

# **BOOK OF ABSTRACTS**

## **SECTION 1: Program - Oral presentations**

*Category: Consumption & Health, Chair: Dr. James Coughlin*

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1. “Keynote: Caffeine Metabolism: Why Are We All So Different?”  
Astrid NEHLIG, PhD. Research Director, INSERUM U 1129
2. [“Genotypogram of Coffee and Caffeine Consumption and Related Genetic Traits”](#)  
[Roseane Maria Santos- Associate Professor at South University School of Pharmacy in Savannah, Georgia, USA](#)

*Category: Biochemistry & Biotechnology of Green Coffee, Chair: Dr. Luciano Navarini*

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3. [“Metabolomic and Proteomic Approaches to Finding Biomarkers in Civet Coffee”](#)  
[Emmanuel Garcia- Director, La Salle Food and Water Institute & Assistant Professor, Chemistry Department, De La Salle University, Philippines](#)
4. [“From Cherry to Cup: Multiphasic Assessment of Wet Coffee Processing Through Beans, Waters, and Cup Quality”](#)  
[Sophia Jiyuan Zhang- Doctoral Researcher, Research Group of Industrial Microbiology and Food Biotechnology](#)
5. [“Demucilaging or Depulping? Implications for Microbiota, Coffee Composition, and Cup Quality”](#)  
[Florac De Bruyn- PhD Researcher, Research Group of Industrial Microbiology and Food Biotechnology, Department of Bioengineering Sciences, Vrije Universiteit Brussel](#)
6. [“The Influence of Coffee Mycobiota From Some Brazilian Regions on the Coffee Beverage: Can These Regions Be Considered Terroir?”](#)  
[Aldir Teixeira- CEO / Diretor Geral Experimental Agrícola do Brasil Ltda.](#)

*Category: Sustainability, Climate Change & Labels, Chair: Dr. Philippe Vaast*

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7. “Keynote: Climate Smart Coffee: From Theory To Practice”  
*Laurence Jassogne, Systems Agronomist- International Institute of Tropical Agriculture*
8. “A Comprehensive Estimate of Global Coffee Farmer Populations By Origin”  
David Browning- CEO, Enveritas
9. [“Emission of Nitrous Oxide in a Coffee Plantation Under Shade, in the Central Valley of Costa Rica”](#)  
[V́ctor Manuel Chaves Arias- Nutrici3n Mineral, ICAFE](#)
10. [“Sustainable Coffee Landscapes in Jinotega, Nicaragua”](#)  
[Saurin Nanavati- Committee on Sustainability Assessment](#)
11. [“Patterns of Adaptive Genetic Variation Across Coffea Canephora”](#)  
[Valerie Poncet- PhD Researcher, UMR DIADE Centre IRD de Montpellier](#)
12. “Observed Climate Trends and Impacts on Global Coffee Production”  
Christian Bunn- Postdoctoral Fellow, Decision and Policy Analysis Research Area, International Center for Tropical Agriculture (CIAT)

*Category: Farm Management, Chair: Dr. Laurence Jassogne*

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13. [“Management of African White Coffee Stem Borer, Monochamus Leuconotus, Pascoe & Outbreak of Invasive Coffee Pests in Tanzania: Could it Be An Effect of Climate Change?”](#)  
[Fredrick Magina- Tanzania Coffee Research Institute \(TaCRI\)](#)

14. ["Economic Feasibility of Six Smallholder Coffee Farming Associations Implementing the Centroamericano Coffee Hybrid in San Pedro Yepocapa, Guatemala"](#)  
[Taya Brown- Ph.D. student in the Horticulture Department at Texas A&M University](#)
15. ["Economic Analysis of Coffee Farming Systems In Tanzania"](#)  
[Leonard Kiwelu- Tanzania Coffee Research Institute \(TaCRI\)](#)
16. ["Use of Plant Growth Regulators to Promote Coffee Flowering to Aid Coffee Harvest and Sanitation For CBB Control"](#)  
[Tracie Matsumoto- Research Leader USDA, ARS, Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center, Tropical Plant Genetic Resources and Disease Research Unit](#)
17. ["The Case for Integrating Soil Science in Coffee Research and Management"](#)  
[Andrew Margenot, PhD- Assistant Professor of Soil Science, Crop Sciences Department, University of Illinois Urbana-Champaign](#)
18. ["Effects of Weed Control Methods in Coffee Crop on Quality of Coffee Beverage After Ten Years."](#)  
[Elifas Nunes de Alcântara- Mina Gerais Agricultural Research Company Brazil](#)

*Category: Roasted Coffee Technology & Processing, Chair: Jim Coughlin*

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19. ["Keynote: The Science of Coffee Freshness"](#)  
[Samo Smrke, Scientific Associate, University of Zurich](#)
20. ["Screening of Parameters Impacting Capsule Coffee Extraction"](#)  
[André EIERMANN- UCC Coffee Switzerland](#)
21. ["Coffee Galactomannans Extraction: From Ground Coffee To Soluble Powders Micronization"](#)  
[Claudia Passos- University of Aveiro Portugal](#)
22. ["The Effect of Water Pulsing Duty Cycle on the Sensory Parameters of Drip Brewed Coffee"](#)  
[Scott Frost- Postdoctoral Scholar, UC Davis Coffee Center](#)

*Category: Coffee Chemistry & Sensory Sciences, Chair: Dr. Valerie Leloup*

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23. ["Keynote: Reinventing the Wheel: the Coffee Taster Flavor Wheel and its Application"](#)  
[Jean Xavier Guinard, Professor University of California, Davis](#)
24. ["Descriptive Sensory Analysis of Drip Brew Fractions to Evaluate Time-Evolution of Coffee Flavor Extraction"](#)  
[Mackenzie Batali- Graduate Student Researcher, University of California, Davis](#)
25. ["Cup of Excellence- Case Study, Trends and Outcomes"](#)  
[Darrin Daniel, Executive Director, Alliance for Coffee Excellence](#)
26. ["Is it Still Necessary: Deepening the Knowledge on 3-alkyl-2-Methoxypyrazines? A Study to Unravel Their Role on Coffee Quality"](#)  
[Dr. Valentina Lonzarch, Illy Café](#)
27. ["Smart Fuzzy Cupper: Employing Approximate Reasoning to Derive Coffee Bean Quality Scoring From Individual Attributes"](#)  
[Javier Livio- Auburn University](#)

*Official poster session – see Section 2 for listing and Section 4 for abstract of Poster Contribution*

28. “Keynote: Coffee Flavor Generation- New Insight in the Role of Precursors”Tomas Davidek, Nestec Ltd., Nestlé Product Technology Centre Beverage, Orbe, Switzerland
29. [“A Flavoromics Untargeted LC/MS Analytical Approach in Determining Key Chemical Markers in Green and Roasted Coffee Beans that Predict the Coffee Brew Cup Score”](#)  
Devin Peterson- Ohio State University
30. [“Methyl Esterified-Components Determined Coffee Flavor Quality”](#)  
Keiko Iwasa- Suntory Global Innovation Center
31. [“Sensory and Analytical Study of Different Coffee Origins as Support for Flavor Creation”](#)  
Maurin Cornuz- National University of Singapore
32. [“Resolving Coffee Roasting Phases By Vacuum Pho-toionization TOF-MS Process Monitoring: Towards Coffee With Optimized Aroma Profile and Antioxidant Capacity”](#)  
Ralf Zimmermann- Full Professor of Analytical Chemistry, Institute of Chemistry, University of Rostock Germany
33. [“Investigating Bitter Substances in Coffee Brews By Analyzing the Response to Taste Sensor”](#)  
Hirofumi Fujimoto- UCC Ueshima Coffee Co, Hyogo, Japan
34. [“Advanced Instrumental Characterization of the Coffee Extracts Produced By Pilot Scale Instant Coffee Process”](#)  
Anna Maria Sulewska- PhD, Industrial Post. Doc.
35. [“Coffee Aroma: Essential Role of Oxidative Maillard Reaction Pathways”](#)  
Anja Rahn- Zurich University of Applied Sciences (ZHAW)
36. [“Keep it Cold: An Investigation of the Shelf Life of Cold Brew Coffee and the Influence of Extraction Temperature Using Chemical, Microbial and Sensory Analysis”](#)  
Samuel Lopane- Researcher and Graduate Teaching Assistant, Food, Nutrition, and Packaging Sciences Dept. Clemson University
37. [“Coffee Brews With Espresso Properties by Modulation of the Carbohydrate Content of Coffee Extracts”](#)  
Guido R. Lopes- PhD Student, Portugal
38. [“In-Bean Experiments: Are They Reliable Enough to Study Coffee Flavor Formation?”](#)  
Luigi Poisson, PhD, R&D Expert, Coffee Flavour Nestle
39. [“New Physicochemical Quality Indicator for Specialty Coffee”](#)  
Toshihide Horiguchi- Horiguchi Coffee
40. “Science: The Power Behind Coffee's Growth”  
Andrea Illy

#### *Coffee & Health*

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41. “Coffee & Human Health”  
Rodrigo Cunha, CNC-Center for Neuroscience and Cell Biology, Faculty of Medicine, University of Coimbra, Portugal

42. “Coffee is THE Health Beverage”  
Dr. James Coughlin, President & Founder of Coughlin & Associates: Consultants in Food/Nutritional/Chemical Toxicology & Safety & Anja Rahn, Research Associate, ZHAW

### *Genetics & Breeding*

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43. “Applications and Regulation of CRISPR System Mediated Targeted Mutagenesis in Agriculture”  
Dr. Agnes Riccroch, AgroParisTech
44. “Conserve Coffee Genetic Resources”  
Sarada Krishnan, Director of Horticulture and Center for Global Initiatives, Denver Botanic Gardens
45. “Battling Coffee Leaf Rust Through Genetics & More”  
Christophe Montagnon, Scientific Director, World Coffee Research
46. “Panel Discussion: Genetics and Breeding Roundtable Discussion”  
Moderator: Peter Giuliano, Chief Research Officer, Specialty Coffee Association. Panelists: Chifumi Nagai, Senior Research Scientist, Plant Breeding and Biotechnology at Hawaii Agriculture Research Center (HARC ), Surya Prakash Nayani, Head of Department, Central Coffee Research Institute, Coffee Board of India
47. [“A Comprehensive Estimate of Global Coffee Farm Populations”](#)  
[David Browning- CEO, Enveritas](#)
48. “Economic Feasibility of Six Smallholder Coffee Farming Associations Implementing the Centroamericano Coffee Hybrid in San Pedro Yepocapa, Guatemala”  
Taya Brown- Ph.D. student in the Horticulture Department at Texas A&M University
49. [“Observed Climate Trends & Global Coffee Production”](#)  
[Christian Bunn, Postdoctoral Fellow, CIAT](#)

### *Science of Quality*

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50. “Post Harvest Theory & Practice”  
Flavio Borem, Full Professor, Federal University of Lavras
51. “Reinventing the Wheel: The Coffee Taster Flavor Wheel and its Application”  
Jean Xavier Guinard, Professor University of California, Davis
52. “From Numbers to Grading Words”  
Javier Livio- Auburn University
53. “Decoding the Journey of Coffee from Cherries to Cup- A Multiphasic Approach”  
Florac De Bruyn- PhD Researcher, Research Group of Industrial Microbiology and Food Biotechnology, Department of Bioengineering Sciences, Vrije Universiteit Brussel & Sophia Jiyuan Zhang- Doctoral Researcher, Research Group of Industrial Microbiology and Food Biotechnology
54. “Humans Making Coffee: On How an Anthropological Perspective Could Help the Coffee Industry”  
Javier Morales Vargas, Anthropology Master Student, Costa Rica University
55. “Cold Brew Shelf Life and Extraction Temperature: What it Means and Why it Matters”  
Samuel Lopane- Researcher and Graduate Teaching Assistant, Food, Nutrition, and Packaging Sciences Dept. Clemson University

*Category: Plant Science, Chair: Dr. Benoit Bertrand*

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56. ["Keynote: Growth and Metabolic Plasticity of Photosynthetic Organisms"](#)  
Ronan Sulpice, Dr., NUI Galway
57. ["Full Genome Sequence Assemblies and Annotations of Coffea Arabica and its Two Diploid Parents"](#)  
[Alexandre de Kochko- Ph.D.- Evolution of Coffea Genomes, UMR DIADE](#)
58. ["Genome-Wide Association Study Identify SNPs and Genomic Regions for Lipids and Diterpenes Contents in Coffea Arabica Related to its Domestication"](#)  
[Caroline Ariyoshi- Universidade Estadual de Londrina](#)
59. ["CRISPR/Cas9-Mediated Efficient Targeted Mutagenesis Has the Potential to Accelerate the Domestication of Coffea Canephora"](#)  
[Jean-Christophe Breitler- Cirad](#)
60. ["Wide C. Arabica Genetic Study Brings New Insights On Movements and Breeding History of the Species"](#)  
[Solène Pruvot-Woehl- Project Leader, World Coffee Research](#)
61. ["Introgression of Coffea canephora Genome in Ruiru 11 Sibs and its Effect on Quality and CBD Resistance"](#)  
[Bernard Gichimu- University of Embu, Embu, Kenya](#)

**Chair: Dr. Andre Charrier**

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62. ["New Assesments of the Coffea Canephora Genetic Diversity and Structure on Wild and Cultivated Accessions Using SSR and SNP Markers"](#)  
[Dominique Crouzillat- Head of Support Science, R&D Nestlé Tours](#)
63. ["Coffee Somatic Embryogenesis, a Model to Decipher Fundamental Mechanisms Associated to Totipotency, Somaclonal Variation and Photo-Autotrophy Acquisition"](#)  
[Hervé Etienne- Cirad](#)
64. ["The Global Conservation Strategy for Coffee Genetic Resources"](#)  
[Sarada Krishnan- Coffee genetic resource conservation, Denver Botanic Gardens](#)
65. ["Is Coffee Flowering the Bottleneck in Climate Change Adaptation?"](#)  
[Eric Rahn- International Center for Tropical Agriculture](#)
66. ["Contributions to the Conservation of Genetic Resources of Coffee in the Democratic Republic of Congo"](#)  
[Piet Stoffelen- Meise Botanic Garden, Belgium](#)
67. ["Association study of tree size and male sterility in a F2 Coffea Arabica population."](#)  
[Lucile Toniutti- World Coffee Research](#)

*Session: Plant Science Continued, Chair: Dr. Maria De Ceu Silva*

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68. ["Determination of the Chemical Composition of Green and Roasted Coffee of Different Coffee Genotypes as a Complementary Method of Phenotyping"](#)  
[Noel Arrieta- Instituto Del Café De Costa Rica](#)

69. [“The Greater Phenotypic Homeostasis of the Allopolyploid Coffea arabica Improved the Transcriptional Homeostasis Over that of Both Diploid Parents”](#)  
[Benoit Bertrand- Cirad](#)
70. [“Physiological Plasticity: A Key Element of Coffee Hybrids to Face Leaf Rust Disease Attack”](#)  
[Juan Carlos Herrera - Nestlé R&D Center, Plant Science Research Unit](#)
71. [“Towards the Identification of Candidate Gene Nucleic Polymorphisms to Predict the Adaptedness of Ugandense C. Canephora Populations to Climate Change”](#)  
[Valerie Poncet- PhD Researcher, UMR DIADE Centre IRD de Montpellier](#)
72. [“Limonene: A Target For Coffea Arabica Aromatic Quality Breeding”](#)  
[Christophe Montagnon- Scientific Director, World Coffee Research](#)
73. “An assessment of the genetic diversity of the cultivated Ethiopian variety, Gesha, grown within the Hacienda La Esmeralda Farm in the Chiriquí Province of Panama”  
Stephanie Alcalá- Graduate Researcher, University of Michigan

