above 30 °C after a sufficient

acclimation time to the new conditions. Adequate temperature value changes with the phenological stage of coffee (DaMatta and Cochicho-Ramalho, 2006). Leaf area expansion and phasic development are faster in the tropics because of higher temperatures during vegetative growth (Rosenzweig and Liverman, 1992). Changes in the sink-source at the final stage of endosperm storage accumulation, associated with the progressive decline in the activity in vegetative apical meristems due to a reduction of air temperature (Barros et al., 1997). In coffee, low positive temperatures are very harmful to photosynthetic structures, and these temperatures provoke chlorophyll loss mostly in the leaf margins and leaf necrosis as well as strong increases in leaf hydraulic conductance due to membrane damage (DaMatta and Cochicho-Ramalho, 2006). Solar radiation and relative humidity influence many physiological processes of the coffee tree (Camargo, 2010).

CLIMATE CHANGE AND COFFEE

Whereas climate variability has always been a major factor responsible for the fluctuation of coffee yields in the world, climate change as a result of global warming is expected to result in actual shifts on where and how coffee may be produced in the future. Current global warming could result in increased respiration and decreased assimilation, altering the carbon balance of tree (Gutierrez, 2002). With regard to the possible effects of global warming and climate change on coffee environment and genetic diversity, nowadays it is not uncommon to observe drying symptoms on new coffee trees due to physiological disorders between vegetative and reproductive growths. This is associated with continued flower blooming and heavy crop load, as a result of change in weather pattern with erratic rainfalls. Under agronomical aspects, some strategies may have on global warming in coffee crop that can attenuate the impact of unfavorable temperatures are agronomical mitigations such as shading management system (arborization), planting at high densities, vegetated soil, correct irrigation, mulching and agronomical adaptation with focus on breeding programs (Camargo, 2010). Artificial shade or shade trees reduce coffee fruit load through their effects on coffee physiology such as longer internodes, fewer fruiting nodes and lower flower induction. Beneficial synchronizing effect of shade through decrease in light intensity and temperature around the berries slows down the ripening of the coffee berry flesh and allows for more time for complete bean filling (Vaast et al., 2005).

CONCLUDING REMARKS

Despite significant research on coffee, there are many gaps that have been identified. The study of different genotypes of *Coffea arabica* L., species and *Coffea* genus that allow identification of photosynthetically efficient genotypes to be used in breeding programs in order to increase productivity. Knowledge of the determinants of yield is important in addressing high cropping efficiency. Identification of traits associated with yield variation under the highly changing tropical environment needs to be done. There are opportunities for research that characterizes the effects of irradiance, temperature, CO_2, and soil and atmosphere water availability on the coffee crop performance.

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Coffee Bourbon Pointu of Reunion Island: How to Define a Terroir to Obtain a «Gourmet» Coffee

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SUMMARY

New ways of coffee consumption have emerged with the development of specialty coffees and terroir coffees. Producing coffee in a European country is a challenge according to the cost of labour. Nevertheless, farmers of the Reunion Island, the French island in the Indian Ocean, decided to grow coffee helped by a development project ("Café Bourbon pointu de la Réunion") with the aim of producing a high value coffee for a niche market. Producing coffee on the Reunion Island is not new: the first coffee plants were introduced in the 18th century from Yemen. Coffee cultivation has contributed to develop the island. "Bourbon pointu" is the result of a natural mutation of a Yemen Arabica coffee plant, discovered in 1771 in a plantation near the village of Sainte Marie. The coffee Bourbon pointu, caffeine low, is well known for its special citrus fruity taste.

MATERIAL AND METHODS

In 2002, 27 coffee Bourbon pointu stocks (*Coffea arabica* [*Laurina*]) are selected among the 2400 coffee trees found in the "creole" gardens. In 2003, 113 plots are planted all around the island to put across experiments in various ecological conditions.

Coffee plots are georeferenced (longitude, latitude, altitude).

Different soil types can be found in the zone of coffee cultivation (according to the FAO World Reference Base for Soil Resources classification):

- type 2: Acrisols,
- type 3: Andic Cambisols on ashes,
- type 4: Silandic Andosols on ashes,
- type 5: Hydric Andosols on ashes,
- type 8: Leptic Cambisols on ashes,
- type 11: Hydric Andosols (Skeletic).

The climate variables have been collected from "Meteo France sites" close to the plots, adjusted according to the altitude for the temperatures[1] and for the sun radiance[2].

[1] Calculated Temperature = Temperature meteo site – (coffee plot altitude-meteo site altitude)/100* variation factor, this factor swings from 0.72 in February (summer) to 0.81 in August (winter).

[2] Calculated decade global radiance in MJ= meteo site decade global radiance in MJ + (coffee plot altitude - meteo site altitude)* global radiance factor according to the altitude for the decade in MJ/100m.

The collected variables are:

- monthly mean temperature,
- monthly minimal temperature (mean, absolute),
- monthly maximal temperature (mean, absolute),
- monthly wind speed (km/h),
- monthly cumulative rainfall,
- monthly sun radiance.

The cherries are collected by the project and are processed in the project workshop. Sensory evaluation of the coffees is done by a 12 coffee experts. The samples are prepared and tested according to the ISO 6668 standard. The sensory attributes are: Aroma strength, Body, Acidity, Bitterness, Astringency, Sour, Metallic, Harshness, Grassy, Dusty, Woody, Persistence and Fruity) completed with 2 hedonic variables (Aroma quality, Preference).

Cup testing done on 3 harvest periods enable the setting up of three marketing classes built from the Preference score. Statistical analyses carried out on all the sensory data show that some correlations exist between Preference and other sensory attributes (Fruity, Aroma quality…) (Table 1). "Grand Cru" is the upper class with a Preference score of 3.50 or more.

Table 1. Links between marketing classes and sensory attributes (from ANOVA and Fisher tests results).

	Authentique	**Sublime**	**Grand Cru**
Aroma quality	Score ≥ 2.75	Score ≥ 2.85	Score ≥ 3.00
Persistence	2.50 ≤ Score ≤ 3.50	Score ≥ 2.50	
Acidity	1.75 ≤ Score ≤ 3.50	1.75 ≤ Score ≤ 3.25	2.25 ≤ Score ≤ 3.25
Body	2.00 ≤ Score ≤ 3.25		2.50 ≤ Score ≤ 3.25
Fruity	Score ≥ 1.25		Score ≥ 2.00
Preference	Score ≥ 2.75	Score ≥ 3.00	Score ≥ 3.50

NB: The scale of the sensory scores is from 0 to 5.

Are downgraded in Original (coffee non marketed), the lots not suiting to all the standards defined for the marketing classes.

The statistical analyses are carried out with XLSTAT® software. Statistical analysis (Analysis of Variance, ANOVA with Fisher tests and Multiple Correspondence Analysis, MCA) crossing the soil data, the climate data and cup testing data enable to highlight the parameters for the production of a "gourmet" coffee and to outline the map of the terroir fit for this cultivation on the Reunion Island.

Table 2. Elements used to carry out the statistical analyses done on the 2006, 2007 and 2008 harvests to define the favourable terroir.

	Harvest 2006	Harvest 2007	Harvest 2008
Number of cities and villages	11	9	6
Number of coffee plots	48	43	29
Altitude (from X to Y)	270 m to 1209 m	453 m to 1209 m	315 m to 1209 m
Number of coffee lots (used for the cup testing)	399	612	579
Number of different soils	6 (2,3,4,5,8,11)	5 (2,3,4,5,8)	4 (3,4,5,8)

RESULTS AND DISCUSSION

The results of the statistical analyses show the impact of altitude on sensory quality of coffee. The statistical analyses (ANOVA, not related here, and MCA: Figures 1 and 2) enable to define the fitted altitudes and soils. All the results enable to define the parts of the Reunion Island adapted to produce "gourmet" Bourbon pointu coffee.

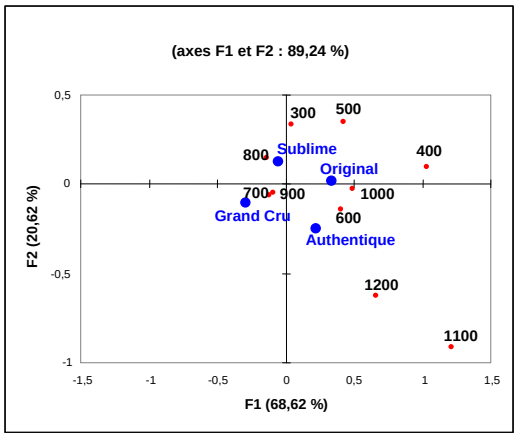

Figure 1. MCA altitude /marketing classes.

Altitude

The study of the impact of altitude on the sensory quality shows that all the marketing classes are located between 700 m and 1000 m. "Grand Cru" is the only class to be located between 800 m and 900 m.

Altitude is linked with climate conditions (Table 3).

The temperature fluctuations and the sun radiance have an impact on sensory quality. Statistical analyses (ANOVA) crossing sensory data and climate data enable to specify the ranges for the Bourbon pointu coffee cultivation on the Reunion Island:

Table 3. Correlations between altitude and the climate variables.

Variables	T° minimum	T° mean	T° maximum	Wind speed	Rainfall	Sun radiance
Altitude	-0.733	-0.863	-0.798	-0.178	-0.019	-0.530

Temperature

- Minimal temperature: between 12 °C and 16 °C
- Mean temperature: between 15 °C and 19 °C
- Maximal temperature: between 22 °C and 24 °C

Sun radiance

- For one month: mean sun radiance between 1100 and 1900 MJ
- For one year: sun radiance between 15500 and 21000 MJ

Rainfall

Rainfall: between 750 mm and 1750 mm, with a maximum of three dry months (with rain fall < 25 mm) along the year.

Soil

The ANOVA shows that the soils of types 4, 8 and 3 give better results for the Preference score than the others soils.

The analysis (MCA) (Figure 2) crossing the soils and the marketing classes shows that the soil of type 4 (Silandic Andosols on ashes) and of type 8 (Leptic Cambisols on ashes) give coffees of better sensory quality (Grand Cru and Sublime are close to these soils).

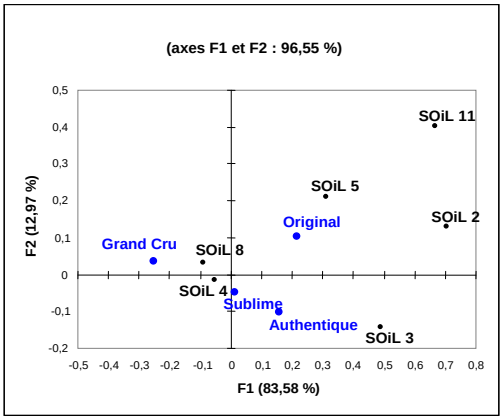

Figure 2. MCA types of soils /marketing classes.

Figure 3 shows that the type of soil has an important impact on the sensory quality of coffee (Soil 4: propitious soil; soil 2: medium soil; soil 9: non propitious soil).

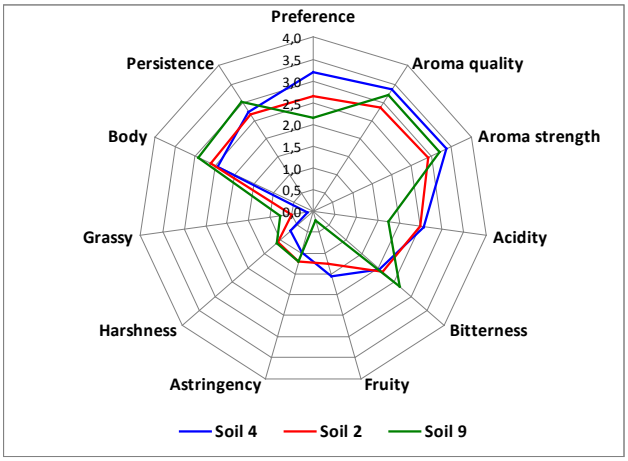

Figure 3. Sensory profile of Bourbon pointu coffee according to the type of soil.

CONCLUSION

Statistical analyses done on three harvest periods enable to highlight the main factors to produce a « gourmet » Bourbon pointu coffee on the Reunion Island (Grand Cru marketing class):

- Altitude : between 800 m and 900 m
- Climatic data
- Soil type 4 (Silandic Andosols on ashes) and type 8 (Leptic Cambisols on ashes/ Brown Andic soil).

Table 4. Adequate climatic data for the highest sores for Grand Cru marketing class.

Variables	T° minimum	T° mean	T° maximum	Wind speed	Rainfall	Sun radiance
Grand Cru	14 °C ± 2	17 °C ± 2	23 °C ± 2	70 km/h ± 20	1450 mm ± 300	17000 MJ ± 300

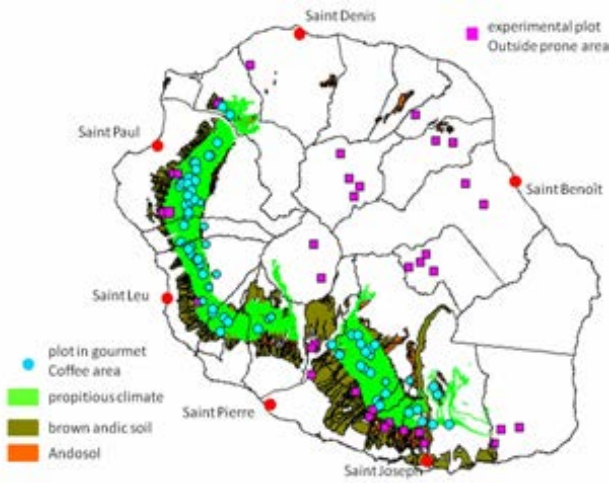

Figure 4. Map of the adequate terroir to produce a Bourbon pointu « gourmet» coffee.

Out of the pedoclimatic conditions and altitude, some other factors are essential to produce a Bourbon pointu «gourmet» coffee:

- Plant material selected for the sensory performances,
- Berry maturity stage when harvesting,
- Post-harvest process…

Scaling-Up LAI in Coffee Agroforestry Systems in Costa Rica

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[5]La Hilda-Doka farm

SUMMARY

Ecosystem Services (ES) provided by coffee plantations may differ according to agricultural practices (monoculture or agroforestry). The leaf area index (LAI) is assumed to be a good parameter to model ecosystem ES. We tested a method to measure leaf area index of coffee agroforestry plantations that display moderate shade or no shade, being valid for a large spatio-temporal scale, relying on a vegetation index (NDVI) derived from satellite imagery (Very High Resolution THRS and medium resolution MODIS). Time series (2001-2009) of LAI were obtained following two steps of calibration: a first calibration between the actual LAI measured in the field and the NDVI THRS image ($R^2 = 0.80$) in order to obtain maps of LAI at high resolution; the second step between the high-resolution LAI map and NDVI from MODIS ($R^2 = 0.66$). We studied the effects of environmental and agronomic factors on LAI. The seasonal dynamics of LAI (2.5 and 4 in the dry season in the wet season) was connected with variations in air humidity and solar radiation. We found that the LAI of some key months was correlated with coffee yield. The dynamic of LAI was also influenced by agricultural practices such as the pruning of coffee. This work is a first step towards spatial modeling of ecosystem services and production.

INTRODUCTION

Coffee systems show large variability in structure (from monoculture to diversified agroforests), or in management (from organic coffee to very intensive systems). These different systems are not equivalent in terms of profitability and provision of ecosystem services (ES) (production, carbon sequestration, erosion control...).

Our hypothesis is to consider that leaf area index (LAI) is an integrated indicator of ES: leaf area determines light interception, gas exchange and hence primary productivity, and LAI can be related to yield, to the annual input of litter to the soil (C cycle and soil OM) and to rain interception (erosion control).

Most studies of coffee LAI were performed at plot scale. In contrast, we propose to explore the plant, plot and farm scales, using remote sensing. Coffee Normalized Difference Vegetation Index (NDVI) has already been studied (Brunsell et al., 2009), but mainly as a vigor indicator and without calibration with true LAI. The aims of our study were (i) to calibrate the relationships between NDVI and LAI for agroforestry coffee systems displaying

low shade levels (ii) to assess some relationships between environmental or agricultural factors and LAI, and (iii) to test the capacity of remotely-sensed LAI to be used as an indicator of yield and provision of ecosystem services.

MATERIALS AND METHODS

Study sites

Two coffee farms were selected in Costa-Rica. Aquiares had no dry-spell and dispersed shade trees whereas La Hilda (Doka) had dry-spell and no shade at all. Aquiares was also the settlement for the Coffee-Flux project (Roupsard et al., 2010, see next ASIC poster).

Remotely sensed images: we acquired a very high spatial resolution (VHSR-2m) image (worldview 2) for each farm in March 2010. We used also a moderate resolution satellite (250 m MODIS) with a high temporal resolution (16 days composite images).

LAI measurement

Light transmittance was measured with 2 LAI 2000 devices on 15 transects across the 2 farms. Effective LAI of coffee and trees was inverted from light transmittance. True LAI of coffee was obtained after measuring the leaf area of 25 coffee plants (60 000 leaves) and compared with the effective LAI, their ratio being the clumping factor.

Calibration of LAI-NDVI

First, an experimental relationship between true field LAI and VHSR NDVI yielded a LAI map at high resolution; second, a relationship was found between the MODIS NDVI and the corresponding area of the LAI map (Figure 1)

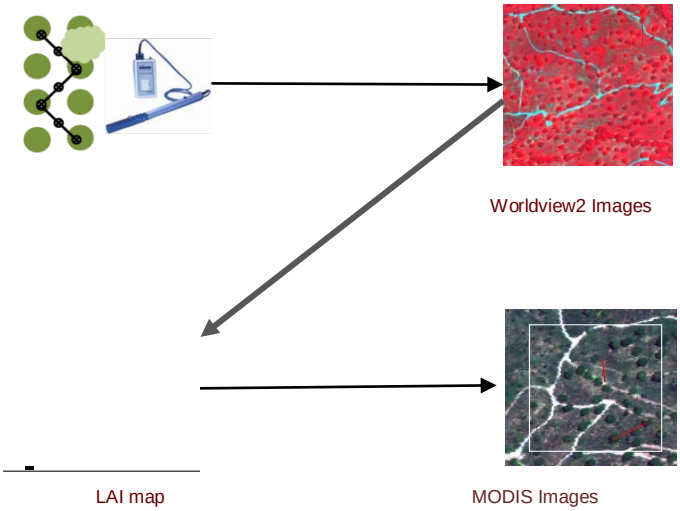

Figure 1. Protocol for calibrating high and moderate resolution NDVI from ground LAI and for mapping LAI.

RESULTS AND DISCUSSION

Remotely-sensed NDVI has been successfully calibrated at high (HR) and moderate resolution (MR) for coffee systems with little or no shade. We derived HR-LAI maps and MR-LAI dynamics (Figure 2):

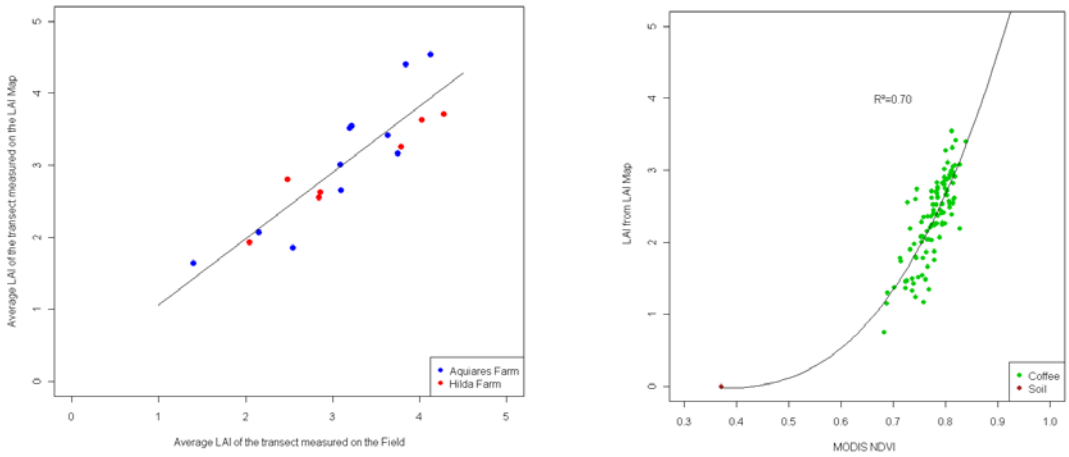

Figure 2. A) Relationship between Field LAI and LAI derived from the HR LAI Map, after its calibration. B) Relationship between LAI from the HR LAI map and MODIS NDVI. The two farms are presented.