*Category: Plant Pathology & Protection, Chair: Prof. Dr. Girma Adugna*

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74. “Keynote: Introduction to the Biology and Evolution of Rust Fungi and the Coffee Leaf Rust Pathogen”  
[M. Catherine Aime, Professor and Director of the Arthur and Kriebel Herbaria, Purdue University](#)
75. [“Pathogenomics of Coffee Rust: New Insights and Future Challenges”](#)  
[Dora Batista- Instituto Superior de Agronomia, Universidade de Lisboa, Portugal](#)
76. [“Early Activation of Defence-related Genes and POD and PPO Activities are Associated with Coffee Resistance to Colletotrichum Kahawae”](#)  
[Maria do Céu Silva- Researcher, CIFC](#)
77. [“Genome-Wide Association Study for Pseudomonas syringae pv. garcae resistance in Coffea arabica L.”](#)  
[Caroline Ariyoshi- Universidade Estadual de Londrina](#)



**SECTION 2: Overview of Posters Contributions**

1. [AMELIORATION OF COLD INDUCED STRESS IN COFFEE \(Coffea arabica L.\) SEEDLINGS BY TiO<sub>2</sub> NANOPARTICLES](#)  
[Robert Acidri, Tottori University](#)
2. [SEEKING TO GENERATE GENETIC DIVERSITY IN ARABICA COFFEA TO OBTAIN BETTER TRAITS OF RESISTANCE TO PESTS AND DISEASES THROUGH THE USE OF MUTATION INDUCTION](#)  
[Noel Arrieta, Costa Rican Coffee Institute - Center Research](#)
3. [GENOMIC SELECTION IN BREEDING POPULATIONS OF Coffea arabica](#)  
[Eveline Caixeta, Universidade Federal de Viçosa - UFV, Instituto de Biotecnologia](#)
4. DNA traceability on Arabica using SNP markers from field to end products  
[Emmanuelle Morel, Nestlé R&D Centre Plant Science Research Unit](#)
5. [ARABICA GENOME MANUAL ANNOTATION USING A COLLABORATIVE PLATFORM : WEBAPOLLO](#)  
[Rudy Aussel, Nestlé R&D Centre Plant Science Research Unit](#)
6. [TECHNOLOGICAL PERFORMANCE OF PROMISING COFFEA ARABICA VARIETIES FOR SPECIALTY COFFEE PRODUCTION IN BRAZIL](#)  
[Gerson Giomo, Instituto Agronômico, Av. Barão de Itapura](#)
7. [Identification of physiological traits and equipments to select Coffea canephora for drought tolerance](#)  
[Juan Carlos Herrera, Nestlé R&D Centre Plant Science Research Unit](#)
8. [EVALUATION OF GENOTYPES OF COFFEA ARABICA L. FROM THE GERMPASM ACTIVE BANK OF MINAS GERAIS TO PRODUCE SPECIALTY COFFEE](#)  
[Marcelo Malta, Universidade Federal de Lavras, Brazil](#)
9. [EVALUATION OF ELITE GENOTYPES OF COFFEA ARABICA L. FROM THE BREEDING PROGRAM OF EPAMIG AIMING TO OBTAIN SPECIALTY COFFEES](#)  
[Marcelo Malta, Universidade Federal de Lavras, Brazil](#)
10. [Analysis of genetic diversity and population structure of Arabica coffee \(Coffea arabica L.\) germplasm available in Indian gene bank using SRAP markers](#)  
[Manoj Kumar Mishra, Central Coffee Research Institute, India](#)
11. [GENETIC PERFORMANCE OF ROBUST COFFEE PROGENIES RESULTING FROM PHENOTYPIC SELECTION](#)  
[Júlio César Mistro, Centro de Café - IAC](#)
12. [SELECTION OF BOURBON COFFEE GENOTYPES IN BRAZIL](#)  
[Júlio César Mistro, Centro de Café - IAC](#)
13. [Coffea arabica var. laurina: in vivo volatile organic compounds \(VOCs\) release under water deficit stress](#)  
[Luciano Navarini, illy caffè S.p. A](#)
14. [GENETIC EXPRESSION OF DEFENSE-RELATED SEQUENCES IN TOLERANT AND SUSCEPTIBLE PLANTS TO Ceratocystis fimbriata.](#)  
[Daniel Ramírez Valerio, Instituto del café de Costa Rica- ICAFE](#)

15. [TECHNOLOGICAL PERFORMANCE OF PROMISING YELLOW BOURBON VARIETY FOR SPECIALTY COFFEE PRODUCTION IN BRAZIL](#)  
[Lucicléia Romano, Instituto Agronômico de Campinas \(IAC\)](#)

Plant Pathology & Protection

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16. [Current spatio temporal dynamics of coffee pests in Kenya](#)  
[Getrude Alwora](#)
17. [BACCHARIS GLUTINOSA \(CHILCA\) ROOTS EXTRACT. EFFECT ON HEMILEIA VASTATRIX.](#)  
[Helena Azinheira, Instituto Tecnológico from Tuxtla Gutiérrez, Chiapas, Mexico](#)
18. [CYTOGENETIC AND TRANSCRIPTOMIC APPROACHES TO UNDERSTAND THE NUCLEAR CYCLE OF HEMILEIA VASTATRIX](#)  
[Helena Azinheira, Instituto Tecnológico from Tuxtla Gutiérrez, Chiapas, Mexico](#)
19. [NOVEL INSIGHTS ON COLONIZATION ROUTES AND EVOLUTIONARY POTENTIAL OF COLLETOTRICHUM KAHAWAE](#)  
[Ana Viera, Universidade de Lisboa, Oerias, Portugal](#)
20. [COFFEE LEAF RUST \(Hemileia vastatrix Berk. & Br\) ON ARABICA COFFEE \(Coffea arabica L.\) AT ITS CENTRE OF ORIGIN, ETHIOPIA](#)  
[Kifle Belachew, Ethiopian Coffee Science Society](#)
21. [RESISTANCE SCREENING OF RELEASED COFFEE VARIETIES AGAINST COFFEE THREAD BLIGHT \(Corticium koleroga\) AT SOUTHWEST ETHIOPIA](#)  
[Kifle Belachew, Ethiopian Coffee Science Society](#)
22. [CARACTERIZATION OF CERATOCYSTIS SPP. FROM THE SIX COFFEE REGIONS OF COSTA RICA](#)  
[María José Cordero Vega, Costa Rican Coffee Institute, Research Coffee Center](#)
23. [PARTIAL RESISTANCE TO NEMATODE Meloidogyne paranaensis IN HÍBRIDO DE TIMOR COFFEE GENOTYPES](#)  
[Dhalton Ito, Instituto Agronômico do Paraná - IAPAR](#)
24. [THE CURRENT COFFEE WILT DISEASE UPSURGE AND OCCURANCE IN HOTSPOTS IN UGANDA IS DUE TO SEXUAL STAGE OF THE PATHOGEN, Gibberella xylarioides](#)  
[Sammy Olal, Uganda](#)
25. [ARABICA COFFEES FROM ETHIOPIA WITH RESISTANCE TO NEMATODE Meloidogyne paranaensis](#)  
[Gustavo Sera, Instituto Agronômico do Paraná - IAPAR](#)
26. [RESISTANCE TO RED MITE IN Coffea arabica GENOTYPE INTROGRESSED WITH Coffea racemosa GENES](#)  
[Tumoru Sera, Instituto Agronômico do Paraná - IAPAR](#)
27. [Assessing the disease resistance performance of compact coffee selections in Tanzania](#)  
[Josephine Urasa, Tanzania Coffee Research Institute \(TaCRI\)](#)
28. [CHARACTERIZATION OF PHYSIOLOGICAL RACES AND ANALYSIS OF EFFECTIVE CANDIDATE PROTEINS IN POPULATION OF HEMILEIA VASTATRIX IN BRAZIL](#)  
[Laércio Zambolim, Universidade Federal de Viçosa - UFV, Brazil](#)

29. [DIVERSITY AND GENE PYRAMIDING FOR RESISTANCE TO COFFEE BERRY DISEASE AND COFFEE LEAF RUST IN HIBRIDO DE TIMOR GENOTYPES](#)  
[Laércio Zambolim, Universidade Federal de Viçosa - UFV, Brazil](#)

#### Farm Management

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30. [Assessment of Refine Granulated Borate \(Granubor\) as Fertilizer Influencing Coffee Productivity in Brazilian Coffee](#)  
[Marcel Barbier](#)
31. [ESTABLISHING AGROFORESTRY SYSTEMS IN HAWAI'I USING ENDEMIC HARDWOOD SPECIES AND SELECTED VARIETIES OF COMMERCIAL COFFEE](#)  
[Juli Burden, Hawai'i Agriculture Research Center](#)
32. [MANAGEMENT OF GENETIC RESOURCES IN THE COMMERCIAL COFFEE PRODUCTION IN BRAZIL](#)  
[Dr. Julio Cesar Mistro, Agronomic Institute, Campinas, Brazil](#)
33. [MANAGEMENT OF INPUTS IN THE AGRICULTURAL COFFEE PRODUCTION IN BRAZIL](#)  
[Dr. Julio Cesar Mistro, Agronomic Institute, Campinas, Brazil](#)
34. [F1 Hibrids response to fertilizer application in the Central Valley of Costa Rica](#)  
[Víctor Manuel Chaves Arias, Instituto del café de Costa Rica- ICAFE](#)
35. [EXPLORING THE INTEGRATED SOIL FERTILITY MANAGEMENT APPROACH OF SAFERNAC MODEL IN A TANZANIAN COFFEE ECOSYSTEM AND THE ROLE OF ORGANIC ADDITIVES](#)  
[Godsteven Maro, Tanzania Coffee Research Institute \(TaCRI\)](#)
36. [SOIL FERTILITY EVALUATION FOR COFFEE IN POTENTIAL AREAS OF EASTERN ZONE, TANZANIA](#)  
[Godsteven Maro, Tanzania Coffee Research Institute \(TaCRI\)](#)
37. [Behaviour of phosphorus in relation to its absorption, translocation and use efficiency in four varieties of Coffea canephora](#)  
[Almas Hamadi, Tanzania Coffee Research Institute \(TaCRI\)](#)
38. [EFFECTS OF SEEDLING MULTIPLICATION METHODS ON GROWTH AND YIELD OF NEW TALL AND COMPACT coffea arabica VARIETIES IN TANZANIA](#)  
[Suzana Mbwambo, Tanzania Coffee Research Institute \(TaCRI\)](#)
39. [RESPONSE FUNCTION OF COMPACT Coffea arabica VARIETIES TO N,P AND K NUTRIENTS AT HIGH PLANTING DENSITY](#)  
[Suzana Mbwambo, Tanzania Coffee Research Institute \(TaCRI\)](#)
40. [Effects of cutting position along mother plants on rooting of hybrid coffee varieties](#)  
[Jeremiah Magea, Tanzania Coffee Research Institute \(TaCRI\)](#)
41. [RESPONSE OF DIFFERENT APPLICATION RATE OF DOLOMITIC LIME ON COFFEE SEEDLING BRANCHES INCREASE IN ACID SOILS OF MBOZI DISTRICT, TANZANIA](#)  
[Dismas Pangalas, Tanzania Coffee Research Institute \(TaCRI\)](#)
42. [Evaluating water stress tolerance among Coffea arabica cultivars](#)  
[Emily Pappo, University of Florida](#)

43. [KNOWLEDGE REPRESENTATION OF THE SPECIALTY COFFEE AGRIBUSINESS SYSTEM AND ITS IMPACTS ON THE IMPROVEMENT OF THE FINAL PRODUCT](#)  
[Eduardo Trauer, Federal University of Santa Catarina, Brazil](#)
44. [A new method for fertilizing coffee trees: the “Fertexpert-Café” system](#)  
[Phillipe Vaast, Cirad,UR Systèmes de Pérennes](#)
45. [IMPACT OF RECENT LARGE SCALE CONVERSION OF INTENSIVE MONOCULTURE COFFEE SYSTEMS TOWARDS SHADED SYSTEMS ON SOIL FERTILITY IN YUNNAN PROVINCE, CHINA](#)  
[Phillip Vaast, Cirad,UR Systèmes de Pérennes](#)

#### Biochemistry & Biotechnology of Green Coffee

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46. [Quantitative Ochratoxin Detection in Green Coffee in 10 Minutes Without Using An Organic Solvent Extraction](#)  
[John Davenport, VICAM](#)
47. [Impact of agro-forestry systems on coffee yield, coffee plant morphology, physical and chemical attributes of green coffee beans and aroma generation of roasted coffee beans](#)  
[Su Xu, University of Nottingham](#)
48. [IMPACT OF STORAGE IN MODIFIED ATMOSPHERES OF GREEN COFFEE BEANS \(Coffea arabica L.\) ON THE QUALITY MARKERS](#)  
[Oscar Gonzalez-Rios, Tecnológico Nacional de México](#)
49. [SENSORY AND BIOCHEMICAL FINGERPRINTING OF COMMERCIAL COFFEE VARIETIES UNDER DIFFERENT GEOGRAPHICAL CONDITIONS IN KENYA](#)  
[Cecilia Kathurima, Coffee Research Institute of Kenya Agricultural Research Organization \(KALRO\).](#)
50. [EFFECT OF VARIETY, SHADE AND ALTITUDE ON SENSORY COMPONENTS OF COFFEE: MURANGA COUNTY, KENYA](#)  
[Cecilia Kathurima, Coffee Research Institute of Kenya Agricultural Research Organization \(KALRO\)](#)
51. [COFFEE CHEMICAL COMPOSITION PRODUCED IN AGROFORESTRY SYSTEMS AND FULL SUN IN SOUTHERN BRAZIL](#)  
[Heverly Morais, Instituto Agronômico do Paraná - IAPAR](#)
52. [INFLUENCE OF THE LEVEL OF SHADING ON THE PROFILE OF CHLOROGENIC ACID ISOMERS AND PHYSICOCHEMICAL COMPOSITION OF COFFEE CONSORTIUM WITH HAVEA BRASILIENSIS](#)  
[Heverly Morais, Instituto Agronômico do Paraná - IAPAR](#)
53. [PROCESSING METHODS IN ROBUSTA VALUE CHAIN IN UGANDA](#)  
[Saleh Nakendo, Africa Coffee Bureau \(ACB\)](#)
54. [OIL BODIES AND OLEOSINS IN Coffea arabica L. SEEDS: EXPERIMENTAL COMPARISON OF TWO METHODS FOR THEIR EXTRACTION AND PURIFICATION](#)  
[Luciano Navarini, illy caffè S.p. A](#)
55. [STUDY OF STRAINS OF THE GENUS Aspergillus SECTION Nigri PRODUCERS OF OCHRATOXIN A \(OTA\) ASSOCIATED WITH THE COFFEE PRODUCTION \(Coffea arabica\)](#)  
[Mirna Leonor Suarez Quiroz, NSTITUTO TECNOLOGICO DE VERACRUZ](#)

56. [Soil carbon dynamics in coffee-avocado intercropping in Coastal California](#)  
[Stephanie Alcala](#)
57. [IMPACTS OF CLIMATE CHANGE IN THE CLIMATE QUALITY INDEX IN ARABIC COFFEE: A CASE STUDY IN THE STATE OF SÃO PAULO, BRAZIL](#)  
[Eduardo Alfonsi, University of Campinas \(UNICAMP\), Brazil](#)
58. [POTENTIAL OF SEVERITY TO COFFEE RUST DISEASE OF COFFEE CROP IN BRAZIL, IN THE SCENARIO OF HIGH CO2 EMISSIONS](#)  
[Waldenilza Alfonsi, University of Campinas \(UNICAMP\), Brazil](#)
59. [PROFILE OF WOMEN IN THE COFFEE PRODUCTION CHAIN IN THE MUNICIPALITY OF BOM SUCESSO, MINAS GERAIS, BRAZIL](#)  
[Luiza Andrade Zenith, Instituto Agronômico de Campinas, Brazil](#)
60. [SUSTAINABILITY LABELS: A COMPETITIVE STRATEGY IN BRAZILIAN COFFEE PRODUCTION](#)  
[Dr. Julio Cesar Mistro, Agronomic Institute, Campinas, Brazil](#)
61. [GREENHOUSE GAS EMISSIONS COMPARISON FOR TWO TECHNOLOGIES TREATMENT OF COFFEE PULP IN COSTA RICA](#)  
[Rolando Chacon, Instituto del café de Costa Rica- ICAFE](#)
62. [THE DYNAMICS OF LAND COVER CHANGES IN BRAZILIAN COFFEE AREAS](#)  
[Priscila Coltri, University of Campinas \(UNICAMP\), Brazil](#)
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## **SECTION 3: Abstracts – Oral Presentations**



## GENOTYPOGRAM OF COFFEE AND CAFFEINE CONSUMPTION AND RELATED GENETIC TRAITS

SANTOS, Roseane M.M.\* , LIMA, Darcy R.A.\*\*†

\*South University School of Pharmacy, Savannah, GA, USA, \*\*Institute of Neurology Deolindo Couto, Rio de Janeiro, BR.

**RATIONALE** Regular coffee intake has been associated with reduced risk of developing important chronic diseases such as type-2 diabetes, Parkinson's, Alzheimer's, liver and colon cancer. Genomic wide association studies (GWAS) on coffee and caffeine consumption have identified single nucleotide polymorphisms (SNPs) at the aryl hydrocarbon receptor region (AHR) and between CYP1A1 and CYP1A2 gene region that present significant association with habitual caffeine and coffee consumption [1]. The association of genotypes found in high frequency within the population of coffee consumers might shed light toward new targets for the treatment of the diseases that regular coffee intake showed to promote preventive effects. Our study was focused in the identification of these associations within a population from a previous pilot study.

**METHODS** Sixteen SNPs with the highest hits of association from GWAS on coffee and caffeine consumption were selected for this study. The study population was 13 DNA samples obtained on previous pilot study with 15 healthy volunteers that received a single cup of coffee and had caffeine plasma levels and CYP 1A2 genotype (rs762551) determined [2]. SNPs were detected by real-time restriction-fragment length polymorphism-polymerase chain reaction (TaqMan Genotyping Assay, Applied Biosystems, Carlsbad, CA). The data analyzed using TaqMan Genotyping Software.

**RESULTS** Our findings are summarized in a novel format to display results from genotyping data of SNPs that showed significant expression for a specific health condition arranged in a color-coded trait. It was named genotypogram, and from our knowledge this is the first time genotype and associated traits are displayed under this unique format. Our data confirmed the GWAS studies on the SNPs in the intergenic region between CYP1A1-1A2 (rs2470893 and rs2472297). It was found to have a direct (positive) relationship between genotypes, meaning that volunteers presented either the same heterozygous genotype (5/13) or mutant (5/13) for both SNPs. Other positive and inverse (negative) associations were found between the 16 SNPs and it was also determined the predominance of wild-type or mutant alleles in the population.

**CONCLUSIONS & PERSPECTIVES** Our study crosses information of genotypes from the same subject with sixteen SNPs previously appointed as significantly expressed during GWAS studies of coffee and caffeine consumption. A novel format to display genotyping data was developed allowing making assumptions about the association between the SNPs that are highly expressed within the coffee consumer's population. We intend to extend this study to a bigger sample population and hopefully this can lead us to the discovery of new targets to treat chronic diseases such as the one's that coffee and caffeine consumption has shown to prevent.

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Emmanuel V. Garcia\* and Carlito B. Lebrilla\*\*,

\*Chemistry Department, De La Salle University, Philippines

\*\* Department of Chemistry, University of California-Davis, USA

\*emmanuel.garcia@dlsu.edu.ph

## RATIONALE

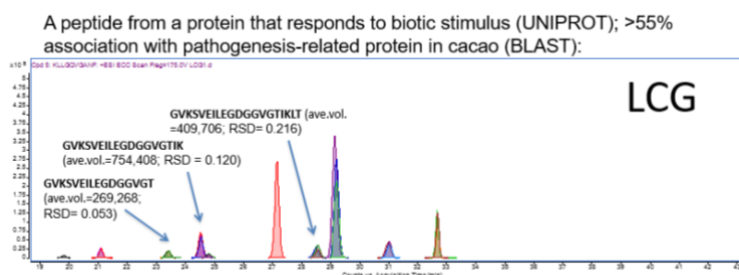
Civet coffee, obtained from the feces of civet cats feeding on coffee cherries, is an exotic, high-priced beverage, whose global demand has been steadily increasing inspite of issues regarding counterfeiting, animal abuse, and disease spread. Thus, physicochemical characterization and authentication studies have become necessary.

## METHODS

In this study, separation with mass spectrometric methods for the analyses of metabolites were carried out using GC-MS, while for endogenous oligosaccharides and peptides, LC-MS methods were developed and used.

## RESULTS

Results showed that two metabolites (2-ketoadipic acid and pentitol) and three peptides associated with a pathogenic-response protein (GVKSVEILEGDGGVGTIKLT, GVKSVEILEGDGGVGTIK, and GVKSVEILEGDGGVGT) may serve as potential bioamarkers.



## CONCLUSIONS & PERSPECTIVES

Metabolomics is very comprehensive in terms of unraveling biomarkers due its nature of covering a wide range of compounds involved in the analysis. However, as was also previously reported, metabolites are far more affected by other factors such as, in decreasing impact, roasting, coffee specie/variety, and geographic origin, than animal perturbation. Also, even if biomarking metabolites are found for civet coffee, it would be again be easy to tamper with beans and make them seem authentic since such small molecules are easy to purchase or make.

Glycomics, as the method development experiments have so far revealed, is interesting but does not hold promise for exposing biomarkers since the variety of oligosaccharides are extremely limited by the existence of only few polysaccharides where these may come from. Unless there are enzymes or microbes in the civet cat's gut that uniquely cleaves polysaccharides, and at much more pronounced impact than thatof roasting, glycan profiles may not be different for civet coffee beans and their non-civet coffee counterparts.

Proteomics and peptidomics, meanwhile, appear to be the most promising methods since these reveal very specific compounds for civet and non-civet coffee beans that are also much more difficult to tamper. However, these are only limited to green (unroasted) beans since practically all protiens, peptides, and amino acids are lost during the roasting process.

The most vital recommendation would be to carry out these methods on as many samples from various, if not all, parts of the world where this exotic coffee can be harvested for at least three harvest season in order for results to be universally conclusive.

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FROM CHERRY TO CUP:  
MULTIPHASIC ASSESSMENT OF WET COFFEE PROCESSING  
THROUGH BEANS, WATERS, AND CUP QUALITY

ZHANG, Sophia J.\*, DE BRUYN, Florac\*, POTHAKOS, Vasileios \*, TORRES, Julio \*\*, MOCCAND, Cyril \*\*, WECKX, Stefan \*, DE VUYST, Luc \*

\*Research Group of Industrial Microbiology and Food Biotechnology, Vrije Universiteit Brussel, Brussels, Belgium, \*\*Nestlé Research Centre, Lausanne, Switzerland.

#### RATIONALE

Wet processing is a commonly applied method to transform Arabica coffee cherries into green coffee beans before they are distributed worldwide. Coffee producers have endeavoured to fine-tune the operational parameters to produce a satisfying green bean quality and delightful cupping experience. However, despite the vigorous empirical tryouts, limited scientific knowledge is available to back up these practices. Therefore, more research is needed to decode the links between the processing practices with coffee bean composition and cup quality.

#### METHODS

A large-scale wet processing experiment was set up in a high-altitude Ecuadorian farm to process Arabica coffee cherries through standard (16 h) and extended (64 h) fermentation. Coffee beans and processing water were sampled along each step. A multiphasic assessment was applied, including an in-depth microbiological evaluation (culture-dependent, amplicon and shotgun sequencing) and a comprehensive metabolomic analysis (based on LC and GC), to monitor the evolution of the microbial diversity and chemical composition in the processing system. The final coffee cup quality was evaluated by a trained panel.

#### RESULTS

Fermentation presented a dynamic interaction between constant nutrient release (mainly from mucilage) and microbial activity in the water. The prevalent microbial groups (mainly lactic acid bacteria) shifted when the fermentation water changed from a sugar-rich to an acidic environment upon extended fermentation, whereby lactobacilli succeeded leuconostocs. Such microbial activities resulted in a fast build-up of metabolites (*e.g.*, lactic acid, mannitol, and esters) in the water as well as gradual accumulation on the beans. Washing and soaking steps tempered such accumulation effects, whereby the compounds partially migrated from the beans to the soaking water and microbial fermentation occurred. Concurrently, the endogenous bean metabolism was active throughout processing, as supported by changes in carbohydrate, organic acid, free amino acid, and  $\gamma$ -aminobutyric acid concentrations. The resulting green coffee beans showed different compositional profiles. Their brewed cups exhibited distinct notes on fruitiness and totally intensity without off-flavours.

#### CONCLUSIONS & PERSPECTIVES

The current study presented a close-up evaluation of each step during wet coffee processing. Through fine-tuning of the parameters at each step, the microbial diversity and endogenous bean metabolism could be altered accordingly, hence providing a great potential to improve coffee quality. Such knowledge will be valuable for all stakeholders of the coffee value chain.

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DEMUCILAGING OR DEPULPING?  
IMPLICATIONS FOR MICROBIOTA, COFFEE COMPOSITION,  
AND CUP QUALITY

DE BRUYN, Florac\*, ZHANG, Sophia J.\*, POTHAKOS, Vasileios\*, CONTRERAS, Gonzalo, F.\*\*,  
MOCCAND, Cyril\*\*, WECKX, Stefan\*, DE VUYST, Luc\*

\*Research Group of Industrial Microbiology and Food Biotechnology, Vrije Universiteit Brussel,  
Brussels, Belgium, \*\*Nestlé Research Centre, Lausanne, Switzerland.

## RATIONALE

Wet-processed coffee is appreciated for its distinct flavor. This flavor results from an interaction between a microbial ecosystem and coffee beans during an underwater fermentation. However, wet processing coffee is resource-intensive. To reduce water usage and boost sustainability of this process, coffee beans can be demucilaged mechanically before fermentation. Still, there is little scientific data on the effect demucilaging has on the microbial ecosystem and how this interplays with coffee bean composition and cup quality.

## METHODS

A large-scale wet processing was performed that consisted of a depulped (with mucilage) and demucilaged (without mucilage) fermentation on an Arabica coffee farm in Yunnan, China. In each process, processing parameters (fermentation and soaking duration) were varied to assess their impact on the microbial ecosystem, coffee bean composition, and coffee cup quality. The microbial ecosystem was followed by selective plating and amplicon sequencing, and the coffee bean composition was analyzed with various chromatography and detection techniques. The resulting coffees were assessed by a trained panel.

## RESULTS

The overall microbial diversity was similar in both fermentation types and predominantly consisted of lactic acid bacteria. Still, specific taxa were preferentially associated with demucilaged or depulped fermentation. Other microbial groups were only transiently present. The coffee bean composition differed in both fermentation types and was affected by fermentation duration, washing, and soaking duration. Yet, differences in the microbial compounds were tempered post-fermentation by washing and soaking. The green coffee beans of each processing variation had different compositions. The resulting sensory contrasts were small, but samples still differed notably in some attributes (*e.g.*, fruitiness and acidity). Compositional and sensory differences were linked with fermentation and soaking duration, rather than demucilaging or depulping.

## CONCLUSIONS & PERSPECTIVES

The present study showed that demucilaging coffee, which can be a more sustainable alternative to depulping coffee, did not significantly alter coffee flavor. Nevertheless, processing parameters should be considered carefully. To gauge if the conclusions of this study uphold in other geographical locations, it would be valuable to perform similar studies elsewhere.

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## The influence of coffee mycobiota from some Brazilian regions on the coffee beverage: can these regions be considered *terroir*?

Teixeira, Aldir A.\*, Teixeira, Regina R.\* Reis, Marcio\*, Turello, Luca\*\*, Bueno, Josiane\*\*\*, Taniwaki, Marta H.\*\*\*

\*Experimental Agrícola do Brasil Ltda – São Paulo – SP, Brazil; \*\**illycaffè* – Trieste, Italy; \*\*\*Food Technology Institute (ITAL) – Campinas – SP, Brazil

**RATIONALE.** Coffee beverage is influenced by several factors, including the species or botanical variety of the beans, climate conditions of the cultivated region, agricultural practices, processing post-harvest and the preparation of the beverage. Furthermore, several studies have demonstrated that fungal contamination can influence the sensorial characteristics of the beverage. The term *terroir* has been used to define a set of all environmental factors that affect a crop's phenotype, including unique environment contexts, farming practices and a crop's specific growth habitat. Some artisanal crops for which *terroir* is studied are wine, tobacco, chocolate as well as coffee. The aim of this research was to analyze the mycobiota of coffee beans and correlate with the sensorial characteristics of the final *espresso* beverage in *terroir* conditions.

**METHODS.** A total of 16 samples of Arabica coffee from different Brazilian regions, with altitude ranged from 850 to 1200 m, was analyzed for fungal infection and sensorial evaluation of the *espresso* beverage. Samples were collected after the coffee beans (natural or natural-polped) were dried (with moisture 10,7 to 11,9%) and prepared. The varieties were Catuaí vermelho, Catuaí amarelo, Mundo Novo e IBC 12. Fungal infection was analysed according to Pitt & Hocking (2009). Coffee samples were evaluated in two different degustation tests: infusion and *espresso* as described in Iamanaka et al. (2014). The sensorial analyses were carried out, evaluating the quality of the beverage in respect to: aroma, body, acidity, bitterness, sweetness and astringency. Additionally, the presence of positive flavors and aromas such as: bread toast, honey, caramel, chocolate, almond, fruit, floral; and/or negative characteristics such as immature, fermented, stinker, woody, rancid, moldy and rioy were also evaluated.

**RESULTS.** Coffee samples showed fungal infection from 0 to 68%. A high diversity of fungi was isolated from the coffee samples and the most common were: *Penicillium brevicompactum*, *Penicillium* sp. nov., *Aspergillus* section *Nigri*, *Fusarium* sp., *Eurotium* species and black molds. Coffee beverage that showed negative sensorial evaluation with attributes such as rioy had a high presence of *A. section Nigri* and *Fusarium*. On the other hand, the most common fungi isolated from raw coffee beans, which presented a good, clean beverage such as caramel, chocolate, honey, fruity, almond, floral and sweetness were: *P. brevicompactum* and *Penicillium* sp. nov.

**CONCLUSIONS & PERSPECTIVES.** These coffee bean samples came from regions which have a particular characteristic that may influence the coffee beverage, including factors such as the soil, microbiota and microclimate. Can these regions be considered as coffee *terroir*?

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## EMISSION OF NITROUS OXIDE (N<sub>2</sub>O), IN A COFFEE PLANTATION UNDER SHADE, IN THE CENTRAL VALLEY OF COSTA RICA

CHAVES, Víctor\*; DELGADO, Carlos\*,  
\*Instituto del Café de Costa Rica (ICAFE).

### **RATIONALE:**

It is considered that the main greenhouse gas (GHG) produced in coffee plantations is nitrous oxide (N<sub>2</sub>O), which largely comes from nitrogenous fertilization; so that the IPCC estimates that 1% of the nitrogen applied as fertilizer in agricultural soils is transformed into this gas. However, this is a very general estimate, so it is recommended to establish emission factors for each region and crop. The objective of this study was to obtain an emission factor of N<sub>2</sub>O in shaded coffee plantations in the Central Valley of Costa Rica, product of the application of increasing doses of nitrogen.