Seasonal dynamics of LAI are influenced by climate (rain) and by agricultural practices (pruning ,..) (Figure 3 and Figure 4)

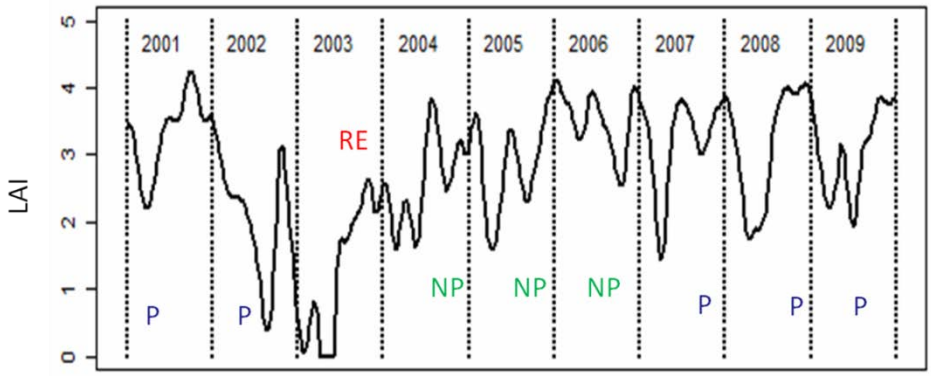

Figure 3. Effect of agricultural practices on LAI time series detected by MODIS and interpreted from farm questionnaire (P coffee pruning, RE coffee renovation, NP coffee not pruned). One MODIS pixel presented as an example.

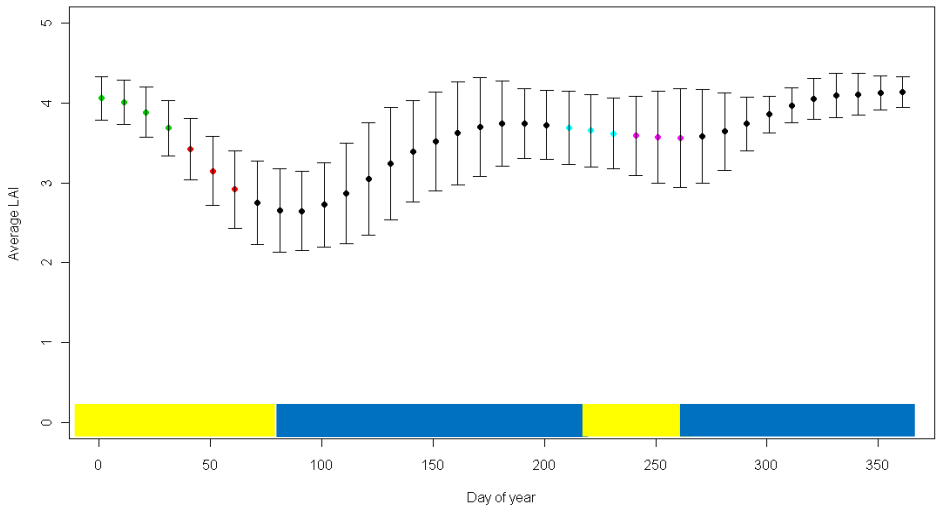

Figure 4. Example of seasonal variations of LAI of a coffee plantation (tree + coffee) derived from MODIS NDVI time-series for one pixel Error bars represent the variability on the 9 year time-series in one entire farm (Aquiares).

Coffee prices during year-N-1 explained 78% of the variability of average year-N LAI, which was interpreted as more pruning for renovation and less fertilization when the coffee prices are low.

LAI of the driest month are linked with yield. (Figure 5)

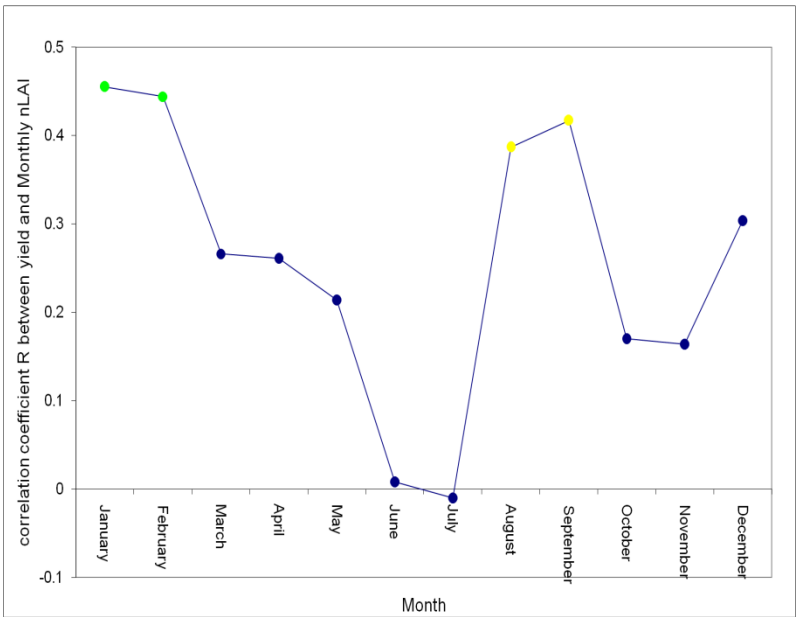

Figure 5. Yearly Evolution of R coefficient correlation between the yield and the average of the month (green pvalue <0.01, yellow pvalue<0.05).

CONCLUSIONS

High (HR) and moderate (MR) resolution NDVI were calibrated using field-truth and we derived HR-LAI maps and MR-LAI dynamics, for full sun coffee or for little shade agroforestry coffee

- Inter-annual variations of LAI are controlled by renovation cycles and fertilizers, which are influenced by coffee prices. Seasonal variations of coffee LAI are influenced by the climate and by the agricultural practices such as pruning
- Coffee LAI is a promising factor for modelling yield. Another output is to model environmental services of entire farms (Gómez-Delgado et al., HESS 2010), according to practices.

ACKNOWLEDGMENTS

This work was supported by the EU-project CAFNET (EuropAid/121998/C/G), Cirad, CATIE, PCP and by the Aquiares farm.

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Sustainable Coffee Production: an Analysis Model for Quality and Certification in the Coffee Agribusiness

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SUMMARY

The present study uses the methodology of the quality pillars proposed by Leme (2007) to analyze four certifications connected to a sustainable production in the coffee agribusiness: (1) the Coffee Quality Program (PQC), a certification established by the Brazilian Coffee Roasters Association (ABIC); (2) the "Café do Cerrado" certification, a geographical indication managed by the "Café do Cerrado" Grower's Federation; (3) the Rainforest Alliance certification; and (4) the Utz Certified certification. Countless labels appeared in recent years to show consumers that a specific agricultural product meets sustainable standards like food safety, social and environmental aspects and also quality standards. In the coffee agribusiness this is also a strong trend, and there are several certifications to attest both agricultural and industrial production. There are many types of labels: with an environmental approach, with socio-environmental appeal and also for quality management. Each certificate has specific objectives and is focused on different kinds of producers. Their benefits vary a lot, depending on the market conditions and certification's requirements. On the other hand, quality is a relative concept; it could be related to the product or to the production process. But what is clear is that the quality concept has to be connected with consumers' demands, in search to fully attend final clients' needs, interests and desires. The consumer needs to feel and desire this special quality issue to pay the premium price that a certificated coffee demands. Unifying both approaches, there are several connections between certification and quality concepts. It is impossible to achieve a certification without a quality control related to the product itself or within the production process. Thus, it is impossible to achieve a premium price if the consumer is not able to perceive that a certification has a worthy specialty value, most of the time, related to quality. The objective of this paper is to unify quality and certification concepts in the coffee agribusiness under the same theoretical model, in a way to allow coffee agribusiness agents to clearly see that to obtain success within a certification process, it has to be strongly tied with quality concepts, whether in the product, in the production process or related with consumers' quality perception. To establish this connection, we used the theoretical analysis model of the quality pillars: product quality, production process quality and the quality signal. As results, the Café do Cerrado and the PQC certifications showed a high quality control and have clear quality features to evaluate coffee. The Café do Cerrado certification and the Utz Certified showed great attention to the quality of the production process, although all certifications analyzed have clear procedures on this subject. On the last pillar, the quality signal, all certifications use public relations to communicate with direct clients and consumers, although the Rainforest Alliance shows more appeal to final coffee consumers. The quality pillars model showed to be very useful to systematize the objects of this study in relation to certification and quality aspects in the coffee agribusiness. For certifiers, the model is useful to analyze how their strategies meet

growers and roasters needs, because it clearly shows that it is not possible to talk about certification without thinking on consumers tastes and demands.

INTRODUCTION

The 21st century brought major changes to the world agribusiness. In a globalized world, consumers demand products that have food safety standards, traceability, certifications and quality.

To attend those demands, a wide number of certifications standards were created. Their objective is to provide consumers with products that have a more sustainable approach. Consumers worldwide, especially in developed countries demand sustainability, regarding environmental issues and social affairs. They also want to have quality in the way of food safety and gourmet products with high differentiation.

In the coffee agribusiness, this trend is strong and there are many certifications to attest not only agricultural practices, but also the roasting process. There are many options: with environmental approach like organic and bird-friendly; with socio-environmental standards like Rainforest Alliance and Utz Certified; with social focus like the Fairtrade; with product intrinsic quality standards like the Coffee Quality Program (PQC) and the Café do Cerrado geographical indication. Each certification works with different objectives and is indicated for different grower`s profiles. The final objective is to achieve consumers, trying to satisfy their demands.

The certification has to establish itself as a different product in the market, focusing on a specific niche that clearly identifies its label as a sign of quality. The added value and a premium price is the goal, since this premium price has to sustain all the supply-chain and the costs related with the certification scheme.

Although quality is identified as a must have item in a certification process, its concept is complex, since quality can be related to distinct process in the supply-chain. Quality can be related to the product itself or the way it was manufactured, obeying rules and previous standards established initially. It also can be related with luxury, special features and attributes, like rare and distinct flavors or materials used. Quality can also be related only with the production process, assuring that there was no failure in the system or that the process respected socio-environmental issues.

Finally, quality concept has to be strongly tied up with the consumers` perceptions. This implies that the consumers can clearly identify the attributes they are searching for, making possible to them to pay more for the perceived added value.

The concepts described above regarding certification and quality are clear: there are many connections between them. It is not possible to achieve a good certification process without a quality control in the product or in the production process. Thus, it is not possible to pay for the certification added value if the final consumer cannot identify that the product has a specific attribute that is worth paying for.

In order to comprehend better those connections, this paper proposes an analysis of four coffee certification schemes: the Coffee Quality Program, the Café do Cerrado certification, Rainforest Alliance and Utz Certified. The objective is to put together certification and quality

concepts under the same theoretical framework. To achieve this objective, a model proposed by Leme (2007), called the “quality pillars” will be used.

THEORETICAL FRAMEWORK AND METHODOLOGY

The Transaction Costs Economics (TCE) theory was systematized by Williamson (1985, 1991), but its origin dates back to 30’s when Coase (1937) showed a new concept - the transaction costs. The transaction cost is the cost of making the economic system work. These are costs associated with economic activities’ coordination, such as *ex ante* costs to acquire market information and to do a business deal, and *ex-post* costs, which are associated with monitoring and contracts execution enforcement (Azevedo, 1997; Farina, 1997, 2000).

According to TCE, contracts are drafted under two behavioral assumptions: people have bounded rationality and can act opportunistically. As transactions differ from each other, Williamson (1985, 1991) used objective and observable elements to characterize them: the transaction specific investments, transactions frequency and uncertainty. Under associating behavioral assumptions with those three elements that characterize transactions, it is possible to identify some transactions that may be more vulnerable to opportunistic actions by one or more parties involved and their respective costs to other parts. (Azevedo,1997).

The most efficient coordination structure for each type of transaction is the one able to minimize transaction costs, ranging from market structures and vertical structures, although hybrid forms are more common. An important point for this work is how economic actors will deal with information asymmetry that can lead to opportunistic actions in their business transactions of buying and selling inputs and products throughout supply chain.

In this context, standards and certification appear as important coordination tools in supply chain. They communicate information to customers and consumers in a consistent and reliable way, reducing transaction costs in buyer vs. seller’s relation since they eliminate and reduce quality uncertainty and create incentives for horizontal and vertical cooperation between firms (Farina, 2003; Machado, 2000; Nassar, 2003).

Quality is a relative concept. Slack et al. (1996, p. 552) proposes a definition that summarizes many quality approaches: “Quality is consistent conformance to customers' expectations”, the word conformance indicates that there is a need to meet a clear specification, ensuring that a product or service complies with specifications originally set. Consistent means that materials, facilities and processes have been designed and controlled to ensure that product or service meets specifications, using a set of measurable characteristics throughout time. Customer’s expectations recognize that a product or service must satisfy customers and that they might be influenced by product’s price (Slack et al., 1996).

The three CQP pillars are the model basis:

1. The first pillar is “product quality”;
2. The second pillar is “process quality”;
3. The third pillar is the “quality signal,”

The quality signal establishes a link between the three quality pillars.

Vertical coordination enters in the model reaffirming that certification through quality attributes requires higher integration between supply chain actors. The last part of the model

refers to the end consumer. Accordingly, a company seeks to meet consumer's quality expectations on coffee. The final link between this theoretical model and practice is that with TCE support, the program objective is reducing information asymmetry between two players: final coffee consumers and growers/roasters.

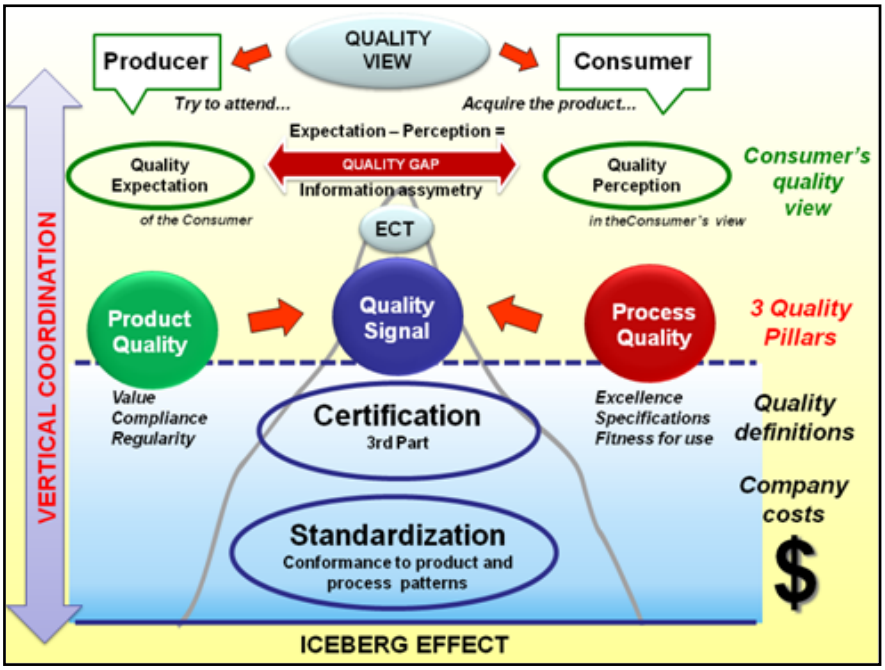

Figure 1. The three CQP quality pillars, the "iceberg effect" and the quality vision.

ANALYSIS AND RESULTS

The Coffee Quality Program (PQC) was created in 2004 by the Brazilian Coffee Roasters Association (ABIC). The PQC has the objective to establish measurable attributes to roast and ground coffee in Brazil. It created a new segmentation in the market, being the unique certification program in the world that separates coffee under 3 categories: gourmet, superior and traditional. The core of the certification is: product quality, assuring a minimal quality and global coffee attributes analysis; production process quality; and, coffee quality maintenance (Leme, 2007). The Café do Cerrado is the first geographical indication for the Brazilian coffee sector. The certification aims to add value to the coffee produced in the Cerrado region of the Minas Gerais state. The certification comprises among others: good agricultural practices, quality evaluation, traceability and standardization. The Rainforest Alliance certification is a socio-environmental certification. The Utz Certified certification is a well known socio-environmental certification, with a focus on management principles.

In order to analyze each certification, they were analyzed under the quality pillars model as follows. An "X" was marked when the characteristic is present and is important for the certification and "0" when the item is absent or not so important for the certification.

Under the product quality Pillar, the PQC and the Café do Cerrado are the only ones that have a clear evaluation of quality aspects. They also established clear quality segments. On the quality process pillar, the Café do Cerrado and Utz Certified are highlighted, since both associate control rules, management and yields improvement.

The quality signal pillar is the hardest one to be evaluated since in order to have a complete analysis, it was required a consumer survey that it was not done in this study. On the other hand, talking with market specialists, it is clear to see that all certifications studied use a strong public relations strategy.

This evaluation, although superficial, show some paths that could be taken by those certifications. The quality concept is highly related with intrinsic product aspects and sensorial attributes. Of course, a better control of the production process is responsible for a quality improvement, but the consumer does not know clearly what all those features mean.

Table 1. Using the quality pillars model to analyze the certifications.

			ABIC PQC	Rainforest Alliance	Café do Cerrado	Utz Certified
QUALITY PILLARS	**Product Quality Pillar**					
	1	Sensorial attributes	**X**	**0**	**X**	**0**
	2	Raw material quality	**X**	**0**	**0-X**	**0**
	3	Product quality control	**X**	**0**	**X**	**0**
	4	Quality segmentation	**X**	**0**	**X**	**0**
	5	Pattern maintenance	**X**	**0**	**0**	**0**
	Process Quality Pillar					
	1	Process control rules	**X**	**X**	**X**	**X**
	2	Traceability	**0**	**X**	**X**	**X**
	3	Manag./Economical gains	**X**	**0**	**X**	**X**
	4	Environmental gains	**0**	**X**	**X**	**X**
	5	Productivity gains	**0**	**0**	**X**	**X**
	Quality Signal Pillar					
	1	Consumer marketing	**0-X**	**0-X**	**0**	**0**
	2	Identification label	**X**	**X**	**X**	**X**
	3	Public relations	**X**	**X**	**X**	**X**
	4	Advertisement	**0**	**0-X**	**0**	**0**
	5	Consumers' recognition	**0**	**X**	**X**	**0**

So, in order to achieve success, certifications must understand consumers` needs and perceptions about quality. What is a sustainable coffee? Why choose a certified coffee? Will the consumers buy certified coffee, with a premium price without outstanding quality cup? These questions are very important, and in order to achieve a better balance between the 3 quality pillars, certifications will have to establish marketing strategies to educate and achieve consumers' minds.

Other important aspect that has to be mentioned is that one of the most important benefits of certifications is to give growers the opportunity to control their production and reduce production costs. All certification analyzed have this potential and this should also be marketed by certifiers as an important management tool.

Finally, this study clearly shows the strong relationship between certification and quality concepts. Both have to work together in order to give coffee growers better management and added value for their coffees.

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Economic Productivity of Coffee Intercropping Practices among Farmers in Kogi State

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SUMMARY

A diagnostic survey of intercropping practices with coffee in three major coffee producing local government areas of Kogi State in Nigeria was conducted. A total of 96 farmers were randomly selected and interviewed using structured questionnaire. Food crops such as Plantain, banana and yam were planted by 90, 81 and 72.8 percent of the farmers respectively. While 74, 70 and 68 percent of the farmers plant cocoa, kola and orange as tree crop intercrops. The analysis of regression equation revealed that intercropping Robusta coffee with food and tree crops were significant at 0.001 and 0.1 percent level of probability. Socio-economic variables such as age, family size and farming experience were not significant in determining the productivity of the intercropping practices. However, farm size is significant at $p < 0.5$ percent. Furthermore, 72.5 percent of the farmers perceived that the productivity of coffee is good with the intercrop and it does not reduce the coffee yield. It is concluded that intercropping of food and tree crops with Robusta coffee is important in this time of climate change for the provision of shade, food and increasing income of coffee farmers. Policy measures to remove constraints on land for the expansion of coffee farms should be put in place.