### **METHODS:**

The trial was established in Naranjo, Alajuela at 1200 m.a.s.l., in a 15-year-old Catuaí planted at a density of 5,848 plts/ha (1.90 mx 0.90 m), under regulated shade of *Inga sp.* The treatments consisted in the application of 0, 100, 225 and 350 kg N/ha based on the use of Urea, divided into 3 annual applications (May, August and October). For the collection of gases, non-flow through non-steady static chambers were used. The gas sampling was carried out from May 2015 to May 2016, the collection of samples was more intensive after each fertilization event, taken at 1,2,3,4,7,8,11,14,18 and 23 days after each fertilization, and then biweekly until the next fertilization. The treatments were laid out in a randomized complete block design, with 3 replicates, placing 2 chambers per plot (one in the fertilization band and another in the inter-row without nitrogen placement).

### **RESULTS:**

There was an exponential response to nitrogen fertilization with an accumulated annual flow of 0.63; 0.92; 1.01 and 1.52 kg N<sub>2</sub>O/ha for applications of 0, 100, 225 and 350 kg N/ha respectively. In all the treatments, the main peak of emission was presented with the establishment of the rainy period, in an increasing way according to the level of fertilization used. The emissions obtained are lower than those reported by Hergoualc'h *et al.*, (2012) and similar to those of Ortiz-Gonzalo (2018). Subtracting the control emission, the average emission factor of the fertilizer was 0.15% of the applied nitrogen, which is much lower than that proposed by the IPCC and closer to that found by Soares *et al* (2015).

### **CONCLUSIONS & PERSPECTIVES:**

It is necessary to increase the studies that allow to have a greater clarity on the GHG emission factors under tropical conditions.

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IONESCU, Kim ; LAVERTY, Molly (Farmer Brothers); PIZA, David and CUELLAR, Olga (S&D); TAYLOR, Meredith (Counter Culture); POPKIN, Colleen (KGM); GOODEJOHN, Kelly; BOTHEREAU, Derek (Starbucks)\*; NANAVATI, Saurin, PERI, Hubert, CALFAT, Sylvia \*\*

\*Coalition for Coffee Communities, USA ([www.coffeecommunities.org](http://www.coffeecommunities.org)); \*\* Committee On Sustainability Assessment (COA- [www.thecosa.org](http://www.thecosa.org)). *This Sustainable Landscapes approach was developed in collaboration with the SAFE platform and SCA as the secretariat.*

#### RATIONALE

Sustainability failures are rooted in diverse factors that exist at a scale that cannot be solved by one group of farmers or one community or one company. The Landscape work COSA co-created for the CCC presents a comprehensive and systematic approach that addresses this challenge.

Productive Landscapes are much more than an ecology, they are shaped by the dynamic tension between social, economic and environmental factors. In this pilot for coffee landscape in Jinotega, Nicaragua, we created an online tech platform that selectively blends big data with local data, allowing interested parties to instantly see and understand issues for better decision making. This promotes well-informed investments and collaboration between different actors, delivering demonstrable and scalable sustainability outcomes.

#### METHODS

This Landscape approach is comprised of primary data collected through household, community and producer organization surveys as well as secondary data from agricultural census and other private, public and spatial data. Our technologies include GIS maps and dashboards, which combine such data to identify bottlenecks that are validated with stakeholders. We then craft and recommend a strategic sustainability plan and how to effectively monitor its implementation over time.

#### RESULTS

Within the data flows made available through the new Landscape platform, we were able to identify hotspots (critical issues) related to coffee production in the department of Jinotega and provide strategic recommendations for roasters sourcing from this key origin. Most importantly, we were able to pilot a technology platform for data visualization and analysis and validate the results with different stakeholders giving us content to further develop the tool in a next phase.

#### CONCLUSIONS & PERSPECTIVES

We look forward to presenting our Landscape Platform technology to ASIC and receive feedback to determine how such tools can be made even more effective for different actors working within a landscape.

#### REFERENCES

Kim Ionescu (SCA)- [kimi@sca.coffee](mailto:kimi@sca.coffee); Molly Laverty (Farmer Brothers)- [MLaverty@farmerbros.com](mailto:MLaverty@farmerbros.com)



PONCET, Valérie<sup>1</sup>; TOURNEBIZE, Rémi<sup>1</sup>; AQUINO, Sinara<sup>1,3</sup>; MARRACCINI, Pierre<sup>2</sup>; MARIAC, Cédric<sup>1</sup>; BETHUNE kevin<sup>1</sup>; ANDRADE, Alan<sup>4</sup>; KIWUKA, Catherine<sup>6</sup>; CROUZILLAT, Dominique<sup>5</sup>; ANTEN, Niels<sup>7</sup>; MANEL, Stéphanie<sup>8</sup>; DE KOCHKO, Alexandre<sup>1</sup>

<sup>1</sup>IRD, UMR DIADE, Montpellier France; <sup>2</sup>CIRAD, UMR IPME, Montpellier, FRANCE; <sup>3</sup>Federal University of Lavras, MG, Lavras, BRAZIL; <sup>4</sup>EMBRAPA Coffee-INOVACAFÉ, Lavras, BRAZIL; <sup>5</sup>Nestlé R&D, Tours, France; <sup>6</sup>NARO, Kampala, Uganda; <sup>7</sup>Wageningen Univ. Wageningen, Netherlands; <sup>8</sup>EPHE, UMR CEFE, Montpellier, France.

## RATIONALE

In the light of adaptation, understanding how organisms respond to their environment, by altering physiological processes, will increase our capacity to make predictions about adaptation to global climate change. Adaptive clines have been increasingly studied in plant species within temperate zones to understand adaptation of organism in natural populations. However, they are still poorly understood in tropical environments. *Coffea canephora*, cultivated as Robusta, is an interesting tropical tree model to investigate adaptation in the tropics as it is largely distributed within the range of the lowland tropical rain forests of Africa.

In particular, modifications occurring in genes related to abiotic stress tolerance make these genes candidate for enhanced resilience to future climate change

## METHODS

We combined the use of both captured regions sequenced for a set of candidate genes related to drought tolerance and whole genome SNP markers.

Leveraging on a robust statistical approach combining multiple neutrality statistics, we provided a comprehensive map of selection signals in the genome of the *C. canephora* both at the species level and within its major genetic groups.

## RESULTS

The genotype-environment association suggests regional adaptation to spatially varying environments of the recent past, with a special focus on the Eastern edge of the distribution, in Uganda. More specifically, we found signals of selection tightly linked to several genes involved in response to biotic and abiotic stress and in caffeine biosynthesis.

## CONCLUSIONS & PERSPECTIVES

Our detection of selection signals support the hypothesis of present ecological gradient contributing to the structure of the genetic diversity. Moreover, assessing the genomic vulnerability of the present populations will help to predict their response to future environmental changes.

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## EFFICACY OF DIFFERENT CHEMICALS FOR MANAGEMENT OF AFRICAN WHITE COFFEE STEM BORER, *MONOCHAMUS LEUCONOTUS*, PASCOE

MAGINA, Fredrick\*, MBWAMBO, Suzana, SHAO, Godbless, TARIMO, Epimaki. and KILAMBO, Deusdedit.

Tanzania Coffee Research Institute, P.O Box 3004, Moshi Tanzania

\*Corresponding author: [fredrick.magina@tacri.org](mailto:fredrick.magina@tacri.org)

### Abstract

The African white coffee stem borer (WCSB), *Monochamus leuconotus*, Pascoe (Coleoptera: Cerambycidae) is an important pest of Arabica coffee in Tanzania. In the past the pest was well managed chemically by use of Aldrin and Dieldrin. The chemicals were banned in early 1970 leaving no option for effective management of the pest. This study investigated efficacy of Fipronil 200 SC and Chlorpyrifos 480g/L in four different coffee ecological zones in the country, including selected coffee estates in Arusha region, and smallholder farms at Mwayaya – (Kigoma region) and Mbinga (Ruvuma region) from March, 2015 to February 2018. Four dosages of Fipronil (15, 20, 25 and 30 mls/20 lts of water), Chlorpyrifos 700mls/20 lts of water (standard) and control (untreated) were banded on coffee stem (90 cm from the ground) two weeks before short and long rains. A completely Randomized Block Design was used for the study. Data taken was number of holes made by the pest before and after the application of treatments (new holes). Data on number of new holes was collected after every three months to allow any new re-infestation by the pest. Data were processed on excel spreadsheet and analyzed by use of GenStat statistical package. All treatments were significantly ( $p < 0.05$ ) different from the control. Chlorpyrifos 700 mls/20 lts of water showed to be most effective with a minimum number of new holes, followed closely by Fipronil at 30, 25 and 20 mls/20 litre. This implies that all the tested dosages of Fipronil have shown a potential for management of WCSB, efficacy increasing with the increase in concentration. Because Fipronil 20mls in 20 lts of water did not differ much from the higher dosages or the standard, it is hereby recommended to be used by coffee farmers for banding/spraying of coffee stems two weeks before short and long rains so as to minimize the re-infestation of the pest in their coffee fields. The information from this study will be useful in the process of pesticide registration.

**Key words:** *Monochamus leuconotus*, Fipronil -, Chlorpyrifos, Arabica coffee, Tanzania.



## OUTBREAK OF INVASIVE COFFEE PESTS IN TANZANIA: COULD IT BE AN EFFECT OF CLIMATE CHANGE?

MAGINA, Fredrick \*, MARO, Godsteven, , MBWAMBO, Suzana, NG'HOMA, Nyabisi, SHAO, Godbless, MUSHI Isaac and KILAMBO, Deusdedit.

Tanzania Coffee Research Institute (TaCRI), P.O Box 3004, Moshi, Tanzania.

\*Corresponding author: [fredrick.magina@tacri.org](mailto:fredrick.magina@tacri.org)

### Abstract

The Tanzanian coffee industry has recently witnessed several outbreaks of “new coffee pests”, hitherto not known to attack coffee. Spectacular cases were reported by farmers in Kilimanjaro (Northern Zone), Njombe (Southern Highlands), Kagera (Lake Zone) and Songwe (Southern Highlands) between 2015 and 2017. The affected areas were visited by a team of researchers from TaCRI to collect the necessary information for facilitating their identification. Adults and larvae of the pests were collected, preserved in 75% Ethanol and sent to ICIPE, Nairobi, Kenya for identification. The pests reported in Njombe were identified as white grub, *Holotrichia serrata* (Coleoptera: Scarabeidae), while those reported in Kagera and parts of Kilimanjaro were identified as black coffee twig borer (BCTB), *Xylosandrus compactus* (Coleoptera: Scolytidae) and the one reported in Songwe is coffee/cocoa bean weevils, *Araecerus fasciculatus* (Coleoptera: Anthribidae). White grubs attacks Arabica coffee roots (and other crops like maize etc.) and destroys feeder roots causing wilting and death to plants. BCTB attacks both Arabica and Robusta coffee twigs by boring underside the twigs and destroys the xylem and pith of the twig, causing yellowing, wilting and death of the affected twigs, and the attack is throughout the year. Bean weevil, known in other countries to attack berries, has now turned to stems and the attack is more serious during the rainy season starting in March every year. Pest incidence, in percentage, was determined by counting the number of farms infested with the pest against the total farms surveyed; and it was 100% for BCTB and white grub, while it was 71% for bean weevils. When farmers were interviewed as to the probable reason for the outbreak of these pests in their respective areas they suspected to be due to climate change and we have taken this up for further exploration. The efforts made by TaCRI, way forward and an integrated pest management practices for each pest are discussed in this paper.

**Key words:** white grub, black twig borer, coffee bean weevil, invasive coffee pests, Tanzania



## ECONOMIC FEASIBILITY OF SIX SMALLHOLDER COFFEE FARMING ASSOCIATIONS IMPLEMENTING THE CENTROAMERICANO COFFEE HYBRID IN SAN PEDRO YEPOCAPA, GUATEMALA

BROWN, Taya\*, RIBERA, Luis\*, LOMBARDINI, Leonardo\*, PALMA, Marco,\* MONTAGNÓN, Christophe\*\*; \*Texas A&M University, College Station, TX \*\*World Coffee Research, Marseille, FR

### RATIONALE

Access to basic economic information (production costs, losses, etc.) is fundamental to evaluating and influencing the survival of any business. The coffee industry has a vested interest in ensuring the wellbeing of its smallholder farmers (Ponte, 2002), who tend to be from lower socioeconomic backgrounds (Bacon, 2005) and do not keep records of spending or income. Through focus groups and Monte Carlo simulation techniques (Mun, 2006) we assessed profitability amongst six smallholder Guatemalan coffee farming communities who received the ‘Centroamericano’ coffee hybrid: a varietal innovation whose impact on profitability has never been studied in this context.

### METHODS

In 2018, using participatory methods (Narayanasamy, 2009), focus groups were held with nearly 200 members of six smallholder coffee farming associations in San Pedro Yepocapa, Guatemala, who received the high-yielding, coffee leaf rust resistant ‘Centroamericano’ coffee hybrid through a development project implemented by World Coffee Research and Anacafé. Cost of living, cost and income from coffee production, for traditional varieties as well as the hybrid, and business structure at both the household and cooperative levels were identified for the six cooperatives. Qualitative data were analysed by the constant comparative method and net cash farm income was the key output variable used to measure economic feasibility through Monte Carlo simulation.

### RESULTS

We show that profitability of the ‘Centroamericano’ hybrid in smallholder systems depends on multiple parameters: it exhibits high productivity, resistance to coffee leaf rust and large bean size but requires more inputs to achieve these. Details, including profitability comparisons of the six cooperatives and between traditional varieties and the hybrid will be displayed in stop-light charts.

### CONCLUSIONS & PERSPECTIVES

We conclude that the ‘Centroamericano’ hybrid can improve profitability for smallholder coffee farmers if the productivity potential is met, but higher inputs and technical assistance are required.

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## ECONOMIC ANALYSIS OF COFFEE FARMING SYSTEMS IN TANZANIA

*Kiwelu, L. \*, Magesa, J.M., Ng'homa, N.M., Mtenga, D.J., Maro, G.P. and Kilambo, D.L.*  
*Tanzania Coffee Research Institute, P.O. Box 3004, Moshi, TANZANIA*

\*Corresponding author, Email [leonardkiwelu@tacri.org](mailto:leonardkiwelu@tacri.org) or [leonardkiwelu@gmail.com](mailto:leonardkiwelu@gmail.com)

### Abstract

The profitability of coffee production in Tanzania was assessed in the context of two farming systems, namely, smallholder pure stand and intercropped farming systems with traditional and improved coffee varieties. The study was conducted in eight districts representing different zones as follows: Rombo, Hai and Arumeru Districts (Northern zone), Mbinga and Mbozi Districts (Southern Highlands) and Buhigwe district (Western zone) for Arabica coffee; and Muleba and Karagwe Districts in Kagera region for Robusta coffee. Simple random sampling technique was used to select 480 growers. A structured questionnaire was administered to the selected growers for collecting data related to production costs in 2015/16 production season (Costs of inputs and labour from the start of a coffee farm until the first harvest of fruits), farm gate price and farm size. Profitability of six different farming systems (washed Arabica, hard Arabica and Robusta as main systems, pure stand and banana intercropped as sub-systems) was calculated based on maximum (highest), minimum (lowest) and average yield scenarios over the extended period of 15 years. Excel Office Package was used to compute Gross Margin, Net Present Value, the Internal Rate of Return (IRR) and, Benefit Cost Ratio (BCR). The analysis showed that coffee growers producing at minimum yield scenario incurred higher costs than the other two scenarios. The results show that all farming systems had positive net present values (NPVs) at 12% discount rate, the IRR were greater than 12% discount rate and the BCR were greater than one, implying that coffee production in Tanzania is a profitable business irrespective of farming system under the maximum and average yield scenarios. With improved varieties, coffee farming is profitable even under minimum yield scenario. Effective systems should be in place to encourage farmers to plant improved coffee varieties, to ensure farmer's access to the right seedlings, adequate and authentic inputs at subsidized price, and to mobilize campaigns on farm rehabilitation.

**Keywords:** *Coffee, cost of production, farming system, profitability analysis, Tanzania*



USE OF PLANT GROWTH REGULATORS TO PROMOTE COFFEE  
FLOWERING TO AID COFFEE HARVEST AND SANITATION FOR CBB  
CONTROL.

MATSUMOTO, Tracie\* and AOKI, Darsen\*

\*USDA ARS DKI PBARC, Hilo, HI, USA

**RATIONALE:** In Hawaii, sporadic rainfall throughout the season results in multiple flowerings result in coffee berries at different stages of development throughout the season which often requiring multiple harvests. This results in increased labor costs in hand-harvested coffee and decreases yield in both mechanically and hand-harvested coffee fields. The extended period of coffee flowering and fruiting also extends the availability of host material for coffee berry borer (CBB). Field sanitation of coffee berries between seasons is an added expense to coffee production that does not generate income for the farmer but is necessary to reduce the amount of CBB at the start of the season. Since CBB is able to reproduce within the protected confines of the coffee berry, it is important to time insecticide and biopesticide sprays, such as Beauveria, when the endosperm of the developing coffee seed is still liquid and the abdomen of the CBB is protruding from the coffee berry. Synchronizing the development of coffee berries in the field so the majority of CBB are at this susceptible stage increases the effectiveness of sprays.

**METHODS:** *Coffea arabica* ‘Typica’ or ‘Yellow Catuai’ on commercial coffee farms located on the island of Hawaii and Kauai were used in this study. Cultural management of the coffee trees were the same as commercial production with the exception of foliar or soil drench applications of ProGibb® 40SG (Valent BioSciences, Libertyville, IL) (GA<sub>3</sub>) and ProTone® SG (Valent BioSciences – SLN Reg. No. HI-130001) (s-ABA). Initial application rates of 100 ppm GA<sub>3</sub> were hand sprayed on foliage at a rate of 0.5 L/tree with 0.05% Tween 20. Soil drench applications of 300 or 1000 ppm ABA were applied as 1 L water dilutions applied around the base of each tree at intervals specified in each experiment. Foliar sprays and soil drench were applied to dormant coffee flower buds prior to coffee flowering period. Harvest data was initially collected from hand harvested individual trees. Data collected from mechanically harvested trees were obtained by taking a subsample of the harvested material and sorting the berries into three categories; green, ripe and over ripe.

**RESULTS:** Coffee trees treated with the GA<sub>3</sub> foliar spray in Kona, HI and Kauai resulted in significantly earlier harvest compared to untreated control trees. Soil drenches and foliar sprays of s-ABA increased coffee yields in trees treated in Kona and Kauai. Foliar applications for both GA<sub>3</sub> and s-ABA resulted in more uniform harvests and higher coffee berry yields during the early season harvests. No differences were detected in cupping scores on beans harvested from treated trees.

**CONCLUSIONS & PERSPECTIVES:** Foliar applications of GA<sub>3</sub>, effectively promotes flowering of buds to reduce the amount of harvests required to harvest ripe coffee berries. This technique has the potential to reduce labor costs during harvest and sanitation of green berries between coffee seasons for CBB control.



MARGENOT, Andrew\*, CHAVEZ NAVARRETE, Eduardo\*\* LOMBARDINI, Leo\*\*\*

\*Department of Crop Sciences, University of Illinois Urbana-Champaign, IL, USA, \*\*Escuela Superior Politécnica del Litoral Guayaquil, Ecuador, \*\*\*Center for Coffee Research and Education, Borlaug Institute for International Agriculture, College Station, TX, USA and Department of Horticultural Sciences, Texas A&M University, College Station, TX, USA

## RATIONALE

Soils are the literal foundation of agroecosystem, but the inherent –and typically uncharacterized– variability of soils in coffee agroecosystems is a persistent information gap that challenges management recommendations. Assessing the pedological context of coffee agroecosystems holds potential to improve their management for productivity and climate change resilience. Evidence from Central America and East Africa indicate that soil nutrient limitation is a major productivity constraint that entrenches the coffee yield gap (1, 2). However, there are no reliable or site specific micro-nutrient norms for Arabica coffee, in part due to strong variability in underlying soil properties (2). Disagreement between soil maps in coffee regions compared to soils observed on-site further limit site-specific soil management recommendations (3).

## METHODS

We combined scientific literature review and field- and lab-assessments of soil types and soil-plant nutrient relationships across four coffee agroecosystems in Panama, Honduras, El Salvador, and Ecuador. The popular (mis)conception of the unique suitability and ubiquity of ‘volcanic soils’ in coffee production is critically evaluated by drawing upon examples from coffee advertising, scientific literature, and soil maps from coffee belt. At the four coffee agroecosystems evaluated, soils classification by USDA Soil Taxonomy and physicochemical analyses demonstrate intra- and inter-agroecosystem variability in soil properties relevant to coffee plant nutrition and climate change resilience (e.g., soil water holding capacity).

## RESULTS

We demonstrate a salient information gap on scientific understanding of soils in coffee agroecosystems, which can may explain contradictory statements on soils and coffee often found in advertising. At the four studied coffee agroecosystems, measured variability in soil characteristics indicates the importance of soil context to develop and implement coffee agroecosystem management strategies. Paired soil-plant analyses demonstrate nutrient-specific soil-plant (de)coupling. Differences in soil fertility and type (USDA taxonomy) within and among the four studied coffee agroecosystems support findings by a limited scientific literature on the necessity of site-specific nutrient management recommendations.

## CONCLUSIONS & PERSPECTIVES

There is a need for soil science to support coffee research and agroecosystem management. Identification of known unknowns on soils under coffee production in the scientific literature and the pedological variability at four studied coffee agroecosystems support the importance of site-specific soil conditions. Missing soils knowledge holds implications for addressing climate-change related threats to coffee sustainability of soil erosion and water storage capacity. The contradiction between soil descriptions in advertising and the disagreement between broad-scale soil maps in developing nations in which coffee is produced represent an information gap and opportunity to improve fundamental knowledge on the biophysical context of coffee production.

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# Effects of weed control methods in coffee crop on quality of coffee beverage after ten years<sup>(1)</sup>.

E.N. Alcântara<sup>(2)</sup>, M.R. Malta<sup>(2)</sup>, A.P. Botelho<sup>(3)</sup>

<sup>(1)</sup>Empresa de Pesquisa Agropecuária de Minas Gerais, Lavras, Minas Gerais, Brazil

<sup>(2)</sup>Coffee researcher of EPAMIG, MG, Brazil, scholarship holder of FAPEMIG

<sup>(3)</sup> Scholarship holder of Brazilian consortium & development of coffee - CBP&D - Café

**Abstract:** Weed control is one important cultural practices in the coffee crop management. Weeds compete with the coffee crop for light, nutrients and water, and interfere with growth and development; weed competition may reduce yield coffee by up to 77%. Coffee crop is more sensitive to weed competition for water on the dry season and for nutrients at the rainy season. This competition, in addition to affecting the production, affects also, other production parameters. This study was made to determine whether different weed control methods between the lines may affect the quality of coffee beverage. An experiment installed at the Agricultural Experimental station research of south of Minas Gerais - EPAMIG - at São Sebastião do Paraíso, MG, Brazil, in a Oxisol clayey, with randomized block design using seven weed control treatments between the lines and three replicates of the coffee cultivar Paraíso MG2. The treatments between lines were: mower, disk harrow, rotary tiller, post-emergence herbicide (glyphosate), pre-emergence herbicide (oxyfluorfen), manual weeding and no weed control. The grain samples were harvested in the treatments and prepared separately during each year. They were, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016 and 2017. The coffee grain samples were sent to Coffee Quality Laboratory in Santo Antonio do Amparo, MG, Brazil, for sensory analysis of tasters. From 2008, through 2010 analyzes were done in the BSCA rating protocol, however, from 2011 through 2017 the SCAA protocol was used. In these ten years the results indicated that, the pre-emergence herbicide treatment, at coffee interrows, presented in all evaluations scores over 80 points. In other words, this weed control method, supplanted all others due to this influence on coffee drink quality, that was classified as special coffee.

**Keywords:** Weed control; coffee beverage quality.

**Acknowledgment:** Brazilian consortium & development of coffee - CBP&D - Café

2017 do Experimento

**Tabela 10 - Médias das pontuações totais das análises de qualidades nos anos 2008 a 2017 do Experimento controle de plantas daninhas em S.Seb.do Paraíso, MG.2017**

Médias seguidas pelas mesmas letras não diferem entre si pelo teste Tukey a 5%

Entrelinhas	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2008-2017
Roçadora	84,6a	75,0b	80,7a	78,2 b	80,0 a	71,6 b	75,7 a	83,94 a	78,67ab	78,67ab	79,02bc
Grade	82,3a	78,3ab	82,0a	78,5ab	80,2 a	77,6 a	76,4 a	83,11 ab	82,53 a	82,55a	79,83ab
E.rotativa	79,6a	76,5ab	83,0a	81,3ab	81,8 a	73,1 b	79,7 a	83,39 ab	78,57 ab	78,56ab	79,74ab
Herb.pós	80,6a	78,3ab	81,0a	78,9ab	79,7 a	79,0 a	78,3 a	81,22 b	80,00 ab	80,00ab	79,77ab
Herb.pre	80,6a	80,3a	82,3a	81,4ab	81,6 a	79,2 a	78,6 a	82,22 ab	82,13 a	82,16a	80,80a
C.Manual	76,0a	75,8ab	82,2a	81,7a	82,4 a	78,7 a	77,9 a	82,44 ab	78,80 ab	78,83ab	79,55abc
Sem capina	78,0a	77,2ab	79,5a	81,7a	75,8 b	71,6 b	77,4 a	83,70 ab	77,57 b	77,55b	77,97c
C.V.%	2,19	2,31	1,90	2,29	0,64	1,03	1,41	0,26	0,94	0,94	2,01



SMRKE, Samo\*, WELLINGER, Marco\*, YERETZIAN, Chahan.\*

\*Zurich University of Applied Sciences, Institute of Chemistry and Biotechnology, Coffee Excellence Center, Einsiedlerstrasse 31, 8820 Wädenswil, Switzerland.

Coffee freshness is one of the core values of high-quality specialty coffee. But why is preserving freshness so important? We maybe strive to maximize coffee's potential to keep its vibrancy as fresh as the day when roasted or we keep coffee fresh to ensure quality and consistency. To keep coffee fresh, understanding the fundamentals of freshness is of utmost importance to be able to apply it in coffee technology, such as packaging or coffee capsules.

Research on coffee freshness has been focused into two fields of interest, which we name chemical (aroma degradation, coffee staling) and physical (coffee degassing) loss of freshness, due to the type of impact the processes have. Loss of aroma during coffee storage is attributed to loss of volatile organic compounds and formation of new compounds through chemical reactions. The dynamics of coffee staling are measured using gas chromatography mass spectrometry (GC/MS) to either identify the compounds formed during coffee aging or through use of freshness indices [1]. The second distinctive process of coffee aging is the degassing of coffee. During coffee roasting a large amount of CO<sub>2</sub> is entrapped within the porous coffee bean structure and this CO<sub>2</sub> is gradually released during coffee storage [2]. This phenomenon has a direct impact on coffee packaging and has recently gained a lot of interest since the degree and control of coffee degassing is important for successful extraction of coffee from capsules.

Studies of coffee freshness have been performed in past using either a single coffee roasted along a specific roast profile, or by observing only relative changes of a coffee sample [1]. Future oriented studies also need to address the dependence of loss of freshness on roast profiles and degrees, and on varieties and origin. The study presented here is the first next step in this challenging field. We have studied the loss of freshness of an Arabica coffee from Guatemala, roasted using different time/temperature roasting profiles (3 different roast times and 3 different roast degrees), by analyzing the loss of aroma and degassing during storage of roasted whole beans at 35 °C. The aroma was analyzed using a headspace GC/MS method[1] and degassing was measured using a newly developed gravimetric method [2].

The results have shown that the commonly used freshness index methanethiol/dimethyl disulfide is sensitive to coffees roasted using different roasting profiles, and represents an valuable alternative to the known methanethiol/2-butanone ratio, which appears to be insensitive to variations in the roast profile. The amount of coffee degassing was strongly impacted by the roast degree and to a lesser extent by roast time. The characteristic time of aroma loss (12 days for methanethiol, > 40 days for other aroma components) is on a longer scale than degassing (8-10 days). The difference in time scale for the release points to different and little known fundamental processes occurring during the release of volatile molecules (chemical degradation, sorption, partitioning, hydrodynamic gas flow). It is therefore currently difficult to establish a link between degassing and aroma loss. The complexity of coffee freshness could be overcome by using machine learning algorithms to predict loss of freshness, which was demonstrated to have the potential to predict coffee freshness, independent of roast profile.

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## SCREENING OF PARAMETERS IMPACTING CAPSULE COFFEE EXTRACTION.

EIERMANN, André\*, GUÉLAT, Loïc-Marco\*, SMRKE, Samo\*\*, WELLINGER, Marco\*\*, YERETZIAN, Chahan.\*\*

\*UCC Coffee Switzerland AG, Bernstrasse 180, 3052 Zollikofen, Switzerland. \*\*Zurich University of Applied Sciences, Institute of Chemistry and Biotechnology, Coffee Excellence Center, Einsiedlerstrasse 31, 8820 Wädenswil, Switzerland.

Single serve coffee capsule represents one of the most convenient and easiest ways to brew coffee. Due to highly controlled extraction conditions, capsule coffee can also be more reproducible when compared to semi-automatic espresso extraction, where the human factor can play an important role in extraction consistency and reproducibility. Obtaining a specialty cup profile from a capsule is yet somewhat challenging, due to the relatively small amount of coffee used in the capsule (brew ratio). Consistent grinding and roasting is needed to successfully extract coffee capsules. In recent years the market size of capsule coffee has been growing rapidly. Over 300 producers of coffee capsule, compatible to the global market leader, have appeared on the

market, offering a unique opportunity to explore the range of roast and ground coffee parameters that are used for capsules and how these parameters impact the extraction and cup profile of coffee. The advantage of studying the extraction of coffee capsule, all compatible to one specific systems, is that the human factor is essentially eliminated from the brewing process and that the brewing unit, brew geometry and coffee bed shape are close to identical for all capsules. Hence, this allows studying the dependencies of the brew process and cup profile on coffee and grind parameters.

The amount of coffee in each capsule, the particle size distribution of ground coffee (laser diffraction and dynamic imaging), and the roast degree by colour were measured for 48 commercial capsules. The brews were prepared to yield 40 mL of beverage. Brew strength was measured by refractometer calibrated to total dissolved solids (VST Coffee refractometer) from which the extraction percentage (yield) was calculated. Sensory analysis was performed on the brews, with the help of *Tastify* software and executed by certified Q-Graders.

The weight of coffee varies considerably between different types of capsules and was measured ranging from 4.8 to 6.2 g. The extraction time varied greatly from 10 s to 46 s. The reason for these variations in extraction time is likely to be linked to different particle size distributions of ground coffee, roast degree of coffee or the pressure inside the capsule (how well the coffee was degassed [1]). Corresponding with large differences in extraction time, the extraction efficiency varies strongly between different capsules types. The brews contained from 2.15 to 3.84% of total dissolved solids, which corresponded to a range of extraction yields from solid ground coffee of 17 to 27.4%.

This study reveals that successful brewing of single serve capsules can indeed be performed with various combination of coffee related parameters, and no simple recipe on how to approach production of capsule coffee can be drawn at this stage. While some general observation and interdependencies did emerge, there is a clear need for further research in order for a more rational approach to emerge. Currently, mostly selective optimization is required for each individual coffee capsule type, in order to find the best combination of conditions.