INTRODUCTION

In Nigeria coffee is a cash crop and a major foreign exchange earner. Both Arabica and Robusta Coffee are grown, however Robusta coffee is more than the Arabica coffee. Robusta coffee is mostly cultivated by about half a million farmers, 90 percent owned by smallholders. Robusta coffee is grown mostly on lowland areas below 1400 meters altitude while Arabica coffee is at higher altitude. Growing coffee forms the economic base for most of the small scale farmers in Kogi state of Nigeria. Intercropping of coffee with food crops is usually carried out to provide food and income for the farmers at the juvenile stage of the crop when its canopies have not completely closed and at old age when rehabilitation is embarked upon. Advantages of multiple cropping systems according to Okigbo and Greenland (1976) include higher yield returns, efficient use of available soil nutrients and better use of labour, weed control, insurance against complete crop failure through diversification and to increase the revenue base of the farmer. In some densely populated areas, banana-coffee intercropping is practiced, but it is not common. Some farmers, however, reported on the advantage of growing coffee under banana such as providing shade, mulch, nutrients and moisture. Some researchers also cite advantages such as reduced erosion in the highlands. In most intercropping, the crops complement one another in terms of socioeconomic benefits to growers and farm families. The intercrop such as banana provides permanent food and income security, doubling as a primary food and cash crop and providing a modest but continuous cash flow throughout the year. Coffee gives a cash boom during the year, helping

farmers acquire funds for more expensive items such as infrastructure, farm inputs, transport equipment and large social events. Coffee is often cultivated with inter-crops like pepper, cardamom, orange, Banana etc. In India it is an age old practice to train pepper vines on shade trees in coffee (Reddy and Rao, 1999). A long term study in India on coffee intercropping systems with bananas, orange and pepper indicates that the income realized from coffee alone was not significantly different from the intercropping systems (Korikanthimath, 1999) and the intercrop could also cushion farmers when coffee is not economical especially during drought years or when coffee price plummets. Nayer (1976) noted that, ginger, yam in young Robusta coffee was a source of higher return per unit area/time, food and employment.

However, the question still arises, how effective is intercropping in coffee farming especially among small holder farmers in Nigeria? Knowledge on cropping system in the tropics is required for various social, cultural, demographic, ecological and economical reasons. A diagnostic survey was therefore conducted in three local government areas of coffee growing areas of Kogi state in Nigeria. The specific objective is to examine the various crops in combination with coffee and farmers perception of the productivity and constraints on the practices.

METHODOLOGY

A multi-stage sampling technique was employed in randomly selecting 8 villages from 3 Robusta coffee growing local government areas of Kabba Bunu, Ijunmu and Yagba East in Kogi state of Nigeria. 96 respondents were interviewed with the aid of well-structured questionnaire. Analytical methods include frequencies, percentages and regression methods.

RESULTS AND DISCUSSIONS

The socio economic characteristics of the coffee farmers revealed that their age ranges between 30 and 60 with a mean value of 60.9, indicating that most of the farmers are old with about 34.4 years of farming experience on the average (Table 1).The farm size is small with a mean of 1.62 hectares and a mean household size of 8.03 of which about 7 of them are assisting on the farm. This indicates the subsistence nature of the farm. Despite the small nature of holding, it is providing work and food for most of the farmer's household.

Table 1. Socio economic characteristics of the farmers.

Variables	Min	Max	Mean	Std Deviation
Age	30	85	60.90	12.23
Household size	2	27	8.03	3.81
No on farm	1	8	7.12	10.88
Yrs of Farming	5	84	34.43	14.73
Farm size (Ha)	0.4	6	1.62	6.59

Source: Survey 2009.

Food crops such as plantain, banana and yam were planted by 90, 81.1 and 72.8 percent of the farmers respectively. While 83.2, 78.7 and 79.1 percent of the farmers plant cocoa, kola and orange as tree crop intercrops (Table 2). Other food crops include Maize (59%), Cassava (51.8%), Okra (43.1%) and Cowpea (21.7%). As with other coffee growing areas plantain and

Banana are the major intercrops. Plantain is planted at the juvenile stage of the coffee plant to act as shade and source of income during the gestation period. However, Banana serves as food and income in a mature coffee farm. Yam, Maize, Cassava and Okra are planted for mostly for the provision of food for the farmers household.

Table 2. Types of Intercrops with Coffee.

Food crop type	**No of farmers**	**Percentage**
Plantain	81	90.0
Banana	73	81.1
Maize	51	59.3
Yam	67	72.8
Cassava	44	51.8
Cowpea	18	21.7
Okra	31	43.1
Tree crops		
Cocoa	74	83.2
Palm tree	62	71.3
Kola	70	78.7
Cashew	32	45.1
Orange	68	79.1
Timber	46	57.5

Source: Survey 2009.

Table 3 shows that despite the fact that Kola and palm trees are planted by majority of the farmers their composition is low, 17.5 % and 11.4% respectively. Intercrops with Orange and Cocoa had 40% and 33.8% respectively in the overall plant population of the farm. This is a reflection of the crops traditionally grown in the area.

Table 3. Percentage composition of tree crop in coffee intercrop.

Tree crop	**Mean**	**Std Dev**.
%cocoa	33.8	24.9
%Palm	11.4	12.1
%Kola	17.9	17.5
%Cashew	24.5	24.5
%Orange	40.0	7.9

Source: Survey 2009.

Farmers perceived that intercropping in coffee is mostly to provide food (88.17%) and income (86.02%). While 69.89 % perceived the intercrop will act as shade. In this time of climate

change shade provision for the establishment of coffee planting is important. 47.31 percent believed that it will provide employment for them. Intercropping with high valued tree crops, such as cocoa, palm trees, kola, cashew, orange and timber are planted for diversification and income security in the face of falling prices and low productivity of coffee. Furthermore, 72.5 percent of the farmers perceived that the productivity of coffee is good with the intercrop and it does not reduce the coffee yield.

Table 4. Benefits and perception of the performance of intercrops in coffee.

Types of Benefits	**No of Farmers**	**Percentage**
Income	80	86.02
Food	82	88.17
Shade	65	69.89
Employment	44	47.31
Others	1	1.08
Perception of Performance of Intercrops		
Good	66	72.53
Fair	17	18.68
Poor	8	8.79

Source: Survey 2009.

The analysis of regression equation in table 5 revealed that intercropping Robusta coffee with food and tree crops were significant at 1 and 10 percent level of probability respectively. Socio-economic variables such as age and household size were not significant in determining the productivity of the intercropping practices. However, farm size is significant at $p < 0.5$ percent. This shows that intercropping will be enhanced with higher farm size Planting Food crops and tree crops will increase the overall productivity of the coffee farm. However, there is the need to look at the effect of the different crops on the overall income of the farm.

Table 5. Regression result of some socio-economic variables and types of intercrop on coffee productivity.

Variable	**Parameter estimates**	**Standard error**	**F- values**	**Probability**
Intercept	7321866	2439463	9.01	0.0066
Age	-190359	150204	1.61	0.2183
Household size	-601968	440985	1.86	0.1860
Farm size	144915	69515	4.35	0.0489**
Arable crops	-902694	151541	35.45	<.0001***
Tree crops	503722	252419	3.98	0.0585*

Note: **, **, *** Significant at 10%, 5% and 1% respectively.*

91% and 79% of the farmers believed that the major production constraints are market price and market information respectively, while the least important constraint is seedling.(Table

6). According to the International Coffee Organization (ICO), in almost all coffee-producing countries, because of poor coffee prices, producers are unable to cover production costs and have led to serious social and economic problems, including increased poverty, indebtedness and abandonment of coffee farms. Other production constraints include lack of finance (75.56%) disease problem (69.62%) and pest problem (68.60%). The crisis facing the coffee industry has been epitomised by massive over production, collapsing prices, deteriorating coffee quality, disease and above all the growing inequality in the coffee value-chain. At the farmer's level price volatility has affected livelihoods of the farmers to the extent that they no longer have reliable income and purchasing power to sustain their livelihoods. The overall negative effect is food insecurity leading to economic and social disorder in the various coffee households.

Table 6. Farmer's perception of production constraints.

Constraints	No constraint	Minor constraint	Major constraint
Market Information	6.98	13.95	79.07
Market Price	5.62	3.37	91.01
Disease Problem	12.66	17.72	69.62
Pest Problem	12.79	18.60	68.60
Lack of seedlings	40.00	27.06	32.94
Lack of Finance	10.00	14.44	75.56
Low yield	14.12	18.82	67.06
Lack of Chemicals	17.86	7.14	75.00

Source: Survey 2009.

CONCLUSION

Intercropping of food and tree crops with Robusta coffee is important in increasing the productivity of coffee especially in this time of climate change for the provision of shade, food and increasing income of coffee farmers. Policy measures to remove constraints on land for the expansion of coffee farms should be put in place. Diversification effort is not a matter of switching individual farmers or farmer groups from one crop to another. Rather, it involves introducing new "high-value" enterprises or helping existing farming system to work towards alternative sources of employment and income.

ACKNOWLEDGEMENT

The authors are grateful to the Director/Chief Executive of the Cocoa Research Institute of Nigeria for the permission granted to publish this work.

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Identification of Soil Organic Nitrogen Fraction as an Indicator of Coffee Plant Response to Nitrogen Fertilizer

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SUMMARY

Application of nitrogen (N) fertilizer by coffee farmers tends to be a must without considering the level of need for this nutrient which causes many of coffee farms are already over-fertilized with urea. Efficiency approach in utilizing N fertilizer, such as determination of fertilizer recommendation, should be done more accurately. Most of coffee fertilizer recommendations are based on of soil inorganic nitrogen content which is more labile compared to organic nitrogen. The objective of this study is to identify a fraction of soil organic N which is very closely related with degree of coffee response to N fertilizer. Hydrolyses were performed on soil samples derived from 30 sites of coffee plantations distributed in Banyuwangi, Bondowoso, Jember, Malang districts, East Java province, Indonesia. Analysis of organic N fractions consisted of total hydrolysable N, ammonium N, amino sugar N, amino acid N and combinations of those fractions. To investigate the level of coffee plants response to N fertilizer, seedlings of coffee were planted in plastic pot treated with and without urea as source of N. Degree of response of coffee plants to N fertilizer was measured based on several growth parameters. Results of this study showed that highest response of coffee was shown by dry weight of leaf, whereas the smallest response was shown by root dry weight. From those of organic N fractions analyzed, hydrolysable ammonium-N showed very significant correlations with leaf number, fresh and dry weights of leaf, fresh and dry weight of stem. The soil organic N fraction which had very significant relation with coffee plant response was hydrolysable ammonium-N and then used as an indicator of coffee plant response to N fertilizer. Using the method of Cate-Nelson, it was revealed that coffee gardens contain hydrolysable ammonium-N less than 535 mg/kg were classified as responsive sites to N fertilizer.

INTRODUCTION

To produce agricultural products, farmers including coffee farmers in the last half century are very dependent on fertilizers, particularly on urea as a main source of nitrogen (N) which is one of the consequences of green revolution movement. Compared to other nutrients needed by coffee plants, N is taken up by coffee plants in larger amount and more essential due to its role the structure and metabolic processes of this plant. Application of urea fertilizer by coffee farmers tends to be a must without considering the level of need for this nutrient which causes many of coffee farms are already over-fertilized with urea. In contrary, availability and price of N-urea fertilizer become the problem for carrying out good agricultural practices in coffee husbandry. Therefore, efficiency approach in utilizing N fertilizer, such as determination of fertilizer recommendation, should be done more accurately. Most of coffee fertilizer recommendations are based on of soil inorganic nitrogen content which is more labile compared to organic nitrogen. An indicator needed for estimating the presence of response of

Arabica coffee (*Coffea arabica*) trees to nitrogen (N) fertilizer has been well understood, however there is still little progress on the work on identification of organic N fraction which regulates the response of coffee to N fertilizer. The objective of this study is to identify a fraction of soil organic N which is very closely related with degree of coffee response to N fertilizer.

MATERIALS AND METHOD

Soil samples from 30 coffee farms distributed in Jember, Bondowoso, Malang and Banyuwangi Districts with various levels of fertility were taken for analysis after air-drying and passing 2 and 0.15 mm sieves. The first size was used for green house study, while the second one for preparing hydrolysates in laboratory study.

Coffee Response to Urea

Each soil sample in amount of 3 kg was placed in two plastic pots each, respectively, with and without addition of urea as source of N. In total there were 46 treatments which were replicated three times. There were three coffee plants in every replication. Seedlings of Arabica coffee var. Andungsari 1 was used as planting materials. All plants were fertilized, except N, as recommended in good agricultural practices, and soil water content was maintained at field capacity. Several plant growth parameters were monthly observed during the first 8 months. Other plant growth parameters were observed at the end of the experiment. Degree of plant response was measured based on the percentage of change of plant growth response after applied with N fertilizer over control.

Analysis of Soil Organic N Fractions

In this experiment, soil organic N forms analyzed were total hydrolysable-N, ammonium-N, amino-sugar-N, and amino-acid-N beside total-N. A method of Mulvaney & Khan (2002) was used to prepare soil hydrolysates and to analyze the soil organic N fractions. The amount of N present was analyzed based on distillation and titration techniques.

Identification of N Response Indicator

To determine the indicator of N response of coffee plants, information related with the amount and type of soil organic N fractions together with the response of coffee plants to urea fertilization in the various soils were collected. Simple linear correlations between every soil organic N fraction and N response in those soil samples were set up. A certain soil organic N fraction which has very high correlation with plant response to N fertilizer is classified as a good indicator of response of coffee plants to N fertilizer.

RESULTS AND DISCUSSION

Among the plant growth parameters observed, leaf dry weight and shoot dry weight had a wider variation in their response to N fertilizer, while stem diameter had the narrowest. Results of this study showed that response of plant growth parameters to N application varied from 20-73%, suggest that N fertilizing is not always followed by positive response by the plants. Figure 1 show that positive response of coffee plants to N fertilizer based on leaf dry weight only found in 63% of the farms tested.

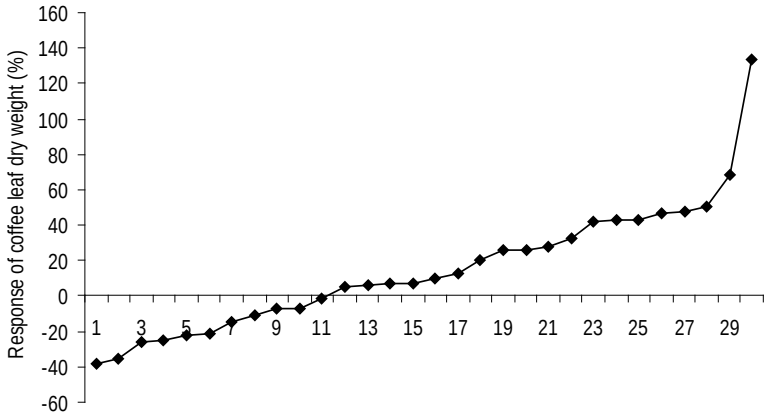

Figure 1. Variation of coffee plant response to N fertilization in term of coffee leaf dry weight in 30 coffee farms.

Table 1. Correlation coefficients between growth parameters and forms of soil organic N.

	N in forms of					
Growth parameter	**Total**	**Total-hydrolizable**	**Ammonium+ amino sugar**	**Ammonium**	**Amino sugar**	**Amino acid**
	R^2 value and significance					
Plant height	0.019 ns	0.004 ns	0.028 ns	0.073 ns	0.019 ns	0.000 ns
Stem diameter	0.156*	0.141*	0.152*	0.151*	0.011 ns	0.045 ns
Leaf number	0.296**	0.256**	0.317**	0.344**	0.011 ns	0.149*
Leaf fresh weight	0.212*	0.197*	0.241**	0.252**	0.011 ns	0.102 ns
Leaf dry weight	0.237**	0.218**	0.261**	0.270**	0.014 ns	0.105 ns
Stem fresh weight	0.151*	0.139*	0.180*	0.176*	0.014 ns	0.106 ns
Stem dry weight	0.064 ns	0.066 ns	0.095 ns	0.178*	0.016 ns	0.009 ns
Root fresh weight	0.131*	0.114 ns	0.138*	0.106 ns	0.032 ns	0.028 ns
Root dry weight	0.061 ns	0.063 ns	0.076 ns	0.093 ns	0.000 ns	0.018 ns
Shoot fresh weight	0.205*	0.190*	0.234**	0.243**	0.012 ns	0.106 ns
Shoot dry weight	0.201*	0.188*	0.233**	0.269**	0.040 ns	0.079 ns

There was a wide variation in correlation coefficients between various forms of soil organic N and plant growth parameters. Plant height and root dry weight did not show any relationship with several fractions of soil organic N measured. While stem dry weight and root fresh weight only showed one or two significant relationship. Leaf number and leaf dry weight showed highly significant relationship with most of the soil organic N fractions except amino

sugar and amino acid. Amino-sugar-N did not show any correlation with any of plant growth parameters. Amino acid-N also showed the same situation, except for leaf number.

As mentioned above that leaf number and leaf dry weight had higher values of coefficient correlation with most of soil organic N fraction, while leaf dry weight has a wider range of responses to N fertilizer, therefore correlation graph based on leaf dry weight was used to determine critical value of plant response using Cate-Nelson method (Figure 2). It was found that concentration of soil ammonium-N at 535 mg/kg is a critical point which means that soil with soil ammonium-N more than that value classified as non-responding site to N application.

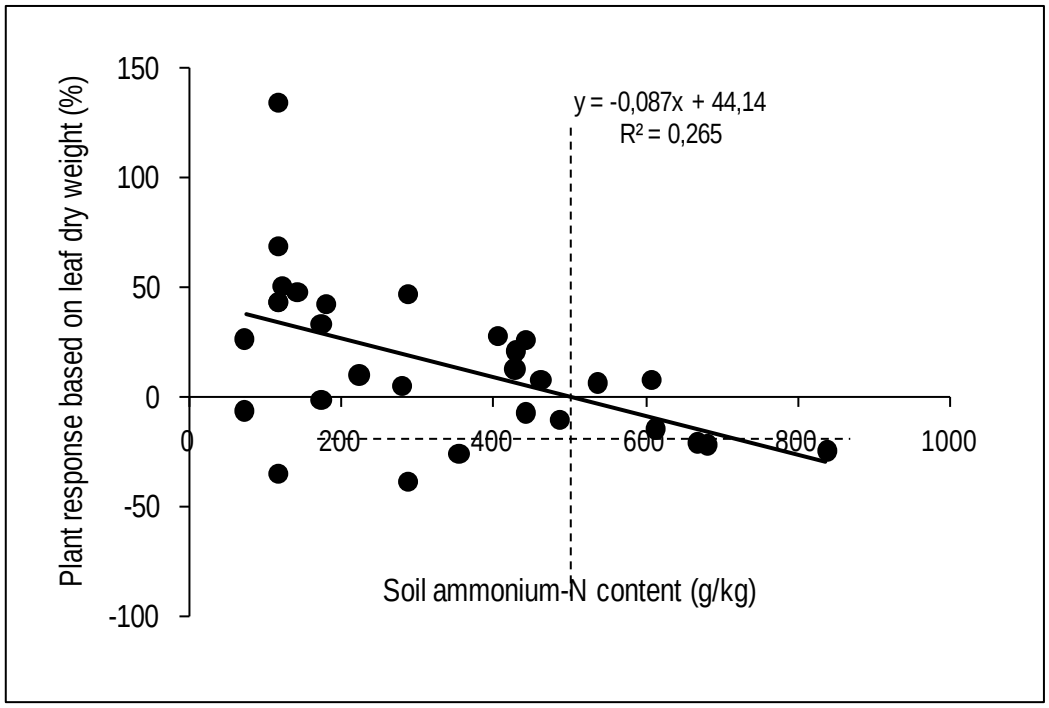

Figure 2. Relationship between soil ammonium-N content (mg/kg) and coffee plant response based on leaf dry weight variable.

CONCLUSION

1. Leaf dry weight and leaf number as variables of plant growth had higher and wider response to N application compared to other variables.
2. Ammonium-N is significant-negatively correlated with leaf dry weight and leaf number.
3. Soil ammonium-N content can be used as an indicator of the response of coffee plants to N fertilizer.
4. Critical value of soil ammonium-N content is 535 mg/kg.

ACKNOWLEDGEMENT

This study was funded by Ministry of Research and Technology through Applied Research Incentive Program Year 2008.

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Volatilization and Efficiency of Urea Fertilization and Growth of Arabica Coffee Seedlings Applied with Filter Press Cake

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SUMMARY

Most of coffee planters use urea fertilizer as the source of N for their crops, nonetheless in soil urea pass through several processes which result in volatilization and cause the inefficiency of fertilization of N. It has been reported that filter press cake disposed from cane sugar factory using sulphitation process may contain sulphonate substance which may inhibit volatilization of urea. The aim of this research is to investigate the influence of filter press cake resulted from sulphitation process on urea fertilizer fertilization, the efficiency of urea fertilization on Arabica coffee growth by supplying filter press cake as well as analyzing the influence of filter press cake and urea application on the growth of Arabica coffee plants. First experiment was undertaken in laboratory to study the effect of filter press cake in hampering volatilization of urea using five levels of period of decomposition of filter press cake, five levels of composition of filter press cake in soil media and four levels of dosage of urea. Ammonium released from the urea was trapped by H_3BO_3 and quantified using $KH(IO_3)_2$. Second experiment was conducted in glass house to study the effect of interaction between five levels of filter press cake and three levels of urea. The research findings showed that filter press cake application could hamper urea release by volatilization process where the longer decomposition period of filter press cake, the higher the volatilization. The increase of the filter press cake dosage resulted in reduction of volatilization, while the high dosage of urea increased the amount of volatilized urea. There was significant difference among treatment combinations between filter press cake and urea fertilizer on the efficiency of urea fertilization. Treatment combinations of application of filter press cake and urea fertilizer provided significantly different influence on the growth of coffee seedlings, where supplying filter press cake by 2.5% of the media could increase growth of coffee seedlings.