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## COFFEE GALACTOMANNANS EXTRACTION: FROM GROUND COFFEE TO SOLUBLE POWDERS MICRONIZATION

PASSOS, Cláudia P.\*, LOPES, Guido R.\*, FERREIRA, Cláudia, P.A.\*, ALMEIDA, M.M.\*\*  
RODRIGUES, Carla\*\*\*, FIGUEIRA, Cláudia\*\*\*, COIMBRA, Manuel A.\*

\* Department of Chemistry, QOPNA, University of Aveiro, Aveiro, Portugal

\*\* Department of Materials and Ceramic Engineering, CICECO, University of Aveiro, Aveiro, Portugal

\*\*\* Diverge, Grupo Nabeiro Innovation Center, Lisboa, Portugal

### RATIONALE

The galactomannans content is a key factor distinguishing coffee quality [1]. During instant coffee processing their content as polysaccharides in the brew is diminished. Optimization of hot water powder extractions can be addressed to prepare soluble coffee with a higher galactomannans proportion. But, the majority are not even extracted, remaining an underused resource of carbohydrates in spent coffee grounds. Pressurized water conditions can be applied to favour their extractability [2]. The applicability however, is dependent on the structural features which should be critically monitored upon extraction.

### METHODS

Ground coffees (Delta, Portugal) were used for optimization of hot water extraction conditions, namely: time, temperature, and coffee/water ratios. The remaining unextracted material was submitted to pressurized microwave assisted conditions evaluating the impact of temperature, time, and use of dilute alkali conditions. Commercial instant coffee, as well as hot water extracted, and pressurized extracted coffee samples were afterwards spray-dried to evaluate microparticles formation and characteristics.

### RESULTS

An optimized hot water extraction at 80°C, 5 min, with a coffee powder/water ratio of 1:10 g/mL achieved the exact 1.2 mannose/galactose ratio existent in the espresso composition. However, the unextracted material remained rich in galactomannans (GM). Under pressurized conditions, the GM extraction was correlated with increasing temperature, however this correlation was dissimulated with an abundant extraction of arabinogalactans (AG) occurring at 170°C, favouring their content over galactomannans ( $\eta_{AG}/\eta_{GM} > 1$ ). Spray-dried powders contained shrivelled, raisin-like shape microparticles with theoretical average aerodynamic diameters of 3-5  $\mu\text{m}$ .

### CONCLUSIONS & PERSPECTIVES

At atmospheric conditions, it is feasible to increase the  $\eta_{GM}/\eta_{AG}$  ratio in soluble coffee. Additional extraction of GM is achievable under pressurized conditions. However, the extraction should not overcome 150°C to favour a higher GM ratio. Micronization of extracts was possible, highlighting the potential use of coffee resources as high value carbohydrate carriers to food and drug delivery applications.

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The effect of water pulsing duty cycle on the sensory parameters of drip brewed coffee.

Lik Xian Lim\*, Scott C. Frost\*, Jean-Xavier Guinard\*, and William D. Ristenpart\*

\*The Coffee Center, University of California at Davis

Drip coffee brewers traditionally deliver hot water to the coffee grounds in a single continuous stream. In contrast, modern brewers are equipped with flow regulators allowing for prescribed water pulses of a desired frequency and duration. Given that little is known about the effect of varied water pulse times on the sensory and chemistry properties of drip coffee, an experiment was designed employing 26 different water pulsing sequences. Using an identical coffee, grind, and brew ratio, the evaluated pulsing sequences produced brewed coffee of significantly different total dissolved solids (TDS) and percent extractions (PE). The duty cycle effect can be interpreted in terms of varied residence times for diffusive mass transfer in the moist coffee grounds in the absence of bulk convective motion. The results imply that a wide range of different tasting coffees can be prepared from the same ground coffee simply by modifying the pulsing duty cycle. Descriptive analysis results spanning the evaluated TDS and PE range will be presented.

### Methods

Pulse sequences were created using paired combinations of five pulse “on” times (20, 25, 33, 50, 100 seconds) and six pulse “off” times (0, 20, 25, 33, 50, 100 seconds). Each pulse on/ pulse off pair would cycle until 200 seconds of total on time was achieved, such that all trials received the same total amount of water. This design produced 26 pulse sequences, from which 20 unique duty cycles were calculated [time on/(time on + time off)]. Using a constant dose (55 g/L), brew water temperature (91°C), and grind, the 26 sequences were evaluated for TDS and PE in triplicate.

### Preliminary results

Figure 1 displays the PE and TDS measures for each replicate of the 26 pulse sequences. Shorter duty cycles yielded increasing TDS and PE.

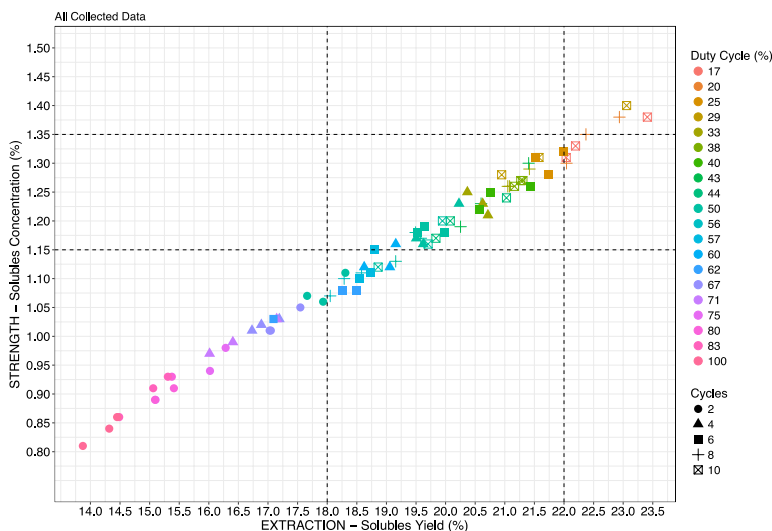


Figure 1. Strength and extraction measures for 26 pulsing cycles. Each unique duty cycle is indicated by color, with the number of cycles required to complete the sequence by point shape.

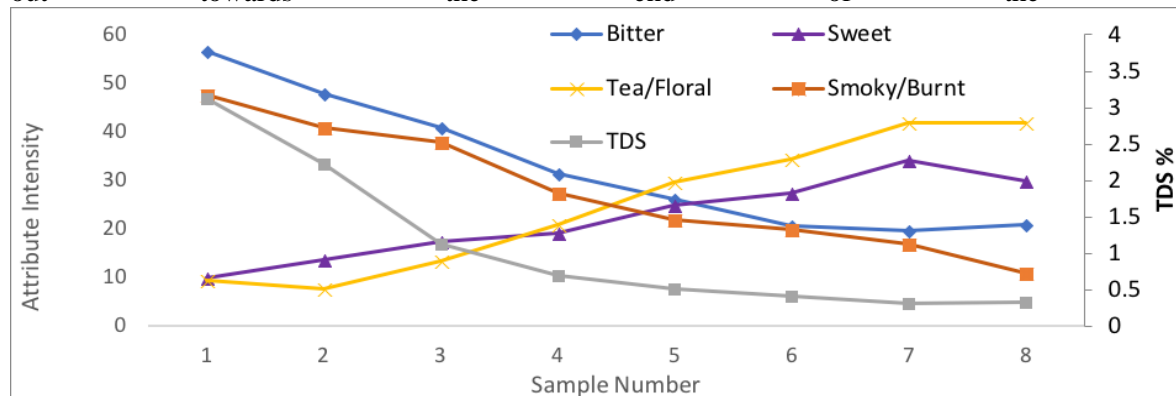


BATALI, Mackenzie, FROST, Scott, RISTENPART, William, GUINARD, Jean-Xavier  
University of California, Davis, CA, USA

Since its publication 20 years ago, the Coffee Brewing Handbook [1] has served as an industry guideline for the many variables that impact coffee brewing and extraction. A primary focus involves the Coffee Brewing Control Chart, which was originally developed by Lockhart in the 1950s using percolators [2]. Despite the widespread use of the Control Chart it contains significant drawbacks, most important of which is the lack of rigorous sensory analytical data specifically quantifying how flavor profiles change with extraction.

With a broader goal to use sensory descriptive techniques to expand and update the Coffee Brewing Control Chart, in this project we investigated how the flavor profile of drip brew coffee varies with time during the brew to identify what flavors are predominant during different points in coffee extraction. Using established descriptive sensory techniques, a team of 12 panelists was trained to develop a common group descriptive language. The experimental design investigated a 4 minute drip brew from an industrial-sized Curtis brewer, with the carafes switched every 30 seconds, resulting in 8 “fractionated” samples of coffee from different time points in the brewing process (0:00-0:30, 0:30-1:00, 1:00-1:30, etc.). These fractions were served to the trained panelists alongside a sample of a full coffee brew under the same brewing conditions. The resulting 9 samples in total were evaluated blindly by panelists in triplicate over 3 evaluation days in a randomized block design to account for sensory fatigue and prevent expectation or systematic bias. Panelists evaluated the 5 basic tastes (sour, bitter, sweet, salty, and umami), as well as 17 flavor attributes they as a group determined during training to be the most relevant to the sample coffees. Attributes were rated on an unlabeled line scale, giving intensity scores out of 100.

The data indicated significant downward trends in sour, bitter, salty, and umami tastes, alcoholic/winey, vinegar, rubber, and smoky/burnt flavors, and astringent mouthfeel over the fractional progression of the brew. Downward trends in flavor intensity were expected in general as the later fractions of the brew are weaker (seen below in the TDS curves for each fraction, along with relation to attribute intensity). Surprisingly, the data also indicated upward trends in sweet taste, as well as tea/floral, honey, and fruity flavors. These results show quantitatively that many of the less desirable attributes are extracted in the beginning of the brewing process, while showing for the first time that some desirable qualities come out towards the end of the brew.



**Figure 1.** Example attribute intensities and total dissolved solids curves for all 8 brewing fractions.

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## IS IT STILL NECESSARY DEEPENING THE KNOWLEDGE ON 3-ALKYL-2-METHOXYPYRAZINES? A STUDY TO UNRAVEL THEIR ROLE ON COFFEE QUALITY

LONZARICH, Valentina\*, MUTARUTWA, Delvana\*\*, COMPAGNONE, Dario\*\*, PITTIA, Paola\*\*, NAVARINI, Luciano\*\*\*

\* Aromalab illycaffè S.p.A. , Area Science Park, Padriciano 99, 34149 Trieste, Italy, \*\* Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Via R. Balzarini 1, 64100 Teramo, Italy, \*\*\* illycaffè S.p.A., via Flavia, 110, 34147 Trieste, Italy.

Among the many volatile organic compounds characterizing the aroma of several food vegetables, one important class is represented by 3-alkyl-2-methoxypyrazines (MPs), including 3-isobutyl-2-methoxypyrazine (IBMP), 3-isopropyl-2-methoxypyrazine (IPMP) and *sec*-butyl-2-methoxypyrazine (*s*BMP). MPs exhibit very low sensory detection thresholds (1-2 ng/L in water) and, in general, are characterised by vegetable-like/herbaceous/earthy notes positively contributing to the aroma of several products. In coffee, IPMP and, particularly, IBMP were suggested to be the main responsables of the natural characteristic odor note of the raw beans. However, more in general, a high amount of MPs has also been considered the cause of undesired off-flavours with negative effects on the product quality. The “peasy defect” or “potato taste defect (PTD)”, found mainly in some Eastern Africa coffee, is a well known example. In this case, MPs presence has been associated to a microbial MPs production induced by a stink bug belonging to the genus *Antestiopsis*, which feeds on coffee cherries. In spite of the importance played by MPs, quantitative data are rather scarce, sometime inconsistent, and cannot permit to draw a conclusive picture on the MPs content in healthy coffee beans when compared to defective ones. In the present work, an analytical method was optimised and applied to determine presence and concentration of MPs in green coffee. The method was preliminary tested on a wide range of food vegetable matrices and then optimized to characterize MPs pattern of green coffee samples of different geographical origin. The experimental data are discussed and compared with those of the literature with a special focus on possible implications on coffee quality.

### METHODS

Several fresh vegetables (bell pepper, pea shells, carrots, cucumbers) were purchased at a local market; samples of six cultivars of *Solanum tuberosum* L. were kindly supplied by Pizzoli S.p.A. (Italy) and samples of *C. arabica* L. of different geographical origin were selected and supplied by illycaffè. MPs were isolated by HS-SPME with a 75 µm CAR/PDMS (Supelco). Analyses were performed with a 6890 Gas Chromatograph equipped with a 5975 Mass Spectrometer (Agilent) and an autosampler MPS2. Quantitation was achieved by using selected ion monitoring (SIM). 2-methoxypyrazine and 3-methyl-2-methoxypyrazine were used as internal standards.

### RESULTS & CONCLUSION

HS-SPME-GC-MS in SIM mode proved to be able to detect and to identify the main MPs in all the vegetable matrices used for preliminary quantification tests. The concentration of MPs ranges from < LOD (e.g. IBMP in potato skin of some cultivars) to 60.53 ng/g (IBMP in green bell pepper). In potato skin samples different MPs content has been determined depending on cultivars. In green coffee, IBMP is the most abundant MP and quantities up to 110 ng/g have been determined. The IBMP content seems to vary with the geographical origin, being more abundant in *C. arabica* from Brazil than from Eastern Africa. IPMP (2 - 3 ng/g) and *s*BMP (2 - 5 ng/g) are less variable with the geographical origin. The present data highlight the needs to further investigate the role played by MPs in both green coffee flavor and the onset of defects and related off-flavours.





Livio, Javier \*, Dr. Richard Chapman \*. \* Auburn University, Samuel Ginn College of Engineering, Department of Computer Science and Software Engineering, Auburn, AL, USA

This paper presents a fuzzy expert system, an enterprise system designed and developed under the category of software as a service (SaaS) to grade specialty coffees from several countries. The system uses approximate reasoning and inner libraries to dynamically construct fuzzy rules, making the system capable of learning as cupping data flows through it. The coffee individual attributes' scores are linguistically expressed through sliders optimally designed to ease data gathering, encouraging the coffee judge to use words instead of numbers (low, medium, high and very high). Results from testing the system show more than 95% of matching results compared to the experts' evaluations.

**METHODS** Use of the domain experts (cupper or judge) knowledge represented in the form of the IF-THEN fuzzy rules [1]. This captures the coffee judges' empirical and heuristic knowledge in our natural way of communication. We modeled the complexity of grading coffee quality beans (the final grade or output) as a set of expressed restrictions based upon certain conditions of the input (coffee bean attributes individual scores).

**RESULTS** A challenge was introduced when expecting the coffee experts to express their finding linguistically. The expert system captured (the attributes' scores) and produced (beans' final grading) with a significant accuracy,. This showed that it is possible to grade coffee utilizing fuzzy expert systems while still following the grading standards of the Specialty Coffee Association [2]. Results from testing are very promising as there was a 95% matching in all the coffee quality grading, meaning that 95% of all the given grades matches human cuppers judgements.

**CONCLUSIONS & PERSPECTIVES** Not only the fuzzy system is able to adhere to these standards but hypothetically, it is capable of alleviating the stress of evaluating coffee using words yet having to express these words with numbers. This system's design was not driven by what the cuppers need to judge the coffee bean attributes, rather it presents a completely new way of recording and reporting the judge's findings, currently based on the SCA cupping form. This system offers the possibility of replacing the use of numerical values for the individual attributes with selection of linguistic terms (words). Nonetheless, the final grading of the coffee bean quality is not the arithmetic addition of the individual scores, it becomes the result of what an underlined Mamdani fuzzy engine infers, offering a level of abstraction between numbers and scores.