INTRODUCTION

Nitrogen (N) plays an important role in determining growth and production of Arabica coffee because most of coffee farm soils contain less available N than required by the plants. One of N containing fertilizers mostly used in coffee farms is urea which contains high concentration of N, non polar and very soluble. However, only around half of the applied N can be absorbed by plants while the rest loss from the soil due to leaching, volatilization and denitrification processes (Katyal and Carter, 1989). Application of urea on soil maybe loss up to 70% by volatilization process which result in low efficiency of urea utilization (Crasswell and Godwin, 1984) and gives no benefit to coffee farmers. Considering that high energy input involving in urea manufacturing and its price getting more expensive needed, the loss of urea should be minimized. Many efforts have been carried out to increase the efficiency of N fertilizers, such as the use of lignosulphonate to reduce the loss of ammonia volatilization (Al-Kanani et al., 1994). Lignosulphonate like substance is predicted present in filter-press cake

(FPC), one of wastes of cane-sugar factory which use sulphite in its refining process. FPC used as source of organic matter for coffee and cocoa plantations increased coffee and cocoa yield by improving physical and chemical soil fertility (Baon and Soenaryo, 1986). The objectives of this study were to investigate the effect of filter-press cake from sugar factory which use sulphitation process on i) ammonia volatilization, ii) urea fertilizer efficiency when applied for Arabica coffee seedlings, and iii) interaction between filter-press cake and urea application on Arabica coffee growth.

Materials and methods

A low gleyhumic soil with clay loam in texture was used in this study and the chemical composition was 0.96% organic C, 0.10% N, 27.6 cmol/kg CEC, 89 mg P_2O_5 (25% HCl)/100 g, and pH 6.8. Filter-press cake was obtained from Semboro Cane-sugar Factory, Jember. Before being mixed with soil, the filter-press cake was composted for 2, 3, 4 and 5 months.

Laboratory Study

This experiment was laid out in randomized completely block design in factorial. First factor was period of composting (fresh, 2, 3, 4 and 5 months) combined with second factor dosage of filter-press cake (0, 2.5, 5,0, 7.5 and 10%) and combined with dosage of urea applied (0, 1, 2 and 3%). Each combination of treatments was replicated three times. Each Erlenmeyer flask was filled with 50 g air dried soil and added with filter-press cake and urea as described in the treatments. Soil water content was kept in field capacity. The Erlenmeyer was tightly close and connected with a plastic pipe at the end of which solution of H_3BO_3 10% added with methyl red was placed to catch volatilized NH_3 from the flask. The amount of N released was quantitatively measured using 0.01N $KH(IO_3)_2$ standard by titration.

Glasshouse Study

Filter-press cake used for this study was composted for 6 weeks before being mixed with soil. Chemical composition of composted filter-press cake was 17.4% organic C, 1.27% N, 1630 mg/100 g P_2O_5 (25%HCl), and pH 7.7. Seeds of Arabica coffee var. Kartika 1 were used to produce seedlings as planting materials for this study. This experiment was laid out in randomized completely block design in factorial. Plant growth medium consisted of 2.5 kg of air dried soil placed in plastic bag. First factor was dosage of filter-press cake (0, 2.5, 5.0, 7.5 and 10%) and combined with dosage of urea applied (0, 0.5, 1.0 and 1.5 g per plant per month). Each combination of treatments was replicated three times. Each replication consisted of four plastic bags. After homogenously mixing of soil and filter-press cake, the 8-weeks old Arabica coffee seedlings were planted in each plastic bags. Soil water content was maintained at field capacity by adding water daily. No drainage hole was made to prevent leaching of urea.

RESULTS AND DISCUSSION

FPC and Ammonia Volatilization

There was no significant effect of period of composting and dosage of FPC on ammonia volatilization when the amount of urea applied was 0 and 0.5 g per plastic bag. Therefore, only the results of 1.0 and 1.5 g urea applied are presented and discussed (Table 1). Based on polynomial orthogonal analysis, there is an interaction between the period of FPC composting and dosage of FPC and urea applied. High amount of volatilized ammonia trapped was found

in the treatment no FPC applied combined with high dosage of urea applied (1.5 g/plant/month). Meanwhile, lowest amount of ammonia volatilized was found in highest dosage of fresh FPC applied combined with 1.0 g urea/plant. Both dosages of urea applied 1.0 and 1.5 g/plant resulted in low amount of ammonia volatilized when higher dosage of FPC applied. There was a significant effect of composting of FPC on ammonia volatilized, where higher ammonia volatilized found in fresh applied FPC compared to those applied with composted FPC. Nonetheless there was no difference in ammonia volatilized among FPC composting for period of 2, 3, 4 and 5 months.

Table 1. The effect of period of FPC composting, FPC and urea dosages applied on ammonia volatilization (mg N).

Composting period (month)	Dose of FPC (g)				
	0	**2.5**	**5.0**	**7.5**	**10.0**
Urea 1.0 g					
0	1,78 aB	1,52 aB	1,49 aB	1,35 aAB	1,11 aA
2	3,10 cD	2,52 cC	1,82 abB	1,37 aA	1,23 aA
3	2,43 bC	2,22 cBC	1,90 bB	1,76 bAB	1,40 aA
4	2,08 abAB	2,29 bcB	1,89 bA	1,79 bA	1,74 aA
5	2,21 bB	2,04 bAB	2,23 bB	1,84 bAB	1,72 aA
Urea 1.5 g					
0	4,50 aC	4,47 aC	3,49 aB	3,21 aAB	2,84 aA
2	6,39 cD	5,71 cC	5,17 cB	5,13 dB	4,49 dA
3	5,35 bD	4,59 abC	4,45 bC	3,70 bB	3,31 bA
4	5,35 bC	4,94 bC	4,99 cBC	4,64 cAB	4,37 cA
5	5,33 bC	4,62 abB	4,58 bC	4,52 cB	4,09 cA

Notes: Figures in the same column followed by the same small letters or figures in the same or followed by the same capital letters are not significantly different according to HSD test at 95%.

Urea Use Efficiency

Application of 0.5 g urea/plant/month resulted in higher urea use efficiency compare to higher dosages of urea. However, there was no difference in that efficiency between urea applied 1.0 and 1.5 g/plant/month. For both concentrations, there was no effect of FPC applied on urea use efficiency. On the other hand, higher the amount of FPC applied reduced the urea use efficiency when 0.5 g urea/plant/month applied to soil.

FPC and Plant Growth

Results of this study showed that most of the plant growth parameters had negative correlation with urea applied, except for leaf number and stem dry weight (Table 2). The negative correlations may be due to high concentration of urea around rhizosphere which cause negative effects to coffee plant growth, especially when combined with application of

FPC. On the other hand, correlations between coffee plant growth parameters and FPC applied in general were negatively quadratic. In these relations, most of the plant growth parameters showed better growth when applied with 2.5% FPC. Although, as source of organic matter, FPC with high content of organic carbon and P which may improve plant growth, when applied in larger amount it may cause detrimental effect to plant growth.

Table 2. Matrix of correlation between plant growth parameters and FPC and urea application.

Growth parameter	Urea	FPC
Leaf number	ns	** (negative quadratic)
Plant height	** (negative linear)	** (negative quadratic)
Stem diameter	** (negative linear)	** (negative quadratic)
Leaf fresh weight	** (negative linear)	** (negative quadratic)
Leaf dry weight	** (negative linear)	** (negative quadratic)
Stem fresh weight	** (negative linear)	** (negative quadratic)
Stem dry weight	** (positive quadratic)	** (negative quadratic)
Root fresh weight	** (negative linear)	** (negative linear)
Root dry weight	** (negative linear)	** (negative quadratic)
Shoot fresh weight	** (negative linear)	** (negative quadratic)
Shoot dry weight	** (negative linear)	** (negative quadratic)
Plant fresh weight	** (negative linear)	** (negative quadratic)
Plant dry weight	** (negative linear)	** (negative quadratic)

*Notes: ns = not significant, * = significant, ** = highly significant*

CONCLUSION

1. Application of FPC reduces ammonia volatilization from urea applied to soil. Higher amount of FPC applied, lower the amount of volatilized ammonia. Higher the amount of urea applied, higher the amount of ammonia volatilized.
2. Higher ammonia volatilized found in composted FPC applied to soil compared to those applied with fresh FPC.
3. Most of the plant growth parameters had negative correlation with urea applied, but had negative quadratic correlations with FPC applied. Better plant growth when applied with 2.5% FPC.
4. Application of 0.5 g urea/plant/month resulted in higher urea use efficiency compare to higher dosages of urea.

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Use of Sub-Surface Soil Water by Wick of Organic Matter to Mitigate Water Stress of Robusta Coffee

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SUMMARY

Coffee trees are susceptible to water stress which occurs during dry season. Inadequate water supply during that period would reduced production significantly. To mitigate negative impacts of water stress, it is justified to find technology for maximum utilization of in-situ available water resources. This research is an effort to optimize soil moisture reserve located in deeper soil layers to minimize yield loss of cofffee due to water stress during dry season. The experiment was carried out on Robusta coffee field of 10 years old located in Kaliwining Experimental Station in Jember District, East Java province, Indonesia. The experiment was conducted during four consecutive years since 2005-2009. The experiment site is a flat area of Inceptic hapludalf soil family. Wick system of organic matter was selected as a method to cope retardation problems of water capillary rise and downward growth of coffee roots. The experiment was set according to randomized completely block design with 4 replications to evaluate depth of organic matter and number of holes to apply the organic matter. Application depth of organic matter was given at 4 levels, namely: 0, 50, 100 and 150 cm, while number of holes was given at 3 levels, namely: 1, 2, and 3 holes/tree. Organic matter was applied in hole(s) of 10 cm diameters located beside coffee trunk. The holes was then filled with organic matter up to the soil surface. Reserch findings revealed that wick system of organic matter effectively mitigated negative effect of water stress on coffee. Wick system of organic matter at 50-150 cm deeps reduced leaf water deficit but increased soil water content during dry season, increased root density, new node formation and coffee production. Wick of organic matter up to 150 cm deep decreased leaf water deficit during dry season from 32% to 22% but increased soil water content of 0-20 cm soil deep from 24.2% to 28.9%, increased root density from 40.1 mg/g soil to 109.8 mg/g soil, increased formation of new nodes from 102.4 nodes/tree to 143.6 nodes/tree, increased number of coffee cheries from 658 cheries/tree to 1172 cheries/tree, and increased production of green coffee from 292 g/tree/year to 523 g/tree/year.

INTRODUCTION

During the last decade, it was noticed a trend of deminishing coffee yield of the existing coffee area in Indonesia. It was supposed due to degradation of environment qulities induced by over-exploitation. To achieve high yield, it had been applied for decades an intensive crop management by application of high external inputs, leading to greater nutrient losses. Application of high external inputs tended to reduce environment quality (Reijntjes et al., 1992).

Coffee trees are susceptible to water strees which occurs during prolong dry season. Coffee production is strongly influenced by actual rainfall one year or two before the crop year. Favorable rainfall warrant high yaield, while unfavorable water supply reduced yield.

Capability of coffee varieties or coffee clones to adapt severe water stess is the desirable characteristic for coffee breeding. Early observation indicated that there are relatively wide variation among coffee varieties and among clones for their tolerance to water streess (Abdoellah, 1983). Experiences indicated that during prolong dry season in 1982, coffee production in Java deminished up to 27% and caused 31,6% death of immature coffee population (Soerotani and Soenardjan, 1983). Watering during dry season increased yield if the quantity of water was at least 100 litre/tree (Pujiyanto and Zaenudin, 1995).

During dry season, soil water content in the uppermost layer droped up to permanent wilting point, meanwhile in the subsurface layer (more than 100 cm deep) was still saturated with water. At this condition, coffee trees in the field had showed wilting symtoms. Observation of coffee plantation in Kaliwining experimental station (Jember, East Java), indicated a retardation of capaliary water movement which caused wilting of coffee trees in the area (Pujiyanto, 1994). This research is an effort to optimize in-situ soil moisture reserve located in deeper soil layers to minimize yield loss of cofffee due to water stress during dry season.

MATERIALS AND METHODS

The experiment was carried out on Robusta coffee field of 10 years old located in Kaliwining Experimental Station in Jember, Indonesia. The experiment was conducted for four consecutive years since 2005 until 2009. The site was a flat area of Inceptic hapludalf soil family with iso-hypertermic soil temperature regime. Organic matter of cowdung was applied in wick system. The wicks was set up by boring the soil to a certain depth.

The experiment was set factorially according to randomized completely block design with 4 replications to evaluate application depth of organic matter and number of holes to apply the organic matter. Application depth of organic matter was evaluated at 4 levels, namely: 0, 50, 100 and 150 cm, while number of holes was given at 3 levels, namely: 1, 2, and 3 holes/tree. Organic matter was applied in hole(s) at 10 cm beside coffee trunk with diametre of 10 cm. The holes was then filled with organic matter up to the soil surface.

Variables of plant growths and soil characteristics were observed periodically, namely: leaf water content, number of new shoot, coffee production, root density, and soil water content. Relative water content was calculated based on the different between leaf water content at field condition and the water content at saturated condition. Root density was determined by weighing coffee roots in a certain volume of soil sample taken from of 0-10 cm 10-20 cm soil deep.

RESULTS AND DISCUSSION

Leaf water deficit

Based on observation during dry season for four consecutive years in the field, there was an indication of better water status in coffee leaves due to organic matter application. Leaf water deficit declined as the depth organic matter application increased. The deeper the organic matter application, the better their leaf water status. Average leaf water deficit at 0, 50, 100 and 150 cm depht of organic matter application were 32%, 27%, 25%, and 23% respectively (Figure 1). At application depth up to 150 cm, organic matter reduced leaf water deficit 28% compared to the control treatment. It was supposed due to better water absortion from the soil profile of the coffee trees. The applied organic matter increased soil water reserve, either directly and indirectly. Reserch findings proved that organic matter had very high water

holding capacity. The capacity of organic matter to retain water is up to 20 times their weight (Oades, 1983), while soil particle is much less. Secondly, organic matter application may improve soil aggregation, formation and stabilization of macro and meso soil pores (Pujiyanto et al., 2003) that enabled coffee to absorb more quantity of water.

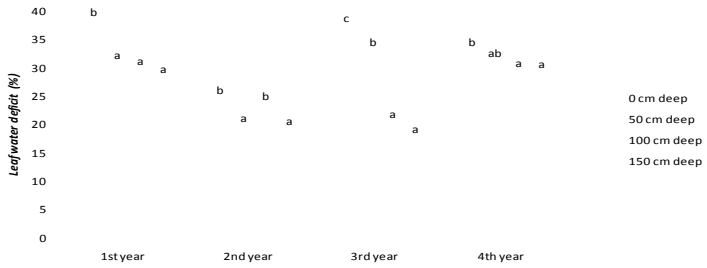

Figure 1. Relationship between depth of organic matter application and average of leaf water deficit of coffee during four consecutive dry seasons.

Soil water content

Figure 3 indicated that water content during dry season at the organic matter treated soil were higher than the control treatment with no organic matter. Average soil water content at 0, 50, 100 and 150 cm depht of organic matter application were 24.2%, 26.7%, 28.8%, and 28.9% respectively. Among the treated soil, there was no significant different among application depth of organic matter at 50, 100, and 150 cm. Relationship between soil water content during dry seasons and depth of organic matter application conformed to an equation of Y = 0.037 X + 24.19 (R^2=0.985**) (Figure 2).

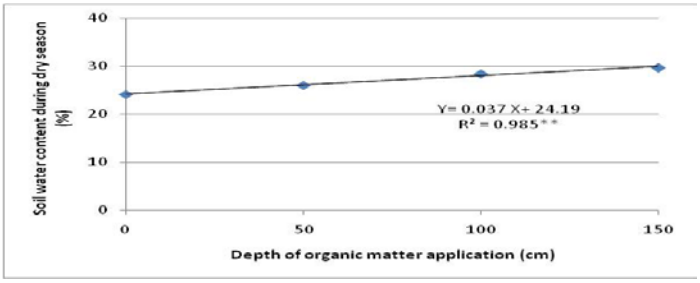

Figure 2. Relationship between depth of organic matter application and soil water content during four consecutive dry seasons.

Root density

Root density was calculated based on root-biomass determination in the soil samples. Observation during four cosecutive years indicated that applied organic matter in the wick increased root biomass around the application site. Average roots biomass for non-treated soil was 40.1 mg/g soil, while in the treated soil samples was 109.8 mg/g soil (Figure 3). Among the tree evaluated depth of organic matter application, the root biomass were camparable. The soil samples were taken from around the holes for organic matter application. Therefore, the composition of the soil samples were similar, giving rise to the similar effect on root growth. Beside induced fovorable soil condition and made available nutrients, organic matter application would increase phytohormones which was assumed produced by microorganisms (Pujiyanto et al., 2004).

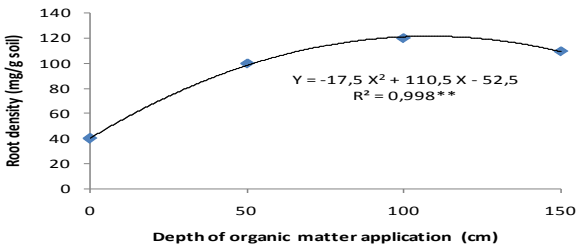

Figure 3. Relationship between depth of organic matter application and average root density of Robusta coffee.

Formation of New Nodes

Production of Robusta coffee is related directly with formation of new nodes at one year before the crop year. Figure 4 showed that the increased depth of organic matter application lineary correlated with number of new-formed nodes. The deeper the organic matter application, the more nodes will be formed. The new-formed nodes on treatment of organic matter wick up to 150 cm deep was 143.6 nodes/tree, while on the control treatment was only 102.4 nodes/tree. Wick of organic matter increased new-formed nodes of 40%. Higher new nodes formation was due to the better soil qualities, in term of nutrients and water supply, and growth condition. Growth acceleration of coffee trees that was indicated by higher number of new nodes was a resultant of improvement effect on soil-media; not only on their chemical properties but also on their physical and biological properties as well (Pujiyanto, 2007). Following decomposition of the organic matter, nutrients and organic acids will be released to soil solution. The nutrients will be available for plant, while the organic acids may favor better condition for coffee growth.

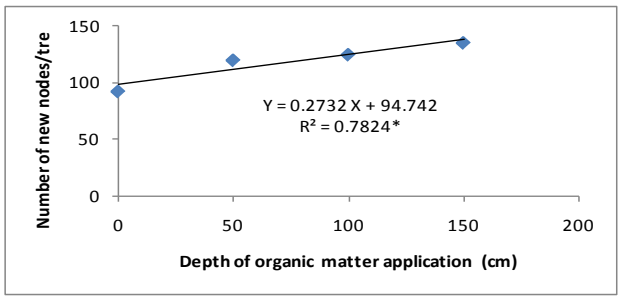

Figure 4. Relationship between depth of organic matter application and average number of new nodes during four consecutive dry seasons.

Number of cheries

The number of coffee cheries increased lineary as the increassed of wick depth of organic matter (Figure 5). It implied that the deeper the wick depth, the better the soil support for coffee. The applied organic matter in the wick would undergo decomposition gradually so that the volume would deminished. The former space for organic matter would be replaced gradually by nearby soil particles from the wick wall to form continuous capilers from the bottom of the wick up to soil surface. Induced by activity of roots and soil organisms, the mixture of organic matter and soil particles will formed stable aggregates that stabilized capillary network in the wick. Therefore, barrier for capillary rise of water from deeper soil layer would be deminished, leading to ease water supply for cofffee roots. Area within the

wick also have lower bulk density than that of bulk soil. Therefore, root penetration within the wick was easier which was indicated by higher root density than in the bulk soil.

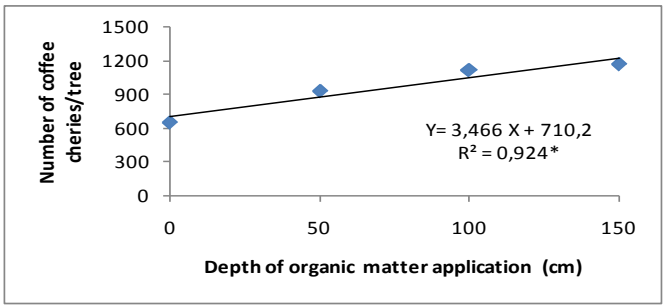

Figure 5. Relationship between depth of organic matter application and average number of coffee cherries during four consecutive crop years.

CONCLUSIONS

1. Wick system of organic matter effectively reduced negative effect of water stress on coffee.
2. Wick system of organic matter at 50-150 cm deeps reduced leaf water deficit, increased root proliferation, new node formation and coffee production. The best result was achieved at 150 cm deep of orgnic matter application.

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Arthropod Diversity on Indonesian Coffee Ecosystems and Its Relationship on Main Insect Pests Infestation

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SUMMARY

The structure and function of arthropods in coffee ecosystem are an important basic knowledge in the implementation of integrated pest management (IPM). Research on arthropod diversity in coffee ecosystems has been conducted on both Robusta and Arabica coffee in East Java and Lampung provinces. Coffee ecosystems without shade trees, with single shade trees, and with shade trees as well as intercropping with other cash crops have been observed on their arthropod diversity. In these ecosystems, arthropods fauna on the coffee trees have been observed using spraying with knock down effect of pesticide of deltamethrin and collected with plastic sheet put on the ground under coffee trees. Identification of arthropods fauna has been done until classification level of family. The results indicated that on Robusta coffee ecosystem, it was found 18-29 families of arthropod and the more abundant groups were family of Formicidae, arachnids (Arachnida), Blattidae, and the order of Orthopthera (Acrididae, Gryllidae, and Tettigoniidae). On Arabica coffee, it was found more abundant families than those on Robusta. There were 22-32 families of arthropod obtained and the abundant groups were Formicidae, arachnids (Arachnida), Blattidae, and several families that belong to the order of Orthoptera. There was a tendency that higher diversity on the coffee ecosystem (coffee planted with shade trees and intercrop with other cash crops) caused higher diversity of arthropod fauna. The more abundant arthropods on coffee ecosystem were the predaceous groups of arachnids, ants, and orthopteran as well as neutral groups from the order of Orthoptera. The phytophagous group known as coffee pests was rare. There was a tendency that the more diverse of coffee ecosystem, the more abundant of arthropod fauna and the less main insect pest's infestation. It is due to more abundant of natural enemies of the pests, especially entomopathogenic fungus groups.

INTRODUCTION

Coffee is the main estate commodity which gives earning of more than two million people in Indonesia. In the year of 2010, it was estimated that total areas of coffee plantation in Indonesia about 1.302 million hectares consisting of smallholders of 95.53%, government estate of 1.72% and private estate of 2.75%. Total production in the same year is estimated about 712,778 metric tons and the smallholders are still dominating with the portion of 95.72% (Anonym, 2010). The characteristics of smallholder coffee plantation in Indonesia is the framers plant the coffee mixed with other cash crops, like banana, black pepper, coconut, citrus, durian, cinnamon, taro, etc. They plant coffee under leucaena, casuarina, erythrina, and other forest trees as well as in some locations without shading trees especially in the higher mountainous areas.

In these several types of coffee habitat, it is interesting to investigate the relationship between arthropod diversity and coffee ecosystems, especially in connection with the infestation of main coffee pests.

MATERIAL AND METHODS

Observations were conducted in Sumberasin Experimental Garden (about 500 m asl), at Malang District, East Java for Robusta coffee and in Andungsari Experimental Garden (1250 m asl) at Bondowoso district, East Java, for Arabica coffee, both gardens were owned by Indonesian Coffee and Cocoa Research Institute (ICCRI). Three types of Robusta coffee habitats have been defined to observe the arthropod diversity, i.e; (1) productive Robusta coffee without shade trees with code of ER-0-SA, (2) productive robusta coffee with single shade using cassia (*Cassia spectabilis*) tree, with code of ER-1-SA, and (3) productive Robusta coffee with mixed shade trees consisting of *C. spectabilis*, leucaena (*Leucaena glauca*), coconut (*Cocos nucifera*) and black peper (*Piper nigrum*), with treatment code of ER-4-SA. Black pepper was planted and climbed on leucaena tree. For Arabica coffee, there also consist of three types of habitats, namely (1). Productive Arabica coffee of catimor variety without shade tree (EA-0-AS), (2). Productive Arabica coffee of catimor variety with single shade tree using leucaena of PG-79 cultivar (*Leucaena diversifolia)*, that is resistant to jumping lice (*Heteropsylla cubana*) (treatment code of EA-1-AS), (3). Arabica coffee of catimor variety with shade tree of leucaena as on (2) and intercropped with Lily flower (*Lilium longiflorum*) (EA-2-AS).

Five trees of coffee were chosen on each habitat for observation of arthropod structure and diversity. Arthropod fauna was killed using spraying of pyretroid insecticide (deltamethrin) and the dead arthropod will fall on the plastic sheet laid on the ground under coffee tree. Identification of collected arthropod using Triplehorn and Johnson (2005) until taxonomic level of family. Observation of main coffee pests were conducted against coffee berry borer (*Hypothenemus hampei*), mealybug (*Planococcus citri*), green and brown scales (*Coccus viridis* and *Saessetia coffeae*) and shoot hole borer (*Xylosandrus* spp.). For these insect pests, natural enemies have also been observed especially for their insect pathogens (mainly fungi), parasitoids and predators.

RESULT AND DISCUSSION

Arthropod fauna on coffee with more shade trees and mixed with other cash crops were more abundance compared with coffee habitat without shade tree (monoculture) or only with single shade tree (Table 1 and Table 2), both on Robusta and Arabica coffee habitats (ER-4-SA and EA-2-AS). On Robusta coffee, there was significantly different on number of arthropod specimen per coffee tree between observation in June and October, especially from the order of Hymenoptera which was dominated by ant (*Dolichoderus thoracicus)*. The highest population occurs on coffee habitat mixed with several shade trees and intercropped with other cash crops (ER-4-SA). The domination of ant species could be explained that observation in October was coincided with the end of dry season in which the populations of mealybug and greenscale usually increase.

Table 1. Arthropod fauna collected from several robusta coffee habitats at Sumber Asin Experimental Garden in Malang District, East Java.

Class	Order	No. Family	Av.No. of arthropod specimen/tree on observation in					
			June			October		
			ER-0-SA	ER-1-SA	ER-4-SA	ER-0-SA	ER-1-SA	ER-4-SA
Insecta	Orthoptera	4	10.2	23.2	21.2	8.4	9.2	13.0
	Blattaria	1	30.8	58.4	55.8	16.2	8.2	5.4
	Lepidoptera	8	1.0	0.8	0.8	4.0	2.6	3.6
	Hymenoptera	3	32.4	81.4	105.2	39.6	670.4	1231.6
	Coleoptera	7	0.6	0.0	0.4	1.4	1.0	1.2
	Homoptera	3	3.8	7.4	0.8	2.8	3.0	4.4
	Diptera	7	4.2	0.2	0.4	2.8	1.2	1.8
	Hemiptera	1	0.4	0.4	0.2	0.0	0.0	0.2
	Dermaptera	2	0.0	0.2	1.6	0.4	0.0	0.0
	Neuroptera	1	0.2	0.8	0.0	0.0	0.0	0.0
	Isoptera	1	0.0	0.4	0.0	0.0	0.0	0.0
Arachnida	Araneae	8	7.4	31.0	12.8	10.0	7.0	7.0
	Opiliones(SO)	1	0.0	0.4	3.0	1.4	0.2	0.2
Malacostraca	Isopoda	1	13.0	0.4	1.6	20.8	0.4	0.2
Total	**14**		**104.0**	**205.0**	**203.8**	**107.8**	**703.2**	**1268.6**
Total Family		**48**	**26.0**	**22.0**	**18.0**	**19.0**	**20.0**	**26.0**

Table 2. Arthropod fauna collected from several Arabica coffee habitats at Andungsari Experimental Garden in Bondowoso District, East Java.

Class	Order	No. Family	Av.No. of arthropods specimen/tree on observation in					
			June			October		
			EA-0-AS	EA-1-AS	EA-2-AS	EA-0-AS	EA-1-AS	EA-2-AS
Insecta	Orthoptera	4	0.6	10.8	5.4	1.0	3.4	0.8
	Blattaria	1	0.0	0.0	0.0	0.0	0.2	1.8
	Lepidoptera	7	1.4	1.6	0.6	1.6	1.4	2.4
	Hymenoptera	7	27.6	0.2	1.4	13.4	13.0	1.2
	Coleoptera	15	0.4	3.0	3.2	0.6	3.6	2.8
	Homoptera	5	1.0	8.0	0.6	0.2	0.8	0.8
	Diptera	14	1.4	2.0	3.6	0.6	0.6	2.8
	Hemiptera	6	0.2	1.6	0.0	0.4	1.4	1.0
	Dermaptera	1	0.0	0.2	0.0	0.0	0.0	0.4
	Neuroptera	3	0.0	0.8	0.0	2.0	0.0	0.4
	Trichoptera	1	0.0	0.0	0.0	0.0	0.0	0.2
	Microcoryphia	1	0.0	0.0	0.2	0.0	0.0	0.0
	Collembola	1	0.0	5.4	0.0	0.0	0.0	0.0
	Ephemeroptera	1	0.0	0.2	0.0	0.0	0.0	0.0
Arachnida	Araneae	8	8.6	23.8	5.2	2.0	10.6	3.4
	Palpatores(S-O)	1	0.8	5.0	0.6	0.0	2.4	3.2
Total specimen	**16**		**42.0**	**62.6**	**20.8**	**21.8**	**37.4**	**21.2**
Total Family		**76**	**22.0**	**31.0**	**30.0**	**23.0**	**28.0**	**32.0**

Table 3. Effect of coffee ecosystems on infestation and natural enemies of main coffee pests.

Coffee Pests	Infestation and natural enemies	Robusta						Arabica					
		June			October			June			October		
		ER-0-SA	ER-1-SA	ER-4-SA	ER-0-SA	ER-1-SA	ER-4-SA	EA-0-AS	EA-1-AS	EA-2-AS	EA-0-AS	EA-1-AS	EA-2-AS
Coffee Berry Borer (CBB, *Hypothenemus hampei)*	Infestation(%)	6.5	3.2	1.1	0	0	0	0	0	0	0	0	0
	Pathogen (%)	0	0.5	2.3	0	0	0	0	0	0	0	0	0
	Parasitoid	0	0	0	0	0	0	0	0	0	0	0	0
	Predator	0	0	0	0	0	0	0	0	0	0	0	0
Mealy bug (*Planococcus citri*)	Pop/brnch.	224.4	106.5	0	200.6	63.5	0.1	0	0	0.25	0	0.2	0.9
	Pathogen (%)	0	31.8	78.8	0	42.6	89.6	0	0	0	0	0	0
	Parasitoid	0	0	0	0	0	0	0	0	0	0	0	0
	Predator	3.1	0.6	0	0.3	0	0	0	0	0	0	0	0
Green scale (*Coccus viridis)*	Pop/brnch	42.1	16.0	3.7	40.8	17.5	1.7	3.8	0	2.4	0	0	0
	Pathogen (%)	1.8	16.3	46.1	30.6	54.2	88.9	42.8	0	84.1	0	0	0
	Parasitoid	0	0	0	0	0	0	0	0	0	0	0	0
	Predator	2.4	1.4	0	0	0	0	0	0	0	0	0	0
Shoot hole borer (*Xylosandrus* spp).	Infestation(%)	6.2	4.1	2.2	4.9	5.7	2.8	0	0	0	0	0	0
	Pathogen	0	0	0	0	0	0	0	0	0	0	0	0
	Parasitoid	0	0	0	0	0	0	0	0	0	0	0	0
	Predator	0	0	0	0	0	0	0	0	0	0	0	0

These insects are usually associated with several species of ant on coffee plantation. Philpott et al. (2006) found that ants and flying pollinators had important role on setting coffee fruits and their weight, especially on the habitat with high shade (high biodiversity). More investigation conducted by Philpott et al. (2008) in Sumatera, Indonesia, revealed that tree, ant, and bird richness was significantly greater in coffee farms planted under forest (Bukit Barisan Selatan National Park) than that in coffee farms outside forest. Effect of high diversity of coffee habitat on the infestation of coffee pests as indicated in Table 3. There was indication that the more diverse coffee habitat, the lighter infestation of several coffee pests. It was caused by more abundance of some natural enemies of the pests, especially in the case of insect pathogen from fungus groups, due to the condition of higher diverse of coffee habitat that had higher relative humidity which was suitable for fungus development.

CONCLUSION AND RECOMMENDATION

It could be concluded that more diverse of coffee ecosystems as shown on robusta coffee with high variable of shade trees and mixed with other cash crops (ER-4-SA) will be followed by more diverse of arthropod fauna. The main insect pests of coffee were also in low infestation due to abundance of insect pathogen that belongs to fungus group. This type of coffee ecosystem should be recommended to the farmers to manage main pest of coffee in sustainable coffee production.

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The Change of Climatology Components of Robusta Coffee Area and Their Impacts on Productivity: a Case Study in East Java, a Coffee Producing Area in Indonesia

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SUMMARY

A study on the change of climatology components of Robusta coffee area and their impacts on productivity have been conducted at Kaliwining Experimental Station, Indonesian Coffee and Cocoa Research Institute, at latitude of 08°15'29"S, 113°36'41"E and altitude of 45 m above sea level. Data used were climatology components, i.e. mean daily temperature, maximum daily temperature, minimum daily temperature, annual rainfall, dry month, wet month, relative humidity, wind speed, sunshine length, evaporation, as well as green bean productivity; respectively. Data were compiled during last 30 years, from 1980 to 2009. Each climatology component was then presented as time series and related to productivity by regression equations. The results showed that there were increases of mean daily temperature, maximum daily temperature, minimum daily temperature, and relative humidity of the observed location during last 30 years, those follow the regression equation of $y = -0.001x^2 + 0.093x + 25.60$ ($R^2 = 0.555$), $y = 0.002x^2 - 0.039x + 32.42$ ($R^2 = 0.558$), $y = -0.008x^2 + 0.419x + 16.04$ ($R^2 = 0.500$), and $y = 0.010x^2 - 0.054x + 82.90$ ($R^2 = 0.870$); respectively. Evaporation decreased by equation of $y = -0.004x^2 + 0.010x + 3.883$ ($R^2 = 0.701$), whereas wind speed followed a quadratic equation of $y = 0.014x^2 - 0.568x + 5.740$ ($R^2 = 0.568$). Productivity of Robusta coffee was dropped by the increase of mean daily temperature, with the equation of $y = 2E + 16e^{-1.18x}$ ($R^2 = 0.771$); but it increased in line with relative humidity according to the equation of $y = 1E\text{-}32x^{17.60}$ ($R^2 = 0.591$). Annual rainfall, dry month, wet month, sunshine length, and evaporation significantly affected coffee productivity by quadratic equation of $y = 0.000x^2 - 3.627x + 3684$ ($R^2 = 0.658$), $y = -102.5x^2 + 895.1x - 1579$ ($R^2 = 0.786$), $y = -143.6x^2 + 1819x - 5431$ ($R^2 = 0.544$), $y = -3.260x^2 + 426.8x - 13608$ ($R^2 = 0.656$), and $y = -5527x^2 + 3949x - 70197$ ($R^2 = 0.853$); respectively. Efforts to reduce the effects of climate change, such as agroforestry, especially on microclimate and coffee production are discussed.

INTRODUCTION

At the last decade, issue of global climate change has been a main topic of discussion of scientists all over the world. Those issue covers increase of air temperature that affect rainfall pattern, occurrence of drought (El Nino phenomenon) and heavy rainfall (La Nina phenomenon); respectively. Those phenomena were predicted will impact on yield of coffee.

As a second highest producer of Robusta coffee in the world, Indonesia paid more interest on those phenomena. Moreover, Robusta coffee is more sensitive to the climate components than Arabica one; because it has a cross pollination character and its growing altitude on lowland rather than Arabica one that has self pollination and highland growing altitude.

To know the detail impact of some climate components on yield of Robusta coffee, a study was done in East Java, Indonesia; using 30 years of data climate components and productivity. The result of that study was presented on this paper.

MATERIALS AND METHOD

This study was conducted at Kaliwining Experimental Station, Indonesian Coffee and Cocoa Research Institute, at latitude of 08°15'29"S, 113°36'41"E and altitude of 45 m above sea level. Data used were climatology components, i.e. mean daily temperature, maximum daily temperature, minimum daily temperature, annual rainfall, dry month, wet month, relative humidity, wind speed, sunshine length, evaporation, as well as green bean productivity; respectively. Data were compiled during last 30 years, from 1980 to 2009. Each climatology component was then presented as time series and related to productivity by regression equations.

RESULTS AND DISCUSSION

The results showed that there were increases of mean daily temperature, maximum daily temperature, minimum daily temperature, and relative humidity of the observed location during last 30 years.

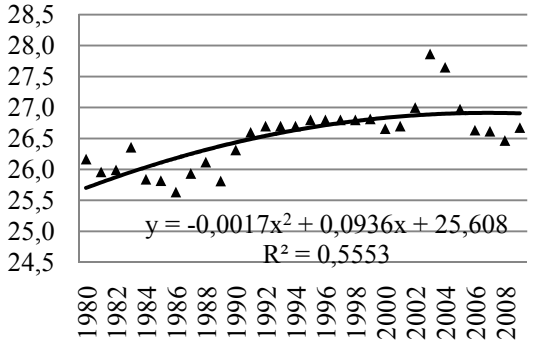

Figure 1. Mean daily temperature (°C) of the observed location during last 30 years.

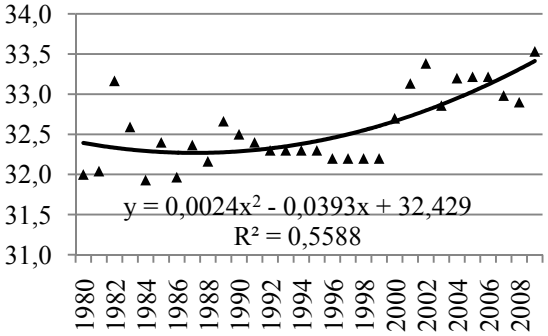

Figure 2. Maximum daily temperature (°C) of the observed location during last 30 years.

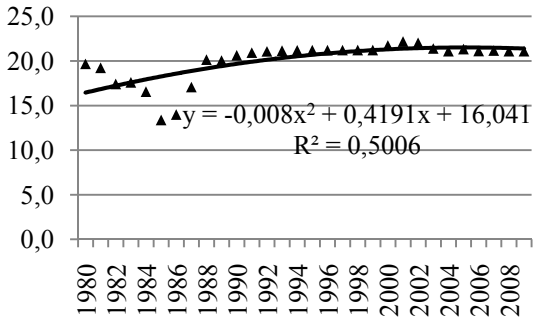

Figure 3. Minimum daily temperature (°C) of the observed location during last 30 years.

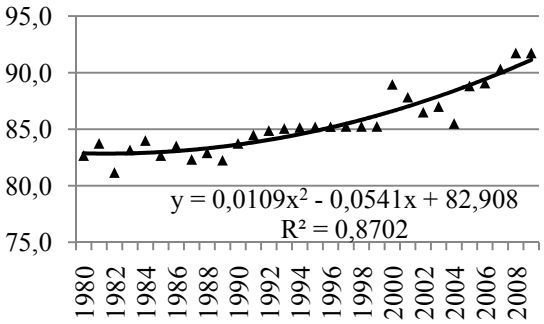

Figure 4. Relative humidity (%) of the observed location during last 30 years.

Evaporation decreased by equation of $y = -0.004x^2 + 0.010x + 3.883$ ($R^2 = 0.701$), whereas wind speed followed a quadratic equation of $y = 0.014x^2 - 0.568x + 5.740$ ($R^2 = 0.568$). Productivity of Robusta coffee was dropped by the increase of mean daily temperature, with the equation of $y = 2E + 15e^{-1.18x}$ ($R^2 = 0.771$); but it increased in line with relative humidity according to the equation of $y = 1E - 32x^{17.60}$ ($R^2 = 0.591$).

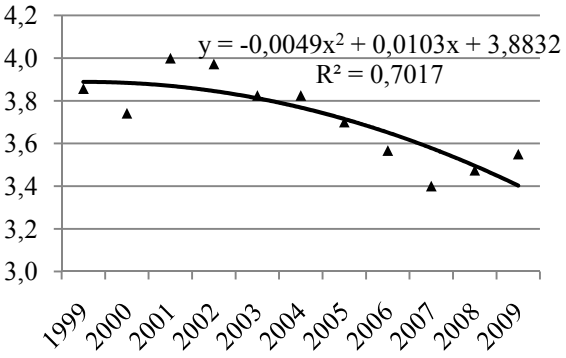

Figure 5. Evaporation (mm/day) of the observed location.