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<sup>1</sup> 1 Based on a paper accepted for presentation at the FUZZ-IEEE 2018 and for publication in the conference proceedings published by IEEE: World Congress on Computational Entelligence, Brazil July 2018



## A flavoromics untargeted LC/MS analytical approach in determining key chemical markers in green and roasted coffee beans that predict the coffee brew cup score

Sittipod, Sichaya\*, Peterson, Devin\*

\*The Ohio State University, Columbus, OH, USA

### RATIONALE

Analytical methods currently utilized by the coffee industry to determine green bean quality do not adequately predict the coffee brew flavor quality. Developing methods that accurately and reliably predict the coffee flavor quality based on green bean chemistry is of strong importance for the industry as consumer demand for premium coffee has been steadily increasing.

### METHODS

The current project utilizes a comprehensive untargeted chemical profiling (flavoromics) approach to identify chemical 'markers' in both green coffee beans and coffee brews that influence coffee brew flavor quality. In brevity, green coffee beans (n=18) of different grade qualities were sourced and their subsequent brews were evaluated for cup quality score by industrial Q-graders using the standard Specialty Coffee Association of America (SCAA) cupping method. Comprehensive LC/MS chemical fingerprints of green coffee beans and coffee brews were collected and modeled to predict cup score and provide information about chemical predictors of the grade quality class.

### RESULTS

High quality models using principle component analysis (PCA) and orthogonal partial least squares (OPLS) were developed with good fit and predictive ability of cup score for green beans and coffee brews ( $R^2 > 0.9$ ,  $Q^2 > 0.9$ ). Highly predictive chemical markers in coffee brews that were negatively and positively correlated to cup scores were subsequently isolated and purified (>90%) by multi-dimension preparative LC/MS fractionation systems. Sensory recombination analysis for the top predictive compounds were reported to impact the flavor profile. Subsequent MS/MS experiments and 2D NMR were used to elucidate the structures of purified markers revealing novel diterpene compounds from the ent-kauran-oic acid family which have not been reported in literature.

### CONCLUSIONS & PERSPECTIVES

An untargeted LC/MS approach was applied to discover chemical markers that drive coffee cup score (flavor quality). Determining quality markers in coffee brew provides crucial information to further identify the related flavor precursors in the green beans. The development of analytical methods to accurately screen for flavor precursors in green beans would improve bean selection and enhance negotiation tools for producers and industry. In addition, this knowledge provides a basis to further optimize brew quality based on green bean chemistry.

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## METHYL ESTERIFIED-COMPONENTS DETERMINED COFFEE FLAVOR QUALITY.

IWASA Keiko\*, SETA Harumichi\*, KANAMORI Takeshi\*, NAGAI Chifumi\*\*, NAKAHARA Koichi\*

\*Research Institute, Suntory Global Innovation Center Limited, Kyoto, Japan,

\*\*Hawaii Agriculture Research Center, Kunia, HI, USA

### RATIONALE

Non-targeted analyses and multivariate analyses firstly revealed that high-quality coffee beans had higher amounts of methyl esterified-components (MECs). It was suggested that MECs were generated during roasting and responsible for the fresh fruity odor, which was distinguishable from the fermentation odor caused by ethyl esterified-components. MECs are known to be in fruits such as strawberries and have fruity fragrance. However, the reports in roasted beans are limited.

. In our lab, to understand the chemical compounds in coffee beans which determine the coffee quality, we have investigated the relationship between the sensorial data and compound information measured by non-targeted analyses. We were successful in identifying specific metabolites in coffee green beans (1, 2). Here we present the identification of MECs as the key quality determinants in roasted beans.

### METHODS

Green beans of thirteen diverse arabica coffee from Guatemala were roasted under the same condition. The relationship between SCA cupping scores and non-targeted GC-MS data was evaluated using multivariate analyses. The candidate compounds to flavor quality were verified using spiking tests. The generation of key compounds and its precursors during roasting were monitored by off-line GC-MS and on-line PTR-MS. The experiments using green beans as a reaction vessel, spiked with  $d_4$  labelled-precursors ( $CD_3OD$ ) were performed (biomimetic in-bean experiment), to validate the significance of the precursor.

### RESULTS

Non-targeted analyses and multivariate analyses revealed that coffee beans with high quality had higher amounts of MECs. Interestingly, spiking of isovaleric acid methyl ester, one of the observed methyl esters, enhanced the flavor quality not only for fresh fruity attribute but also for cleanness, acidity and bitterness. In addition, monitoring and biomimetic in-beans experiments suggested that MECs and precursor methanol as the methyl moiety donor were developed during roasting. Precursor methanol was a key factor for the chemical formation for MECs. This process resulted in the flavor quality of the roasted beans.

### CONCLUSIONS & PERSPECTIVES

Our unique approach identified MECs and their precursors which impacted not only the aroma but also the taste quality of roasted coffee. Further study is ongoing to explore the precursors of methanol which might be a key factor, leading to the high-quality coffee beverage products.

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Sensory and analytical study of different coffee origins as support for flavor creation.

Cornuz Maurin\*, Lau Hazel\*\*\*, Bin Yu\*, Lassablière Benjamin\*

\*Strategic Development, Mané South East Asia, Singapore

\*\*Food Science and Technology Programme, Department of Chemistry, National University of Singapore, Singapore

## RATIONALE

As specialty coffee gains popularity around the world, particularly in countries where the coffee market is dominated by products such as instant coffee mixes and ready to drink coffee beverages, which often contain flavors, the need arises to create coffee flavorings with a new, single origin inspired, specificity. This study proposes to confront the sensory evaluations of four coffee origins performed on one hand by a team of CQI coffee Q-graders and on the other by a group of trained flavorists.

In parallel, aroma extract dilution analysis of the four coffee origins give chemical insight about the impact odorants of those specific coffee origins (1). Combined together, the sensory and analytical information allows flavorists to recreate coffee flavoring preparation specific of each origin.

## METHODS

Four iconic and popular arabica coffee origins, Brazil Santos Arabica, Colombia Supremo, Ethiopia Yirgacheffe and Guatemala New Orient were selected; samples were obtained and roasted to achieve the desired flavor profile.

The standard Specialty Coffee Association cupping protocol was used for the sensory evaluation by the flavorists and for the formal evaluation by q-graders.

Solvent Assisted Flavor Evaporation (SAFE) was used to produce the aroma extract used in the GC-O/AEDA technique.

## RESULTS

Having coffee professionals and flavorists discuss and evaluate coffee together helped the latter understand and differentiate desirable traits in coffee and get insight from the consumer point of view. The result from volatiles analysis is then used as a backbone for the flavor creation and feedback from coffee professionals serves as validation. As proven by Mayer *et. al.*(2), the mix of about 20 key odorants is sufficient to recreate the aroma of ground coffee, however the challenge faced by flavorists is to integrate constraints of taste and stability to their formulation.

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## RESOLVING COFFEE ROASTING PHASES BY VACUUM PHOTOIONIZATION TOF-MS PROCESS MONITORING: TOWARDS COFFEE WITH OPTIMIZED AROMA PROFILE AND ANTIOXIDANT CAPACITY

ZIMMERMANN, Ralf\*, HEIDE, Jan\*, EHLERT, Sven\*\*, CZECH, Hendryk\*, WALTE, Andreas\*\*, KOZIOROWSKI, Thomas\*\*\*.

\*University of Rostock, Joint Mass Spectrometry Centre, Rostock, GERMANY, \*\*Photonion GmbH, Schwerin, GERMANY, \*\*\*PROBAT GmbH, Emmerich am Rhein, GERMANY.

### RATIONALE

On-line process monitoring of the coffee roasting process enables a better control of the product quality, in particular the flavor profile and likely also antioxidant capacity. In the consortium, a rugged photoionization mass spectrometer (PIMS) for on-line, real-time process monitoring and optimization is developed.

### METHODS

The use of laser- or VUV-lamp based single-photon ionization (SPI) and resonance-enhanced multiphoton ionization (REMPI at 227 nm, 248 nm and 266 nm) time of-flight mass spectrometry covers a broad range of flavor compounds and antioxidants. The benefits of PIMS are demonstrated by roasting experiments of Arabica and Robusta coffees with different roasting profiles. Statistical data analysis such as non-negative matrix factorization (NMF) was applied to identify and group temporal evolution profiles of relevant compounds or molecular masses.

### RESULTS

NMF analyzed PIMS results (four factor solution) allow to recognize roasting phases which represent 'evaporation', 'early roast', 'late roast' and 'over-roast' status. Each roasting phase features a different molecular fingerprint, which was further investigated due to the high selectivity and softness of the ionization process, simplifying the interpretation of the factor loadings. Validity of the NMF results was proven by recovering known markers in the NMF loadings, e.g. pyridine for over-roast. Additionally, in micro probe PIMS experiments high molecular weight aromatics, representing important antioxidants in coffee (polyphenols) were observed. In order to explain the formation processes, also the results of PIMS from single beans roasting [2] or thermal analysis Fast-GC PIMS [3].

### CONCLUSIONS & PERSPECTIVES

Photoionization mass spectrometry allows to monitor the chemistry of coffee roasting at appropriate time resolution. This research is strengthening the chain of process understanding, monitoring and finally on-line process control for product/process optimization. The new PIMS process monitor uses industry-standard laser technology (Excimer) and an innovative orthogonal acceleration TOF technology for ruggedness, superior sensitivity and improved mass resolution.

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## INVESTIGATING BITTER SUBSTANCES IN COFFEE BREWS BY ANALYZING THE RESPONSE TO TASTE SENSOR

FUJIMOTO, Hirofumi\*, IWAI, Kazuya\*, NARITA, Yusaku\*, HANZAWA, Taku\*, KOBAYASHI, Tsukasa\*, KAKIUCHI, Misako\*, WU, Xiao\*\*, MIYAKE, Kazunari\*\*\*, TAHARA, Yusuke\*\*, IKEZAKI, Hidekazu\*\*\*\*, FUKUNAGA, Taiji\*, TOKO, Kiyoshi\*\*  
\* Innovation Center, UCC Ueshima Coffee Co., Ltd., Hyogo, Japan, \*\* Research and Development Center for Taste and Odor Sensing, Kyushu University, Fukuoka, Japan, \*\*\* Graduate School of Information Science and Electrical Engineering, Kyushu University, Fukuoka, Japan, \*\*\*\* Intelligent Sensor Technology, Inc., Kanagawa, Japan.

### **RATIONALE**

Taste sensors have been used in food industry owing to their global selectivity that determine tastes, such as bitterness, sweetness, saltiness, acidity, umami, and astringency. Especially, in the coffee industry, taste sensors are applied for products comparison, analysis of compatibility cooking and beverages, and quality inspection, among others. Previous study reveals that the bitterness response value of taste sensors strongly correlates with results of sensory tests by humans for bitterness<sup>1</sup>. However, since taste sensors have wide a range of selectivity, it is not clear that which substances in the coffee brew are responding to the taste sensors.

This research aims to investigate the bitterness substances in coffee brew by analyzing responses of a taste sensor.

### **METHODS**

The coffee brew was obtained from coffee beans (*Coffea arabica*, Brazil) with different roast degree (light, medium, high, and dark roast). Coffee brew was fractionated into four fractions by liquid-liquid extraction using organic solvents. Each fraction was analyzed using the taste sensor. The bitterness response of each fraction was calculated. Substances contained in each fraction were relatively quantitated using liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) and liquid chromatography-photodiode array (LC-PDA). Results obtained from the taste sensor, LC-MS/MS, and LC-PDA were analyzed by partial least squares regression (PLS-R) and substances contributing to the taste sensor were investigated.

### **RESULTS**

It was found that the bitterness response value of the taste sensor increased with an increase in the degree of roasting. This result is in agreement with ones obtained from the previous studies. PLS-R analysis revealed that nicotinic acid and nicotinamide strongly contributed to the bitter response value of the taste sensor. The bitterness response value of coffee having nicotinic acid and nicotinamide were larger than that of control coffee. Sensory testing of coffee having nicotinic acid or nicotinamide gave a more bitter taste.

### **CONCLUSIONS & PERSPECTIVES**

This research proves the value of taste sensors in coffee industry. It is expected to make it easier to make coffee brew having desired taste using taste sensors.

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SULEWSKA, Anna Maria\*, HOLMEN, PEDERSEN, Anders\*, KRYGER SØRENSEN, Jakob\*,  
HOFMANN LARSEN, Flemming\*\*

\*GEA Process Engineering A/S, Soeborg, DK, \*\*University of Copenhagen, Frederiksberg, DK.

## RATIONALE

The world consumption of instant coffee (IC) has increased by over 17% between 2012 and 2017 reaching 1.66 million tonnes (data according to Euromonitor International). Due to consumers demand for convenience and changing consumption patterns, further growth is predicted.

One of the major challenges during the IC production is to retain the aroma and taste of the freshly brewed coffee [1]. The organoleptic properties of the final product may be influenced by any of the process steps (i.e. roasting, bean treatment, extraction, extract treatment, concentration and drying). Literature on this topic is very limited, as it requires either access to industrial scale facilities, or specialized equipment to perform pilot scale experiments. Recently characterization of industrially produced aroma concentrate were published [2], but more research is still necessary to better understand the influence of processing parameters on the taste and aroma of instant coffee.

## METHODS

Roasted coffee was extracted in pilot scale (400 g coffee per extraction) with deionized water at temperatures ranging from 140 °C to 190 °C. Resulting extract was further treated and freeze dried to yield the final IC product. Freshly brewed coffee and instant coffee obtained from the same roasted beans were compared by sensory evaluation and advanced analytical methods, such as <sup>1</sup>H NMR and GC-MS. Additionally, samples taken from individual processing stages were characterized.

## RESULTS

It was possible to identify and quantify numerous compounds including, but not limited to: caffeine, trigonelline, acetic acid, formic acid, 3-mercapto-3-methylbutyl formate, furfurylthiol; and follow them through the processing steps involved in the production of instant coffee. It was shown that the composition of organic acids and other compounds differs between the freshly brewed and instant coffee.

## CONCLUSIONS & PERSPECTIVES

Our approach to follow the compounds important for coffee aroma and taste during the processing will allow for identification of the most crucial processing steps. This in turn will facilitate their improvement in order to retain more of the valuable characteristics of the freshly brewed coffee in the instant coffee product.

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RAHN, Anja, SMRKE, Samo, GLOESS, Alexia, YERETZIAN, Chahan  
Zurich University of Applied Sciences, Institute of Chemistry and Biotechnology, Coffee Excellence  
Center, Wädenswil, Switzerland

Roasting is an essential stage of coffee manufacturing during which coffee's characteristic texture, colour and aroma are generated. The physical and chemical transformations taking place during roasting, including the Maillard reaction, are considered to be governed by the time-temperature sequence, otherwise known as the roast profile. The roast profile itself is regulated in part by the roaster's airflow. Oxygen delivery has received negligible attention regarding its influence on the coffee aroma formation during roasting despite there being evidence that oxygen can participate in the Maillard reaction as well as polysaccharide degradation.

Using an Arabica coffee originating from Guatemala the current study examines the influence of coffee roasting under an aerobic (air) versus anaerobic (N<sub>2</sub>) atmosphere. PTR-ToF-MS was used throughout roasting in order to monitor the real-time evolution of coffee aroma. Coffee aroma compounds were then compared between the two atmospheric conditions in order to determine the impact of oxygen in the roasting air on aroma formation. To complement the PTR-ToF-MS data, roasted ground coffee was analyzed using HS-GC/MS. In addition to these measurements, prominent differences in aroma were observed.

PTR-ToF-MS experiments demonstrate that roasting under air (of which 21% is oxygen) significantly increases the intensity of a variety of VOCs. Conversely, hydrogen sulfide is the only compound observed to be significantly higher under anaerobic conditions. Amongst the VOCs enhanced under aerobic conditions is benzaldehyde a known degradation of phenylalanine, suggesting the oxidative modification of amino acid side chains. Distinct sensory differences were perceived between aerobically and anaerobically roasted coffees, in particular for the aroma attributes "coffee" and "fishy". This provided further evidence towards oxidative modification and promotion of oxidative Maillard reaction pathways during roasting. Moreover, the prominent sensory differences between roasting under air and nitrogen exposes the importance of air flow in a roaster towards coffee quality.