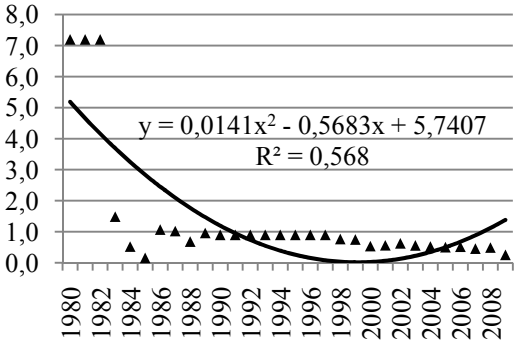

Figure 6. Wind speed (m/sec) of the observed location during last 30 years.

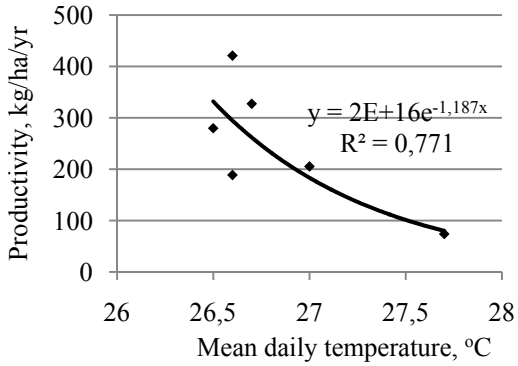

Figure 7. Relationship between mean daily temperature and productivity of Robusta coffee.

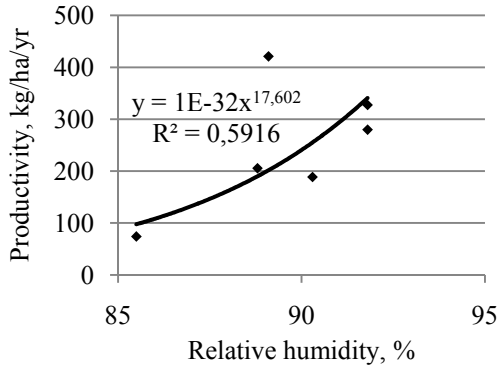

Figure 8. Relationship between relative humidity and productivity of Robusta coffee.

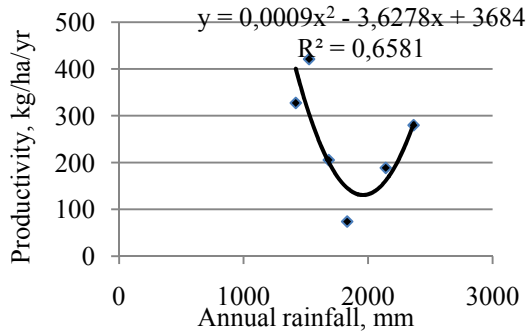

Figure 9. Relationship between annual rainfall and productivity of Robusta coffee.

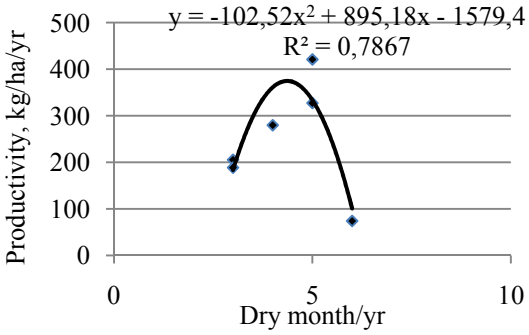

Figure 10. Relationship between dry month and productivity of Robusta coffee.

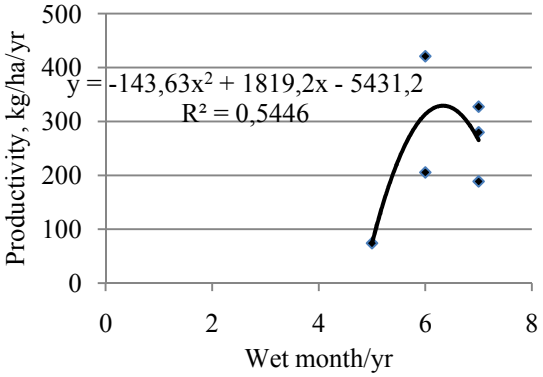

Figure 11. Relationship between wet month and productivity of Robusta coffee.

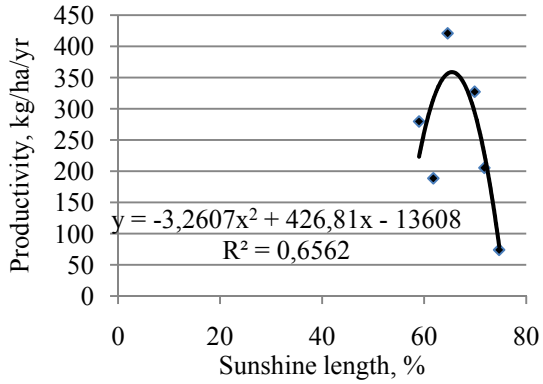

Figure 12. Relationship between sunshine length and productivity of Robusta coffee.

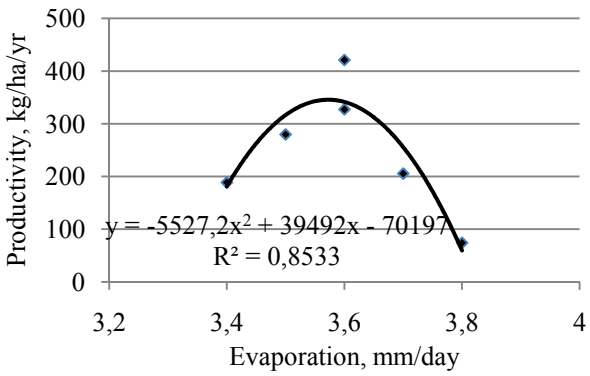

Figure 13. Relationship between evaporation and productivity of Robusta coffee.

Annual rainfall, dry month, wet month, sunshine length, and evaporation significantly affected coffee productivity by quadratic equation.

From all data could be seen that many of climate components have changed significantly during last 30 years. Some of those components affect the productivity of Robusta coffee. To reduce the negative impact of climate change, at least microclimate, implementing agroforestry is one of alternatives, because by that, the microclimate will be more stabile.

CONCLUSION

From this study can be withdrew conclusions as follows:

- Many of climate components have changed significantly during last 30 years.
- Some of those components affect the productivity of Robusta coffee.
- The ideal components of climate those will give highest productivity of Robusta coffee are 26.5 °C of mean daily temperature, 92% of relative humidity, 1500 mm of annual rainfall, 4-5 dry month per year, 6-7 wet month per year, 65% of sunshine length per day, and 3.5-3.6 mm/day of evaporation; respectively.

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Livelihood Strategies of Smallholder Coffee Farmers in South Sulawesi and East Nusa Tenggara (Flores)

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SUMMARY

The way coffee production is inserted within social and agro-ecological systems will affect the willingness of farmers to engage in quality upgrading initiatives. A key objective of ACIAR-supported research in Eastern Indonesia is to understand the varying livelihood strategies of smallholder coffee farmers across the islands of Sulawesi and Flores. How do livelihood strategies affect decision making processes that shape smallholder engagement with the growing specialty coffee market?

Our research interviewed a total of 803 smallholder households engaged in coffee production across South Sulawesi and East Nusa Tenggara. Coffee from these regions is produced by smallholder farmers, whose livelihood strategies are highly variable (Figure 1). For smallholders in Flores, coffee is the primary source of household income but is of secondary agricultural importance compared to livestock production and food production. Farmers here may be reluctant to invest in risky quality improvement initiatives. In the Enrekang district of Sulawesi, farmers maintain diverse and highly intensive cash crop production with minimal food production. In Toraja, smallholders are the least dependent on coffee for their livelihoods despite the fact that farm-gate prices here are the highest of all study sites and few other commodities are produced for external markets. Local economies in Toraja are strongly remittance-driven.

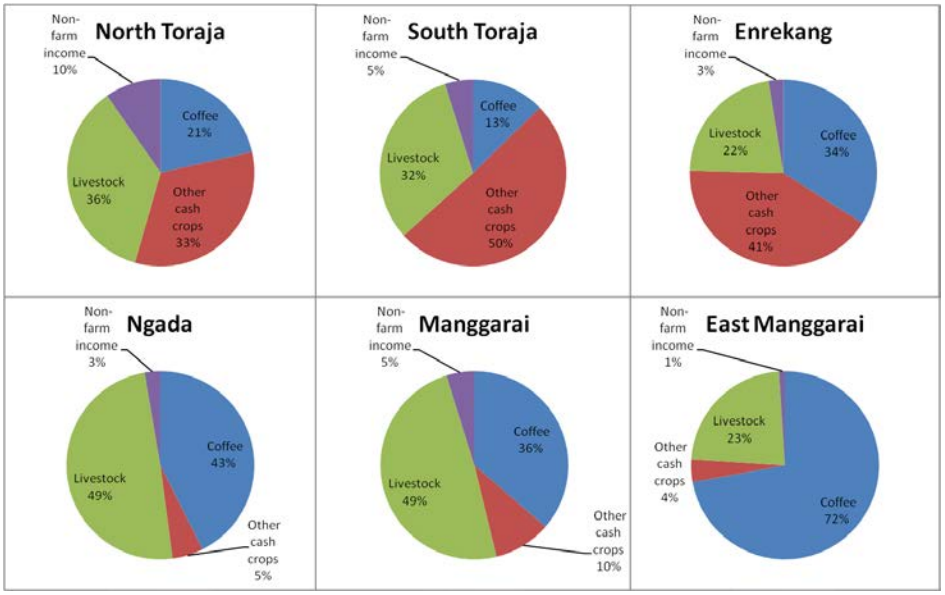

Figure 1. Livelihood strategies of farm households.

Talble 1.

Region	Districts	Coffee's share in household income	Livelihood strategies	Share of farmers' producing food crop (rice or corn)
South Sulawesi	Enrekang (n=199)	56 %	Coffee and cocoa, intensive horticulture and goat farming	16 %
	Toraja (n=199)	38 %	Coffee, rice, pig farming, off-farm wage labor and remittances	61 %
East Nusa Tenggara (Flores)	Ngada (n=201)	87 %	Oriented towards food security	75 %
	Manggarai (n=97)	90 %	Oriented towards food security	92 %
	East Manggarai (n = 100)	96 %	Oriented towards food security	75 %

These preliminary results indicate different roles of coffee in smallholder farmers' livelihood strategies across the region. The results also suggest a need to tailor interventions that consider varying smallholder farmers' livelihood strategies in order for smallholder farmers to fully benefit from the upgrading of coffee quality in value chain.

INTRODUCTION

Sulawesi and Flores are among the key Arabica coffee producing regions of Indonesia. Majority of coffee farmers in Indonesia are smallholders. Their livelihood strategies are the combination of activities that not only include productive activities, but also investment and reproduction. Households choose different strategies to meet their ever changing needs. In a farming households, livelihood strategies are often not only confined to agriculture but also include non-farm activities. This diversifies household income and ensures households to pursue their goals. Migration, both seasonal and permanent, is also a part of rural households' livelihood strategy. It is often an important source of cash income for smallholder households.

METHODOLOGY

The focus of the study is on household as the basic unit of economic decision making. We carried our household survey in South Sulawesi (Enrekang, South and North Toraja districts) and Flores (Ngada, Manggarai and East Manggarai districts) between 2008-2009. We selected a total of 803 smallholder households that are engaged in coffee production in six districts.

RESULTS AND DISCUSION

Table 2. shows that average smallholder farmer income in South Sulawesi is higher than in Flores. This is largely due to South Sulawesi farmers' involvement inintensive horticulture and livestock raising (e.g. goat, pigs), and off-farm wage labor. In contrast, farmers livelihoods in Flores is generally oriented towards food security.

Table 2. Household characteristics.

Region	Districts	Income (Rp/year)	Income only based on coffee	Farmers growing staple crop
South Sulawesi	Enrekang (n=199)	12.9 million	7 %	23 % (rice)
	South Toraja (n=65)	14.5 million	0 %	2 % (rice)
	North Toraja (n=135)	11.8 million	0 %	56 % (rice)
Flores	Ngada (n=207)	9.2 million	4 %	17 % (rice) 57 % (corn)
	Manggarai (n=97)	3.9 million	38 %	23% (rice) 89 % (corn)
	East Manggarai (n=100)	8.2 million	54 %	7 % (rice) 79 % (corn)

Table 3. Farm characteristics.

Distrcts	Average farm land	Forms of coffee produced and sold	Average price of coffee (GBE)	Farmers with access to credit (incl. informal credit)	Farmers with access to extension services	Membership to farmers' group
Enrekang	0.66 ha	Parchment (100%)	24,005 Rp	21 %	74 %	66 %
South Toraja	0.57 ha		23,819 Rp	9 %	34 %	26 %
North Toraja	0.78 ha		25,434 Rp	8 %	34 %	27 %
Ngada	0.43 ha	Greean bean (49%) Cherry (50%)	17,309 Rp	43 %	57 %	65 %
Manggarai	0.88 ha	Greean bean (94%) Cherry (6%)	16,632 Rp	23 %	45 %	51 %
East Manggarai	0.81 ha	Greean bean (15%) Cherry (85%)	17,141 Rp	88 %	22 %	40 %

The main staple in Flores is essentially a mixture of rice and corn, Table 2 shows that in Flores majority of smallholders grow staple food in contrast to Sulawesi, where majority purchase their staple crop, rice (with the exception of North Toraja).

Our study also indicates differences of farm characteristics across the study sites. In Flores, farmers use little agricultural input for coffee production (i.e. application of fertilizer,

pesticide and herbicide etc.). In Enrekang, farmers are engaged in intensive horticultural production. In addition, some farmers raise goats and pigs.

Table 3 indicates that despite the fact farmers in South Sulawesi are producing parchment coffee, the farm gate prices of Arabica coffee are higher compared to Flores. In Flores, farmers sell coffee in different forms.

Cases from Toraja in Table 3 also indicates that access to extension services and memberships to farmers' group are not necessary a condition for attaining high farm gate price.

Figure 1 shows that in Flores the contribution of coffee income to household farmers income in is higher than Sulawesi. it's also indicate that in Flores coffee is important income for households farmers than Sulawesi.

CONCLUSION

Preliminary results indicate that coffee consists only a part of smallholders' livelihood. Income from coffee is highly variable across the region. Farmers can upgrade quality of coffee, but this is conditioned by their access to basic resources and their capacity to reallocate resources across the spectrum of livelihood activities. Another critical element in upgrading involves development of close market relationships with buyers of specialty coffee. Building mutual trust and exchanging information is particularly essential. This suggests a need to reconsider the role of formal institutions supporting farmers such as credit, extension services and farmer organisations as a vehicle for promoting quality upgrading in the global value chain.

Efficiency Determinant Factors of Arabica Coffee Farming in Enrekang District, South Sulawesi

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SUMMARY

Arabica coffee is one of important income sources for farmers in Enrekang District, South Sulawesi. Technical efficiency is one of indicators in Arabica coffee farming performance. Attainment of high efficiency is very important in the effort to improve level of competitiveness and advantage of Arabica coffee farming. Technical efficiency is one component of economy efficiency. A coffee farming can be classified as economically efficient if technical efficiency has been reached. The aim of this research is to study the level of technical efficiency of Arabica coffee farming in Enrekang district and to investigate factors influencing technical efficiency of Arabica coffee farming. This research was carried out in 2008-2009 in which the number of respondents was 199 farmers using random sampling method. Analysis of data obtained using frontier production function and Maximum Likelihood Estimation method of estimation with assumption that the Arabica coffee production in Enrekang district follow Cobb–Douglas function. The result showed that production of Arabica coffee was not influenced by the all variables measured. Arabica coffee production was not affected by NPK fertilizer and labor. Technical efficiency of Arabica coffee in Enrekang district was 62.34 percent. Age and experience of farmers are important factors that can influence technical efficiency in Arabica coffee farming.

INTRODUCTION

Arabica coffee is one of important income source to farmer in Enrekang District, South Sulawesi. Arabica coffee prices received by farmers recently increased significantly. The production function is often defined as a function that describes the physical relationship between the number of inputs with a maximum output produced. One model that can explain the physical relationship is the frontier production function. The frontier production function has been applied in various fields, such as agriculture, fisheries, and financial economics. The frontier production function can be used to analyze the efficiency and inefficiency (Sukiyono, 2004).

Technical efficiency is one component of overall economic efficiency. A farming system can be said economically efficient if the technical efficiency have been achieved. Kumbhakar and Lovell (2000) said that there are three ways to maximize profits. The first way is to maximize the output (production) often called technical efficiency. Second, the maximum benefit can be obtained through an appropriate combination of inputs at a certain level of input prices (input allocative efficiency). The third way is to produce the right combination of production at a certain level of production prices (allocative efficiency of production). This study aims to estimate the level of technical efficiency achieved by Arabica coffee farmers in Enrekang and to identify the factors that influence the level of technical efficiency.

RESEARCH METHOD

Technical efficiency measures the extent to which a farmer to change inputs into outputs at the level of technological and economic factors. This means that two farmers who use the amount and type of inputs and the same technology would probably produce a different amount of output.

Arabica coffee production in the research location is assumed as a function of labor (X1), urea (X2), SP 36 (X3), KCl (X4), NPK (X5), ZA (X6), organic fertilizer (X7), pesticides (X8), and herbicides (X9). Mathematically can be written as follows: Yi = F (X1, ..., X9) or in the form of the natural logarithm linear econometric model, the production of Arabica coffee farming frontier can be written as follows:

$$Log(Yi) = ß_0 + ß_1 \log X_{1,i} + ß_2 \log X_{2,i} + ß_3 \log X_{3,i} + ß_4 \log X_{4,i} + ß_5 \log X_{5,i} + ß_6 \log X_{6,i} + ß_7 \log X_{7,i} + ß_8 \log X_{8,i} + ß_9 \log X_{9,i} + V_i - U_i$$

The main factors to be included in the model inefficiency Arabica coffee farming techniques on the attributes of farmers such as age and experience of Arabica coffee farming. These factors will affect the farmers in managing their agribusiness. Data used in this research is survey data conducted in Enrekang, South Sulawesi Province in 2008-2009 with respondents consisting of 197 farmers. The data were collected randomly and used the interview technique. Data collected include Arabica coffee plant production data, the number of input / inputs used by farmers such as labor, chemical fertilizer, organic fertilizer, and pesticides.

RESULTS AND DISCUSSION

Input use by farmers in this location is good. Results of analysis has been done shows that the average productivity of coffee Arabica 389.08 kg / ha / year (Table 1).

Table 1. Production function variable of Arabica coffee in Enrekang District, South Sulawesi.

Variable	Coefficient	Std.deviation	T Test
Constanta	98.407	41.202	2.388
Urea	0.326	0.282	4.099
ZA	0.168	0.97	1.857
KCL	2.127	0.340	6.249
SP 36	0.601	0.209	3.307
NPK	-0.004	-0.001	-0.020
compost	0.060	0.083	1.798
Labor	-3.210	-0.253	-4.111
Pesticide	0.735	0.067	1.399
Herbicide	8.434	0.051	0.867
Land holding	155.596	0.354	6.170
R^2	0.777		

Table 2 shows that all variables except labor and NPK affected significantly to total production of Arabica coffee. The table also shows that the highest elasticities is land area variable it means that the land area has the greatest influence on the production of Arabica coffee produced. The variable that gives the smallest effect is compost variable. Provided R square = 0777 or 77.7%, meaning that as many as 77.7% regression models of Arabica coffee production function in Enrekang district can be explained by usng of urea, ZA, KCL, 36 SP, NPK, compost, labor, pesticides, herbicides, and land holding, while the remaining 22.3% is explained by other factors not taken in this research.

Table 2. Frontier production function variable estimation of Arabica coffee in Enrekang District, South Sulawesi.

Variable	Coefficient	Std.deviation	T Test
Intersep	0.705	0.356	1.981 ***
Urea	0.139	0.047	2.954 ***
ZA	0.021	0.038	0.548 ***
KCL	0.038	0.039	0.957 ***
SP 36	0.007	0.039	0.181 ***
NPK	-0.037	-0.068	-1.006 ***
Compost	0.980	0.089	1.489 ***
Labor	- 0.216	-0.203	-1.066 ***
Pesticide	0.092	0.079	1.172 ***
Herbicide	0.453	0.254	3.589
Land holding	0.254	0.185	1.379 ***
λ	0.357	0.409	2.385
σ	0.598	0.305	10.305***

The result of the estimated frontier production model analysis shows that almost all of the variables in the frontier production function has significantly positive effect, unless the using of herbicides. The NPK and labor variables have a negative sign, thus it conflicts with the theory of production.

Table 3 below shows a summary of the expected level of technical efficiency of the production function frontier. Technical efficiency level of the lowest Arabica coffee farming in the research area obtained is 0.071 or 7.1 percent and the highest is 94.7 percent. Overall, the average technical efficiency achieved by Arabica coffee farmers in the research area is 62.34 percent. Figures efficiency of 62.34 percent gave meaning that the average farmer can achieve at least 62.34 percent of potential production derived from the combination of production inputs.

Arabica coffee production is influenced by the amount of input and will be also influenced by the productivity of farmers in managing of Arabica coffee farming. The productivity of a farmer is often influenced by age and experience of farmer.

Table 3. Arabica coffee farming efficiency in Enrekang District, South Sulawesi.

Statistic	Efficiency level
The number of samples	197.00
Mean	0.6234
Standar deviation	0.2554
Minimum	0.071
Maximum	0.947

Age and farmers experience was inserted into the model to understand the effects of both variables on the techniques efficiency of Arabica coffee farming in Enrekang. Table 4. shows that the farmers experience variable may provide greater leverage than the variable farmers age. Thus, the analysis showed that farmers age and farmers experience is the important factors that could affect the Arabica coffee production in Enrekang. The results of the analysis indicate that the age and farmer experience has positive correlation with the level of efficiency.

Table 4. Age and experience of Arabica coffee farmer in Enrekang District, South Sulawesi.

Variable	Coefficient	Std.deviation	T Test
Intersep	9.223	2.141	0.088
Farmers' age	3.990	0.129	1.864
Farmers experience	13.080	0.274	3.946 ***

CONCLUSION

- The frontier production function showed that almost all variables have positive signs, except for NPK fertilizer and labor variables.
- Herbicides variable did not provide a statistically significant effect on the production of Arabica coffee in Enrekang.
- Arabica coffee farming efficient level of the lowest 7.1 percent, the highest 94.7 percent.
- The average farm efficiency is 62.34 percent
- Age and farmers experience variables is important factors and have influence on Arabica coffee production.

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Cost of the Use of Legumes as Source of Nitrate Fertilizers in Coffee Trees

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SUMMARY

Experiments led in Ivory Coast, introduced two legumes (*Gliricidia sepium* and *Albizzia guachapele*) in the systems of culture of the *Coffea canephora*. Over against of high costs of artificial fertilizers, these legumes are used as a source of nourishing elements for coffee trees. The efficiency of the nitrogen resulting prunes was demonstrated at the level of the development and of the production of coffee trees. Indeed, the profit of productivity of both legumes is superior to the witness without fertilizer and without legume of the order of 45%. Seen the convincing profits, which indicate that these legumes allow to reduce the use of nitrogenous mineral fertilizers and to produce an environment-friendly good quality coffee, it turned out necessary to specify the economic performances of this innovation. The various costs connected to the technique were thus estimated. It emerges that production costs connected to the introduction of legumes are 1, 4 - 2 times month when those some mineral fertilizer. The margin after refund of fertilizers, plot of land of coffee trees associated to legumes, is from 1,3 to 2 times superior to that of the coffee plantation using the urea. This innovation allows of what a valuation of the working day family.

INTRODUCTION

Since the beginning of 1990s, following the example of the other African producing countries, Ivory Coast saw its production of coffee declining because of the weaknesses of the courses, some degradation of the quality, and the retraining of the exploitations in cocoa, rubber plant and palm tree. While they were of the order of 300 000 tons a year in the 1980s (Ministry of Agriculture, 2001), the exports of coffee Robusta of Ivory Coast are spent unless 150 000 tons a year from 1990s (OIAC, 2004).

Over against of this situation, several programs of coffee production boost were introduced by the Ivory Coast government. These programs recommended, among others the use of technical routes more adapted, in particular, the use of selected varieties and fertilization.

The use of nitrogenous mineral fertilizers in the optimal conditions of culture allows having profit of production of the order of 30 in more than 100% with regard to the cultures without fertilizer. However, the rate of adoption of fertilizers in coffee plantations remains low (less than 15%), this because of the high costs of these fertilizers. As alternative, two legumes were introduced into the systems of culture as a source of nourishing elements for coffee trees.

The performances of these legumes for the development of the production of coffee trees were established, the profit of productivity of both legumes are superior to the witness without fertilizer and without legume of the order of 45% (N'Goran and Amani, 2004). The

present study aims at estimating the economic performances of this innovation over the first seven years of driving of the test.

MATERIALS AND METHODS

Site of the study

The study is led to the station CNRA of Divo (Figure 1), in Ivory Coast within the framework of the realization of the project AEIA no: IVC / 5 / 022 and IVC / 5 / 025.

The average rainfall is relatively low amounting to 1354 mm / year against approximately 1500 mm / year on a national scale (Figure 2) grounds are weakly acid, with a report carbon / high nitrogen (9.92), sign of a good on-surface microbial activity. They contain, altogether, three individualized well horizons (Cestac and Snoeck, 1982). These soils are saturated and have a low capacity of exchange in base. They suit particularly in the culture of Coffea canephora, variety Robusta (Coste, 1989). The physical and chemical characteristics of this soils are: “argile+limon” at the end: 28 % - %C: 1.19 - %N: 0.12 - C/N: 9.92 - That: 3.20 méq / 100g - Mg: 0.86 méq / 100 g - Mg: 0.86 méq / 100g - K: 0.30 - pH: 5.9.

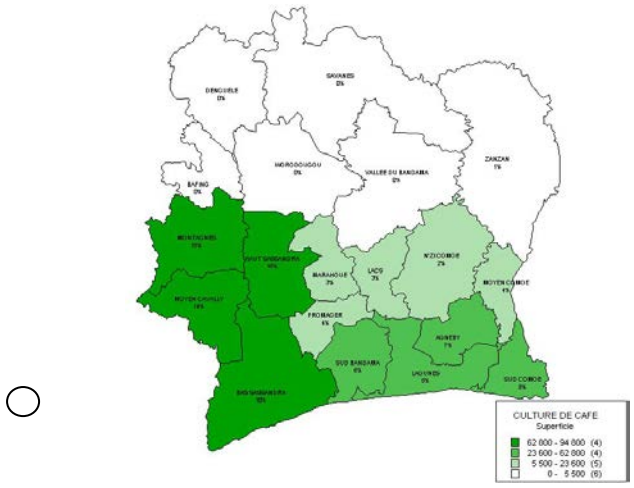

Figure 1.

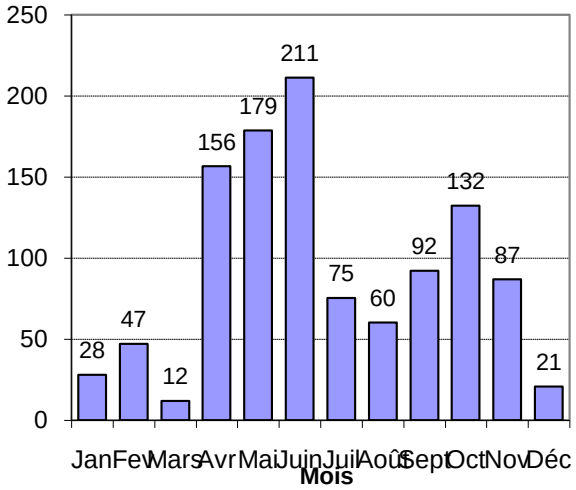

Figure 2.

Experimental device

The test was led according to a device in blocks of Fischer with 4 treatments and 5 repetitions. Two legumes were associated with coffee trees: *Gliricidia sepium* and *Albizzia guachapele*.

T1: witness without legume and without fertilizer;
T2: association coffee tree / Gliricidia;
T3: association coffee tree / Albizzia;
T4: urea at the rate of 100 kg N / ha.

Coffee tree Robusta used for the test is clonales selections reproduced by taking of cuttings and organized in the field after 8 months of breeding in tree nursery. They are planted in the density of 1333 trees in the hectare with a space of 3 m x 2.5 m. Legumes are planted in the line spacing of coffee trees, and in the same density with the same space as the coffee trees. On one year after plantation, legumes are cut. On one year after plantation, legumes are cut at a height of 1.50 m of the soil, and the new branches are cut every 3 months for mulch coffee trees. Prune them are weighed before the mulch in the feet of coffee trees.

Methodology

The collected data concerned, for each of 4 treatments, on one hand, on time of works and on the other hand, and the flows of money.

Time of works is expressed in the working days by activity and by treatment. The flows of money consisted in raising products (receipts) and loads (expenses) by technique.

The economic profits were estimated from income statements and through two indicators: the margin after refund of inputs and the net valuation of the work. In this case, the cost of inputs corresponds mainly:

- in the purchase of the plant material, the plantations of coffee trees, stubs of *Gliricidia sepium* and plantations of *Albizzia guachapele*: the cost of a plantation of coffee tree is considered at 300 F CFA, the cost of a stub of *Gliricidia* to 25 FCFA, the cost of a plantation of *Albizzia* stemming from seeds 150 FCFA and,
- in the purchase of the urea: cost of a kilogram: 300 FCFA. For an average application of 128 g by coffee tree a year.
- The working day is paid in 1 000 F CFA. The production of coffee trees is expressed in kg of trade coffee.

RESULTS AND DISCUSSIONS

Net valuation of the work

The cost of stake places coffee trees in the hectare over the first seven years (purchase of plantations, plantation …) rise 1 108 400 F CFA among which 41 % for the purchase of plantations. This cost is associated with the 198.4 working days. The costs of the other treatments vary of 1 334 880 F and 1 650 700 F (Table 1) among which 67 % and 82 % are in the works of effective implementation of coffee trees.

Table 1. Estimation of cost per technical per ha.

Traitements	Année 0 à Année 7		
	Quantité	Homme/jour (HJ)	F CFA
T1	1 500	198,40	1 108 400
T2		305,28	1 344 880
T3		307,83	1 566 130
T4		233,60	1 650 700

Margin after refund of inputs

The profitability compared by four techniques over the period of 7 years (1 cycle of production of coffee) shows a profit margin (Table 2) which varies between 1 033 000 of 2 257 250 F. The strongest margins are observed in the case of the association of coffee trees with Albizzia and the technique using the nitrogenous mineral manure.

Table 2. Estimation of benefits after inputs reimbursement per ha for 7 years.

Traitements	T1	T2	T3	T4
Coût des travaux à l'ha	1 108 400	1 344 880	1 566 130	1 650 700
Quantité de café marchand récoltée en Kg	4 282	6 315	7 271	7 816
Prix de vente unitaire en F CFA	500	500	500	500
Recette totale	2 141 000	3 157 500	3 635 000	3 908 000
Marge bénéficiaire	1 033 000	1 812 620	2 069 350	2 257 250

In case of the nitrogenous mineral use of the fertilizer, the costs are superior of the order of 5% with regard to the treatment *Albizzia*. The profit margin of coffee tree + urea is superior no than of 8 % with regard to *Albizzia*. The profit margin coffee tree + urea are superior of 20 % with regard to coffee tree + *Gliricidia*, for costs of superior production of 18%. The average of profit margin of Coffee trees + Legumes is superior with regard to the witness (T1) of more than 46 % for only approximately 20% of additional expenses. The profit margin of these two legumes is thus upper furthermore of twice with regard to the witness without fertilizer, and without legumes. The urea is superior no than of the order of 1.3 with regard to the average profit margin of *Gliricidia* and *Albizzia*.

CONCLUSION

Legumes, with regard to the witness and to the use of the urea, value better the working day while protecting the environment. These works show the interest of the system of culture of *C. canephora* in association with legumes. This device offers to the producers the possibility of planting, during several years, trees in the line spacing of coffee trees while preserving a density of plantation of coffee trees equal to that usually adopted.

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Quality of Robusta Coffee in Côte d'Ivoire: Importance of the Shade

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SUMMARY

In Côte d'Ivoire, the project «Café Terroir/CFC/ICO-05» was implemented with the objective of identifying types of coffee having particular taste qualities and offering market opportunities to the farmers. Several types of coffee were identified according to the cultivated varieties, the climates and the soil conditions, following collection of samples of coffee and soils. To take into account the influence of the farmers' practices on these types of coffee, a survey was conducted. The farming system (farms with or without shade) seems to have an influence on the quality of the coffee. Indeed, in Aboisso where more than 56% of plantations are under shade of diverse trees, the coffee is of better quality.

INTRODUCTION

Coffea canephora, the species of coffee tree cultivated in Côte d'Ivoire, is a shade tolerant tree well adapted to the tropical climate of Africa, where it is especially cultivated in low altitude. The best productions, in quantity and quality, are obtained in humid zones where the rainfall is between 1300 and 1800 mm a year, but with a regular distribution.

The introduction of the new varieties, which support full sunshine and resist to the coffee leaf rust, profoundly modified the farming systems. Indeed, the traditional system, where the coffee tree is planted under the existing forest with little change of its original setting, evolved towards a monoculture or a pure culture system where the shade is totally eliminated (Moguel and Toledo, 1999). However, the systems of mixed cropping where the coffee trees are associated to other tree species and\or food crops (banana, etc.) still remain very wide-spread.

The shade trees bring some nitrogen, recycle nutriments, increase the infiltration of rainwater, and reduce the erosion of soils. They also interact, in a positive or negative way according to the situation, with the pests and the diseases. The shade was also quoted among the factors which affect the quality of the coffee (Avelino et al., 2002). The quality of coffee includes all the characteristics desired by the consumer. These include, besides the caffeine contents, the physical and chemical properties of the product (absence of other plant parts, foreign bodies and pesticides residues), the bean size grading (Gnadré, 2003).

Within the framework of the implementation of the project «Café Terroir/CFC/ICO-05» in Côte d'Ivoire, the parameters which affect the quality of coffee were investigated. The present paper reports the results obtained. The genetic factors and the effect of the soils will be mentioned. Emphasis will be put on the effect of the shade.

MATERIAL AND METHODS

The coffee and soils samples were collected on-farm with 27 coffee growers in three major production areas (Figure 1) according to three levels of technicalities:

- Level 1: improved plant material with good agricultural practices,
- Level 2: improved plant material with bad agricultural practices,
- Level 3: traditional plant material.

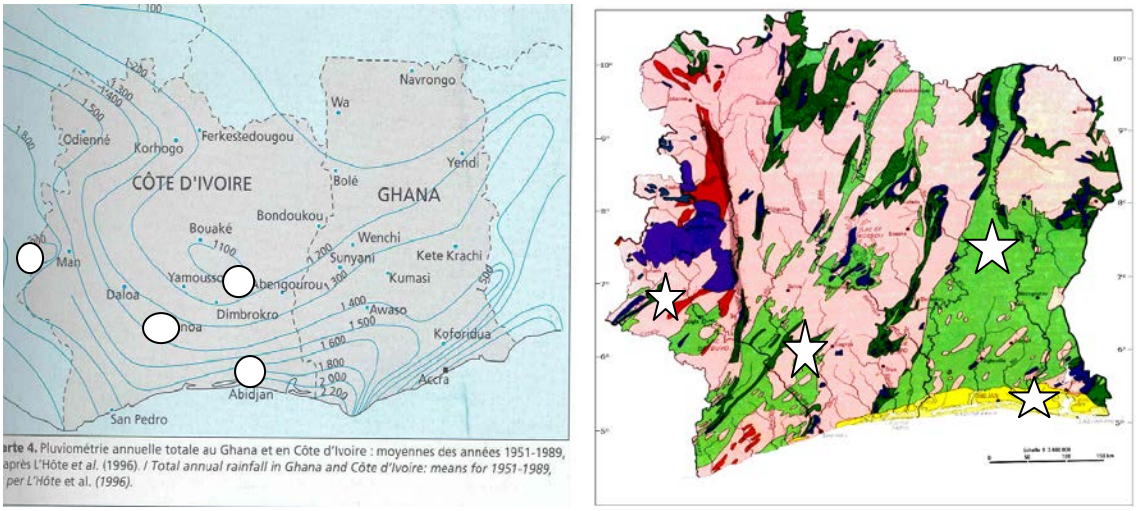

Figure 1.

Two post-harvest techniques were applied for the coffee samples. These were wet shelling before drying and direct drying before shelling. After drying, the samples of coffee beans were sent to CNRA, OIAC and CIRAD for processing and tasting.

With regard to the assessment of the agronomic parameters, quantitative and qualitative information were collected with the growers, in particular the means of production, the mode of creation of new farms and the cropping systems. At farm level, the observations consisted in the description of the plots, the evaluation of the management level, the identification of the planting design and the material planted, the estimation of the size of the plot, the identification of the farming system (no shade or shade), the counting of the shade trees and the identification of the species of shade trees.

RESULTS AND DISCUSSIONS

Characteristics of the sites of study

The analysis of the climatic and soil conditions of the three regions gave the following essential indications:

- The annual average rainfall varies between 1200 mm and 1600 mm;
- Coffee plantations are established on hillsides with altitudes which do not exceed 250 m.

The lowest altitudes are found in Aboisso (Table 1). Overall, the soils of the middle-hillside*s* are the most used for growing coffee.

Table 1. Characteristics of the climate and the soils of the study sites.

Parameters	**Divo - Gagnoa**	**Abengourou**	**Aboisso**
Rainfall (mm)	1320	1250	1600
Altitude (m)	149-246	164-227	53-110
Source rock	Granite Migmatite	Schist	Tertiary sand, Granite

Characteristics of plant material collected

More than 65% of the plant material was improved coffee clones distributed to the farmers. Cuttings of traditional varieties were collected and established in the nursery on-station for future characterization.

Table 2. The type of the collected plant material.

Plant material	**Southeast**	**East**	**West central**	**Average**
Improved varieties	67	67	67	67
Traditional varieties	33	33	33	33

Typology of the coffee according to the varieties planted and the soils

According to the type and the characteristics of the soils in the different regions, the coffees were classified in each in the following groups:

- Abengourou Coffee: a good rating. Fruity and good aromatic quality. Some coffee samples of this locality have rather a bitter taste, or are astringent.
- Aboisso Coffee: They present overall the same characteristics as those of Abengourou, with in addition a slightly acid taste. But, no bitter, green or astringent taste.
- Divo Coffee: ligneous, earthy, bitter or strong. This type is in positive connections with the silt and sand contents of the soil in this locality.

Influence of the cultural practices on the quality of the coffee: role of the shade

More than 78% of the plantations of coffee trees of Aboisso were established under the “forests”, while most of the farms of two other localities were established on fallows. This is the case of Divo and Lakota where more than 78% of coffee plantations are established on fallows of *Chromolaena odorata*. The highest densities are found in Aboisso (more than 60% of plantations). In addition, plantations established under shade of forest trees are dominant in the Aboisso region (56%). Plantations without shade are much diversified (coffee + diverse food crop). The other parameters did not vary from one locality to another. The establishment of coffee trees on forest land with managed shade seems to have a positive influence on the quality of Aboisso coffee. Recent studies conducted in Guatemala and in Costa Rica confirmed these findings (Guyot et al., 1996). Indeed, Guyot et al., in 1996, showed that the

presence of shade trees in the coffee farm lengthened the period of fruit maturation and guaranteed a better quality of coffee.

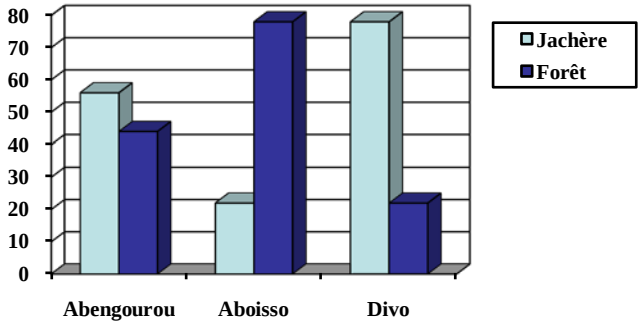

Figure 2. Mode de création selon forêt ou jachère.

Table 3. Cropping systems.

Zone of production	No shade	Low shade (< 10 trees/ha)	Relatively heavy shade (>10 trees/ha)
Aboisso	44	23	33
Abengourou	67	11	22
Divo - Gagnoa	56	11	33

CONCLUSION

An increase in the level of shade associated with coffee trees established on forest lands lead to the production of coffee with good ratings, fruity, aromatic, absence of bitter taste and without astringency. The quality of the coffee seems to depend on the mode of establishment of the coffee trees. This study must be extended to the entire coffee growing area of Côte d'Ivoire in order to confirm the relationship between plant material, rainfall, soil and the farming system on the quality of the coffee.

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Biofertilizers – Effective Enricher for Sustainable Nutrition of Coffee Seedlings in Nursery

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INTRODUCTION

Dawn of the green revolution has brought impressive gains in food production but unfortunately with abysmal concern for sustainability. The tryst with the self sufficiency with respect to food grain production in India is realized upon the combinations of high yielding varieties, irrigation, chemical fertilizers, insecticides and pesticides. Excessive and unscrupulous use of nitrogenous fertilizers without proper emphasis on phosphate and potash fertilizers besides plant protection chemicals for enhancing production has resulted in deterioration of physical, chemical and biological health of agricultural soils. The current availability and affordability of fossil fuel based chemical fertilizers at the farm level have been ensured only through imports and subsidies. Dependence on chemical fertilizers for future agricultural growth would mean further loss in soil quality, possibilities of water contamination and unjust onus on the fiscal system. At this juncture, bio-fertilizers are vital for intensive agriculture owing to its crucial role in augmenting optimum nutrient supply through exploration of biological N fixation, P solubilisation, decomposition and recycling of organic wastes.

Inherently, the coffee growing soils of India are poor reserves as far as P replenishment is concerned. The intrinsic characters like soil acidity, low base saturation, abundant Al and Fe render P unavailable for the growing plants. The higher organic pools in these soils ought to be decomposed properly in order to ensure balanced nutrition. Humus formed after thorough decomposition adequately addresses the soil flocculation and better physical environment. Coffee being rightly called as 'century plant' needs to be nurtured right from the nursery stage. Conventional practices of nursery mixture preparation with enriched forest soils are moving into oblivion owing to the dearth of resources. In this scenario, focus is on the possibility of exploration of bio-fertilizers in introducing vigour in the seedlings which can resist the attack of pest and disease and last long in the field with sustainable yields. Proper and adequate nutrition of seedlings is of pivotal importance in improving vigor of the growing plants. Combined application of vesicular arbuscular mycorrhiza (VAM) and *Azospirillum brasilense* to coffee seedlings has been reported to improve biometric parameters besides total P uptake (Glory Swarupa, 1997, Salakinkop et al., 2003). Similarly, enhanced uptake of N and micronutrients (Fe, Cu, Zn and Mn) were reported by Merina Prem Kumari and Balasubramanian (1993). Inoculation of *Azotobacter* in S 795 Arabica coffee was found to influence positively on shoot dry weight, root spread and girth of the seedlings (Mir Azizuddin and Krishnamurthy Rao, 1996). With these perspectives a study was planned to outline the effects of different bio-fertilizers on the growth and performance of young Arabica and Robusta seedlings.

MATERIALS AND METHODS

Recently released Arabica variety from CCRI 'Chandragiri' (a hybrid derived from Villa Sarchi, a semi dwarf mutant of Bourbon coffee and Hibrido de Timor) and Robusta coffee variety 'CxR' (an inter-specific hybrid of *Coffea canephora* × *Coffea congensis*) were selected for the study. The Chandragiri is known for its high tolerance to leaf rust disease besides superior bean quality. The CxR is high yielding and drooping nature facilitates high density planting which is being popular among the planters. Coffee seeds were sown in raised nursery bed and later on attaining 'button stage', transferred to polythene bags containing nursery mixture (Forest soil: FYM: Sand :: 6:2:1) after 45 days. The seedlings were treated with bio-fertilizers (5 X 10^8 CFU per seedling) during this period. The following seven treatments with four replications and forty seedlings per treatment were included in the trial.

T_1 – Un-inoculated control
T_2 – *Azospirillum*
T_3 – *Azatobacter*
T_4 – Phosphobacteria
T_5 – *Pseudomonas fluorescens*
T_6 – AM fungi
T_7 – Microbial consortia (all the above five)
Design – Randomized Block

Growth parameters monitored periodically were subjected to statistical analysis to draw valid inferences. The variations in growth attributes among the treatments remained identical over the period of observation. The data pertaining to growth parameters of Arabica and Robusta coffee seedlings recorded on 150^{th} day after sowing are depicted in Tables 1 and 2 respectively. The soil nutrient status (Table 3) at the initiation of the experiment and after 150 days of sowing of seeds is recorded for the nutrient budgeting.

Table 1. Biometric parameters of Arabica seedlings (150 DAS) as influenced by microbial inoculation.

Treatments	Plant height (cm)	Stem girth (cm)	Tap root length (cm)	No. of leaves	Bush spread (cm)	Shoot weight (g/plant)	Root weight (g/plant)
T_1	16.8^{c}	3.9	21.8	11.0	19.9^{bc}	10.68^{c}	3.39^{d}
T_2	21.8^{ab}	4.3	26.5	13.5	27.0^{ab}	14.30^{ab}	4.79^{ab}
T3	23.5^{ab}	4.2	28.8	13.0	29.8^{a}	14.30^{ab}	4.79^{ab}
T4	24.0^{ab}	4.2	27.5	13.5	30.0^{a}	14.71^{a}	4.72^{ab}
T5	26.3^{a}	4.1	25.3	13.5	33.5^{a}	15.53^{a}	4.60^{bc}
T6	25.3^{a}	4.1	25.8	14.0	33.8^{a}	15.71^{a}	4.90^{ab}
T7	29.5^{a}	4.4	26.5	14.0	33.0^{a}	15.84^{a}	5.15^{a}
sem+/-	1.5	0.16	1.85	0.47	1.77	0.56	0.08
CD @ 5%	4.36	NS	NS	NS	5.14	1.68	0.24

DAS – Days After Sowing.

RESULTS AND DISCUSSION

The biometric parameters of Arabica seedlings (Table 1) reflected significant influence of microbial inoculation in general and consortium in particular when compared to the control. The highest seedling height (29.5 cm) was recorded in consortium treatment (T_7) followed by T_5 (26.3 cm) and T_6 (25.3 cm) which were treated with *Pseudomonas fluorescens* and AM fungi, respectively. All the three (T_5, T_6 and T_7) treatments remained on par and were statistically superior over rest of the treatments. Enhanced production and subsequent utility of IAA, IBA, NAA, GA 1 to 3, phytohormones, vitamins, and other plant growth promoters (PGP) in growth media by different constituents boosted the growth and dry matter production (Mishra, 1993). In the next stratum T_2, T_3 and T_4 remained comparable and were statistically superior over the control. The stem girth, tap root length and number of leaves recorded remained inconclusive to draw inference on the effect of microbial inoculation, however numerically superior values were recorded in the consortium treatment over the rest. The bush spread was significantly higher in all the treatments compared to the control with numerically superior values in AM fungi and consortium treatments. Similarly the root and shoot weight were significantly higher in microbial inoculants in general and consortium in particular when compared to the control.

Table 2. Biometric parameters of robusta seedlings (150 DAS) as influenced by microbial inoculation.

Treatments	Plant height (cm)	Stem girth (cm)	Tap root length (cm)	No. of leaves	Bush spread (cm)	Shoot weight (g/plant)	Root weight (g/plant)
T_1	19.0^{bc}	3.9	25.0^{b}	11.0	33.3	11.5^{b}	4.9^{d}
T2	21.3^{bc}	4.2	31.8^{a}	12.5	33.0	12.6^{a}	6.5^{ab}
T3	23.0^{ab}	4.0	30.3^{a}	12.5	37.5	12.6^{a}	6.4^{bc}
T4	23.8^{ab}	4.0	32.0^{a}	13.5	33.8	12.8^{a}	6.9a
T5	23.3^{ab}	4.1	30.8^{a}	13.0	33.0	12.7^{a}	6.8a
T6	25.5^{a}	4.1	29.3^{a}	13.0	35.5	12.7^{a}	6.6^{ab}
T7	27.8^{a}	4.1	32.0^{a}	13.0	34.8	13.0^{a}	6.9a
sem+/-	0.99	0.099	1.35	0.57	1.93	0.04	0.09
CD@ 5%	2.88	NS	3.94	NS	NS	1.35	0.27

DAS – Days After Sowing.

The data pertaining to the growth parameters of Robusta seedlings is made available in Table 2. The effect of AM fungi was conspicuous as either solitary or in consortium in modulating the growth pattern of the young seedlings as evidenced by significantly higher plant height in T_6 and T_7 compared to rest of the treatments. Similarly, the tap root length, root and shoot weight were found to be significant in microbial inoculated treatments over the control.

The prominent effect of AMF in both the varieties may be attributed to increased uptake of available soil phosphorus (P) and other non labile minerals essential for plant growth as earlier reported (Smith and Read, 1997) and now evidenced in the higher nutrient status after inoculation (Table 3). The indirect effect of AMF in stabilizing the soil aggregates (Miller and

Jastrow, 1990) by production of glycoprotein glomalin (Wright and Upadhyaya, 1996) is more important in considering it as one of the components in the consortium. In addition, the prominent role in alleviating plant biotic (Guillemin et al., 1994; Linderman, 2000) and abiotic stress (Rosendahl and Rosendahl, 1991; Goicoechea et al., 1997; Auge, 2000) render it inevitable for the sustainable cultivation.

Table 3. Soil Nutrient status as influenced by microbial inoculation (150DAS).

Treatments	Initial status				After 150 days			
	pH	OC(%)	Av. P Kg ha^{-1}	Av. K Kg ha^{-1}	pH	OC(%)	Av. P Kg ha^{-1}	Av. K Kg ha^{-1}
	Arabica							
T_1	7.0	2.89	16	556	6.1	3.73	80	230
T_2	7.1	1.72	18	700	5.8	3.73	76	200
T_3	6.9	2.29	15	696	5.2	3.73	77	220
T_4	6.8	2.58	17	572	5.5	3.15	71	220
T_5	6.8	2.58	21	516	5.8	4.01	74	130
T_6	6.8	2.29	22	560	5.5	3.73	83	200
T_7	6.7	2.89	26	592	5.8	3.73	85	190
Robusta								
T_1	6.8	2.89	26	608	5.4	3.44	71	210
T_2	7.4	1.72	25	648	5.4	3.15	88	250
T_3	7.4	1.28	27	664	7.0	4.01	75	270
T_4	7.4	1.72	24	672	7.3	3.15	87	210
T_5	7.5	2.58	26	676	7.4	4.01	70	210
T_6	7.5	2.58	27	636	7.2	3.15	74	200
T_7	7.5	2.58	26	652	7.4	4.01	92	250

CONCLUSION

The vigor of the seedlings could be effectively improved through bio-fertilizers besides imparting traits to combat biotic and abiotic stress expected in the later periods. Considering the constraints of coffee growing soils, P solubilizers or to be more precise, AMF needs to be incorporated to enhance nutrient assimilation of the whole system.

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Temporal Variations in the Abundance of Three Important Insect Pests of Coffee in Kilimanjaro Region, Tanzania

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SUMMARY

Temporal variation in abundance of white coffee stem borer (WCSB), antestia bug and coffee berry borer (CBB) were investigated between September 2007 and August 2008 at medium altitude (1200-1600 m.a.s.l) and high altitude (1600-2100 m.a.s.l) areas in Kilimanjaro region. A multistage random sampling method was used to select farms and trees for sampling in the two locations making a total of 810 trees. Insects were counted every month to establish the population size. High populations of antestia bugs and CBB were recorded during the short and long rains, during flowering and fruit development. WCSB increased gradually during short and long rains. Populations of WCSB were high at high altitude compared to medium altitude and occurrence of CBB at medium altitude was observed where it was not common in the past. Since the population size of WCSB was high in all locations and it is the most damaging insect pest, it is recommended that more attention should be focused on management of this pest.

INTRODUCTION

Economically important, insect pests of Arabica coffee in Tanzania are: antestia bug (*Antestiopsis* spp.), white coffee stem borer (WCSB) (*Monochumus leuconatus* Pascoe), coffee berry borer (CBB) (*Hypothenemus hampei* Ferrari), coffee leafminer (*Leucoptera* spp.), scales (*Coccus* spp.) and mealy bugs (*Planococcus kenyae* Le Pelley) (Magina, 2005). Koul and Cuperus (2007) noted the importance of spatial and temporal distribution of insect pests in different agro-ecosystems as a prerequisite for developing sustainable pest control strategies. Available information of this kind, for major insect pests of Arabica coffee in Tanzania, dates back to the 1950s and has not been updated recently. The objective of this study was to study the temporal variations in distribution of coffee insect pests in two altitudes in Kilimanjaro region, so as to update the available information.

MATERIAL AND METHODS

Study area

The study was conducted in Hai and Moshi Rural districts in Kilimanjaro region located between latitudes 3˚00’ and 3˚15` S and longitudes 37°00’ and 37°45’E. Two wards, namely, Machame East ward located at medium altitude (1200-1600 m.a.s.l.) and Kilema Northern ward located at high altitude (1600-2100 m.a.s.l.) areas were involved. The sites were selected

because of availability of weather data (rainfall, temperature and relative humidity). The area has bimodal rainfall pattern (long and short rain seasons).

Study procedures

Assessment of insect population size was conducted for a period of 12 months, from September 2007 to August 2008. Two factors were investigated to establish their effect on population dynamics of the three insect pests which includes: altitude (high and medium), and seasons (wet and dry). Multi-stage sampling techniques were used. Sampling was done randomly and total sample size was 810 trees (Table 1). Data for pest infestation were collected from unsprayed coffee farms carefully located by means of Global Positioning System (GPS).

Table 1. Summary of the sampling protocol in two districts.

District	No. of wards	No. of villages per ward	No. of farms per village	No. of sampled tree per farm	Total samples
Moshi rural	1	3	15	9	405
Hai	1	3	15	9	405
Total	2	6	30	18	810

The farms were divided into equal grids. The nearest coffee tree to the point at which the two transects crossed was sampled. Therefore, in every farm, a total of 9 points/trees were sampled regardless of farm size. The length of transects varied depending on the size of the farm. The outermost lines of coffee were not sampled and were regarded as a guard row.

Data collection and analysis

Data collected was: total number of the three pest species WCSB, antestia bug and CBB per coffee tree and fruit phenology. Counting of the insects was done on a monthly basis to allow for changes in life cycles for some of the insect pest species. Assessment of the number of each pest type was carried out as prescribed below:

WCSB

The lower trunk, up to 0.6 m above the collar level, was closely examined for any signs of stem girdling or boring by white coffee stem borer. The number of insects was represented by the number of bores per coffee tree.

Antestia bug

The tree was careful examined from all angles for the presence of the pest without disturbing the tree canopy. The total number of adult antestia bug and nymphs was recorded.

CBB

A primary branch bearing coffee berries was randomly selected in the middle third of the bearing head and two medial berry clusters were examined for the presence of the pest and recorded in a standard sheet (Kamanywa et al., 2006).

Data analysis

The mean numbers of insect pests per location were determined and population dynamics curve were drawn to show the occurrence of insects at different periods of the year.

RESULTS AND DISCUSSION

Relative abundance of WCSB, antestia bug and CBB as influenced by altitude, weather and fruit phenology.

The population size of WCSB was higher at high altitude (1600-2100 m.a.s.l.) than medium altitude (1200-1600 m.a.s.l.) (Figure1). This is in contrast with findings made in 1950 by Tapley (1960) which showed that the upper limit of the 'borer belt' in Kilimanjaro was approximately 1,524.3 m at Mkuu and Kilema, 1,341.5 at Kirua, Old Moshi, Uru and Kibosho, 1,250 m at Lyamungu, 1,189 m at Machame and up to 1,463 m at Sanya. This might be a result of climatic change, particularly an increase in temperature at Kilema. The population of antestia bugs was lower at the higher altitude (Kilema) where the temperature is relatively low compared to medium altitude (Lyamungo) (Figure 2 and 3). An increase in temperature is known to shorten the life cycles of the pest, resulting in many generations of the pest within a short period of time (Le Pelley, 1968). CBB were only present at Lyamungo (medium altitude) (Figure 4). The pest has been reported to prefer low-altitude Arabica coffee, being seldom serious over 1370 m.a.s.l and not found beyond 1680 m.a.sl (Tapley, 1960).

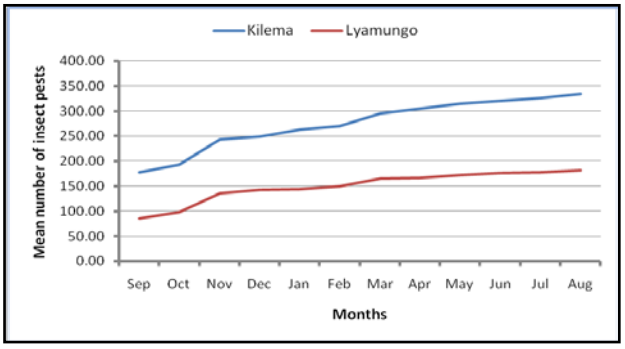

Figure 1. Population dynamics of WCSB at Lyamungo and Kilema.

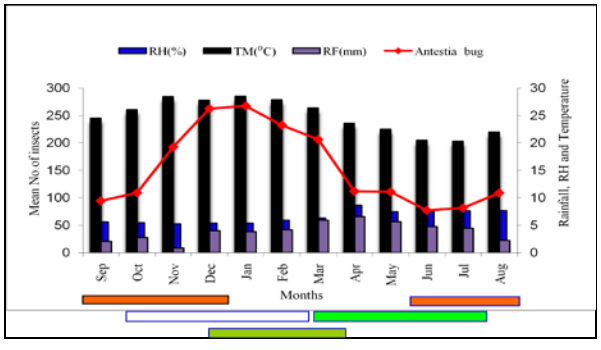

Figure 2. Population dynamics of antestia bugs at Lyamungo.

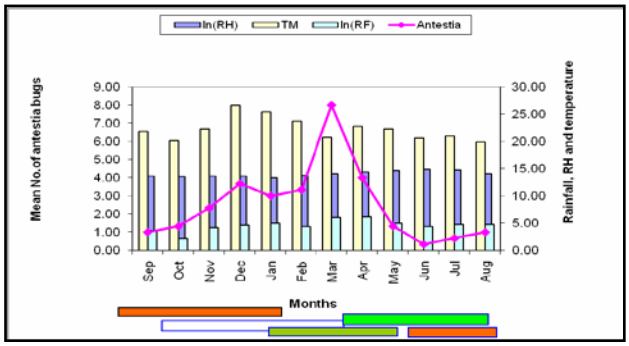

Figure 3. Population dynamics of antestia bugs at Kilema.

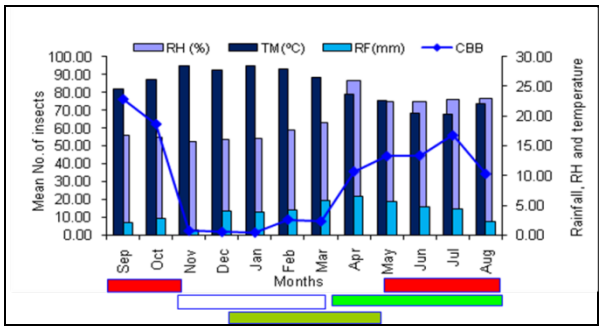

Figure 4. Population dynamics of CBB at Lyamungo.

The availability of flowers and fruits is an important factor for the increase of antestia bug and CBB. WCSB normally emerges from coffee stem during the onset of short and long rains (Le Pelley, 1968). The infestation of WCSB was observed to persist throughout the cropping season. However, a gradual increase of WCSB occurred in November and February (short rains) to March (on set of long rains) in both medium and high altitudes (Figure 1). Le Pelley (1968) reported that activities of these pests particularly reproduction, and ultimately an increase in population occurs with the onset of the rains. The population level of CBB was high in April to September, and decreased from October to March due to availability of fruits. The insect thrives and breeds in the hardened maturing fruits (Le Pelley, 1968). The absence of CBB at Kilema suggests that the high altitude (as therefore low temperature) is inhibitive to further proliferations of the pest. On the other hand, antestia bug was abundant from November to March, which is the period for flower formation and fruit setting. This appears to be related to occurrence of coffee berries in the field which are sources of food for the pest. These results are consistent with the report by Le Pelley (1968) that insects that breed in the coffee and attack flowers and those that attack the berries (CBB and antestia bug) can be expected to maintain themselves in high population if flowers or berries in the right stage are available especially over long periods. This is because the pests depend on fruits (young and ripe cherries) for food.

CONCLUSION

1. The study observed that altitude is a major factor that greatly affect the population of coffee pests
2. Rainfall was observed to have strong influence on the abundance of coffee pests.
3. CBB was found to be an important pest in medium altitude areas

4. WCSB was abundant and well distributed in all locations and observed be the most damaging coffee pest in the study areas. More attention should be focused on management of this pest.

ACKNOWLEDGEMENTS

This is part of MSc. dissertation work. The authors wish to thank the coffee farmers in Tanzania and Regional IPM CRSP in East Africa for financial support to the study.

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