## KEEP IT COLD: AN INVESTIGATION OF THE SHELF LIFE OF COLD BREW COFFEE AND THE INFLUENCE OF EXTRACTION TEMPERATURE USING CHEMICAL, MICROBIAL AND SENSORY ANALYSIS

LOPANE, SAMUEL\*, MCGREGOR, JOHN\*

\*Clemson University, Clemson, SC, USA

### RATIONALE

Cold brew coffee is a product with growing market share – it offers the potential for high quality, a unique flavor profile, a position within the growing specialty coffee world, and RTD (ready-to-drink) convenience. However, there is little published information around the exact chemical characteristics of cold brew to verify producers' claims and allow for prediction of shelf life. There is a proliferation of beverages on the market, and a dearth of data surrounding them. This study examined the shelf-life of cold water extraction (cold brew) coffees based on sensory and chemical profile and microbial growth, while also investigating the influence of extraction temperature on the chemical and sensorial profile of cold water extraction coffee. Because of the dual purpose of this study, the experiment was structured as a split-plot design – meaning there were multiple independent variables (time and extraction temperature) that affected a large number of dependent variables that were measured. This experimental design, as well as the statistical techniques used, allowed the study of both the effect of time and extraction method on the coffee beverages.

### METHODS

In order to evaluate the shelf life of cold brew, investigate the influence of extraction temperature, and contribute to the body of knowledge around cold brew, a number of chemical, microbial, and sensory tests were conducted on three distinct extraction methods of cold coffee over a 42-day storage period. The three treatment groups in this study were cold water extract (CWE) coffee, ambient water extract (AWE) coffee, and hot water extract (HWE) coffee. The analytical tests that were conducted on these groups were %TDS via refractive indices, caffeine and chlorogenic acid concentration via HPLC, pH and titratable acidity, headspace analysis via SH-GC/MS, and microbial growth via aerobic plate counts and psychrotrophic counts. The sensory analysis was conducted using sensory descriptive analysis with a trained panel of coffee professionals to evaluate thirteen sensory attributes over the same period of 42 days.

### RESULTS

There were significant differences in chemical and sensorial profile between the three extraction groups, illustrating the role extraction temperature has on modulating the characteristics of a coffee beverage. Chemically, the cold brew treatments (AWE and CWE) had higher pH, TA, and headspace intensity than coffee brewed at traditional (hot) temperatures. Sensorially, the cold brew treatments had significantly higher mean sweetness scores and significantly lower mean bitterness scores. In the shelf life evaluation, there were also significant changes in all treatments over the 42-day period: decreased pH, increase in mean sour and papery defect scores, and finally a decline in mean acceptability score to an unacceptable level. There was no microbial growth found in the coffee extracts.

### CONCLUSIONS & PERSPECTIVES

Based on the findings of this study, the shelf life of cold coffee is limited not by microbial stability, but rather by deterioration in sensory attributes. A 42-day organoleptic shelf life for refrigerated cold brew is suggested. Further work is recommended to elucidate the mechanisms of coffee

staling in a refrigerated environment, with particular interest in the degradation products of chlorogenic acid, as a significant decline in chlorogenic acid concentration was found over the storage period. Cold extracted coffees were found to be chemically and organoleptically different beverages from coffees extracted at high temperatures.

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## COFFEE BREWS WITH ESPRESSO PROPERTIES BY MODULATION OF THE CARBOHYDRATE CONTENT OF COFFEE EXTRACTS

LOPES, Guido R.\*, PASSOS, Cláudia P.\*, RODRIGUES, Carla,\*\* TEIXEIRA, José A.\*\*\* COIMBRA, Manuel A.\*

\*QOPNA – Department of Chemistry, University of Aveiro, Aveiro, Portugal \*\*Diverge, Grupo Nabeiro Innovation Center, Lisboa, Portugal \*\*\*Centre of Biological Engineering, University of Minho, Braga, Portugal

### RATIONALE

Espresso, instant or filtered coffee exhibit distinct physico-chemical properties due to the nature of the coffee used (*i.e.* coffee powder or soluble coffee powder) or to the conditions used to prepare the brews (*i.e.* pressure or filter). All brews contain carbohydrates, proteins, lipids or volatile compounds, but their different amount and composition contributes to diverse physico-chemical and organoleptic characteristics (body, color, taste, viscosity or foam). Thus, modulating coffee extraction should allow to obtain more instant, filtered or espresso-like extracts according to the desirable brew characteristics.

### METHODS

Response surface methodology (RSM) was used to study and optimize the coffee extraction process using a conventional hot water extraction at atmospheric pressure (solid-liquid extraction) and with pressurized water via microwave-assisted extraction. The influence of time of extraction, temperature, mass-to-volume ratio, and the grinding grade of the roasted powder was studied concerning the overall extraction yield, carbohydrate content and sugar composition. The optimized conditions regarding espresso similarity was scaled up and the freeze-dried extract was compared to commercial espresso and instant coffee extracts regarding sugar composition and glycosidic linkages (GC-FID, GC-MS),  $K_{mix}$  280, 325 and 405 nm, lipid content (Soxhlet extraction), caffeine and chlorogenic acids content (HPLC), viscosity (Cannon-Fenske viscometer), pH and foamability properties (CO<sub>2</sub> injection).

### RESULTS

Temperature is the most important factor for extracts differentiation (overall yield, carbohydrates content). The use of pressurized water at high temperatures (180 °C) favoured the increase of overall yield mainly through the extraction of arabinogalactans over galactomannans, resulting in extracts resembling instant coffee. Scaled up extract exhibited a sugar composition similar to espresso, with predominance of galactomannans over arabinogalactans, contrary to instant coffee. Viscosity and pH of the coffee extract solutions were also more similar to espresso. Visually, these coffee brews were less opaque than espresso and instant coffee. All extracts were able to produce foam when submitted to CO<sub>2</sub> injection.

### CONCLUSIONS & PERSPECTIVES

Mild extraction conditions allowed to obtain coffee brews with sugar content and composition as well as viscosity properties similar to espresso coffee, which open the possibility of the development of instant coffee products chemically and organoleptically closed to those obtained with espresso coffee.

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## In-bean experiments: Are they reliable enough to study coffee flavor formation?

POISSON, Luigi\*, AUZANNEAU, Noémie\*, SCHAEERER, Ania\*, PITTET, Jonathan\*, BLANK Imre\*\*, DAVIDEK, Tomas\*

\* Nestec Ltd. Nestlé Product Technology Centre Beverage, , 1350 Orbe, Switzerland

\*\*Nestec Ltd, Nestlé Research Center, CH-1000 Lausanne 26, Switzerland

### RATIONALE

Coffee roasting is a very complex process with many chemical reactions proceeding in parallel, while the physical environment changes all over the roasting course. The necessity to investigate the formation of flavor components in a real food system on top of model systems was discussed and corroborated by comparative studies in e.g. cereal products and roasted coffee. Therefore, the so-called in-bean approach was developed to maintain the integrity of the coffee bean structure permitting to have the similar physical and chemical changes during the roasting course in recombined bean like in the original real bean matrix<sup>1</sup>. Labelling studies using <sup>13</sup>C and <sup>15</sup>N labeled precursors as well as the Carbon Module Labeling (CAMOLA)<sup>2</sup> technique are very potent tools in gaining a more precise insight into the formation pathways of key flavor components. The combination with kinetic studies assessing the flavor formation during the roasting course allow for a quite complete picture taking into account not only the depletion of precursors but also the intermediate transformations<sup>3</sup>.

However, the question remains whether the described approach is sufficiently reliable and robust to reflect the above said complex mechanisms during coffee roasting.

### METHODS

The representativeness of the in-bean approach was investigated by comparing formation kinetics of selected aroma compounds in recombined and reference coffee bean. Therefore, key aroma compounds were quantified by means of Stable Isotope Dilution Assay (SIDA) in combination with Solid-Phase Microextraction and Gas Chromatography-Mass Spectrometry (SPME-GC-MS/MS) throughout the roasting course at 10 sampling points.

### RESULTS

The results highlighted similar formation kinetic for most of the evaluated volatile compounds like Strecker aldehydes, alkyl pyrazines or cyclic enolones. In addition, the intermediate and final quantities of key odorants in both samples were mostly quite comparable. Higher variability was found for sulfur compounds like dimethyl sulfide or  $\alpha$ -diketones (2,3-butanedione).

### CONCLUSIONS & PERSPECTIVES

The developed and refined bio-mimetic approach was validated as a valuable approach in studying different aspects of flavor formation upon coffee roasting. Important is a very rigorous sample preparation avoiding strong bean degradation and/or deficient incorporation of precursors.

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## New Physicochemical Quality Indicator for Specialty Coffee

HORIGUCHI, Toshihide\* FURUSHO, Tadasu\*\*

Dept. of Ecological Symbiotic Science, graduate schools Tokyo University of Agriculture.

\*papa@kohikobo.co.jp \*\*tfurusho@nodai.ac.jp

### RATIONALE

To judge the quality of green coffee beans, determination of physicochemical indices are as important as the physical and sensory evaluations. Therefore, this study aimed to analyze the level of acid (pH, titratable acidity) that strongly affects flavor, lipids (total lipids and the acid value), and sucrose, which are some of the various components present in green coffee beans and to create new quality indices from the correlation between the results obtained and the SCA sensory evaluation.

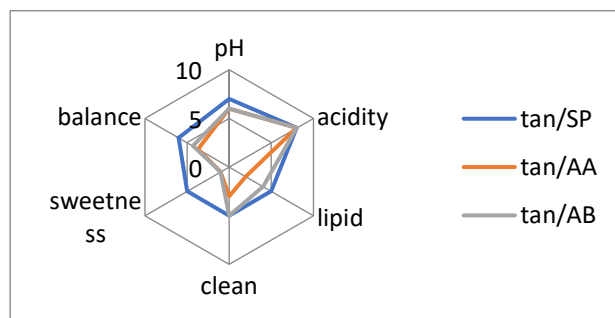
### METHODS

The 2015–16 and 2016–17 specialty coffee (SP) and commercial coffee (CO) crops were analyzed upon entry into the Japanese port.

The beans were pulverized and the total amount of lipids was measured by the chloroform-methanol method. Lipids extracted by the ether method were titrated against KOH, and the acid value, which signifies lipid deterioration, was measured. Medium roasted coffee beans were pulverized, and the acidity was measured using a pH meter. After neutralization titration using NaOH, the titratable acidity was determined. In addition, the amount of sucrose was analyzed by HPLC. Furthermore, 13 individuals with cupping skill performed the sensory evaluation in a blinded state using the SCA cupping form.

### RESULTS

When the lipid content in the beans was  $\geq 15\%$ , it was considered to be higher, and when the acid value was  $\leq 3$ , freshness was considered to be maintained. Moreover, sucrose content is normally in the range of 5–8, and values  $\geq 7$  suggested a high sucrose content. The pH of roasted coffee beans ranges from 4.75 to 5.2. In this study, the titratable acidity ranged from 5.5 to 8.5. In addition, among the 50 samples, the score for SP and CO was  $\geq 80$  and  $\leq 79$  points, respectively. There was a correlation between total score in the sensory evaluation and acid value, acidity in the sensory evaluation and pH, and body (mouth feel) and lipids. These results demonstrated that these values can likely be utilized as quality indices of green coffee beans.



SP/Tanzania Blackburn Estate

### CONCLUSIONS & PERSPECTIVE

A quality indicator was created from the analysis values.

By applying the analysis values to the indicator, judging the quality of a coffee bean becomes possible from the physicochemical aspect. The use of these findings will contribute to maintaining the quality of green coffee beans in the producing as well as in the consuming countries.



## A COMPREHENSIVE ESTIMATE OF GLOBAL COFFEE FARMER POPULATIONS BY ORIGIN.

David Browning, Carl Cervone, Maria Burkiewicz, Maike von Heymann, Alexandra Hilble\*

\*Enveritas, a non-profit founded in 2016 to overcome systemic barriers that are preventing proven solutions from being applied to end poverty for smallholder coffee growers, New York, USA.

### RATIONALE

There are many sustainability initiatives, current and future, that seek to address social, economic and environmental challenges in the coffee sector. A critical underpinning to scientific inquiry in this arena is an accurate estimate of coffee farmer populations and characteristics by origin. However, to our knowledge there has never been a rigorous undertaking to answer this question. Commonly cited estimates of 20-25 million farmers rely upon a self-reported exercise by coffee nations 20 years ago, whose original source data, definitions and methods have been lost to history.

### METHODS

The population of coffee farmers for 20 major origins is analysed with the use of a consistent, three-step methodology. The first step involves building a data set from literature reviews, interviews with stakeholders, and, for most countries, primary data collected from farmers (i.e., over 20,000 farmer surveys). The second step involves building a data model that takes a rigorous and consistent statistical approach for estimating farm size and production distribution characteristics for each country. It includes statistical inference, scenario analysis and bootstrapping methods. In the last step, the results from the analysis are compared with official statistics and any differences – if any – are explained.

### RESULTS

Such a large (in scope) and rigorous assessment of the coffee farmer population has not been carried out before. We present a robust, methodologically-consistent data set of productivity and farm size estimates, and also summarize the main characteristics of coffee producers in 20 major countries. Finally, the study answers the fundamental question – “how many coffee farmers are there in the world?” with a new estimate that can underpin future research and policy work.

### CONCLUSIONS & PERSPECTIVES

We believe that this study will become a great source of knowledge and information about the unique production characteristics of the coffee sector in each of the countries researched, with important policy implications. At the same time, we hope that it will open a broader discussion testing and revisiting the assumptions made for the purpose of further analyses and updating the results obtained.

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International Coffee Organization, United States Department of Agriculture, primary data collected by Enveritas.



BUNN, Christian\*, LUNDY, Mark\*, CASTRO, Fabio\*, LADERACH, Peter\*\*

\* International Center for Tropical Agriculture (CIAT), Cali, Colombia, \*\*International Center for Tropical Agriculture (CIAT), Hanoi, Vietnam

Coffee stakeholders from across the globe report perceived irregularities in weather patterns that result in reduced yields or increased production costs. Using global climate models, it was projected that coffee production will be negatively affected by climatic change in coming decades. Efforts to adapt coffee production to these projected changes may benefit from understanding the impacts to date. We used a database of geo-referenced coffee occurrence locations and data on production quantity, area and yield to develop RandomForest regression production functions from historical weather anomalies between 1961 and 2016. These production functions were projected on a counterfactual of detrended climate anomalies. We then compared observed production, modeled production on observed climate and modeled production on detrended climate.

We found that recent climate trends had diverging effects on production across the globe. Compared to a detrended climate, climate trends resulted in significant declines of coffee production in Central America, East Africa and parts of South East Asia. At global scale however, this decline was offset by yield increases in Brazil. Because of the importance of Brazilian production for total global volumes in sum there was no net effect on global production. An incorporation of market effects might challenge this finding as world market prices may already be higher because of the negative production impacts. Our findings support the perception by stakeholders that coffee production is already affected by climatic change. To date, the effect on consumers has been limited but projections suggest that this will change in coming decades. Private and public sectors should consider investments in climate smart coffee to avoid a further decline of production.

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## FULL GENOME SEQUENCE ASSEMBLIES AND ANNOTATIONS OF COFFEA ARABICA AND ITS TWO DIPLOID PARENTS.

de KOCHKO, Alexandre\* and CROUZILLAT, Dominique\*\* on behalf of the Arabica Coffee Genome Consortium (ACGC) 14 countries, 25 institutions, 63 researchers

\* UMR DIADE, IRD, BP 64501, 34394 Montpellier CEDEX 5, France. \*\* Nestlé R&D Centre, Plant Science Research Unit, 101 Av. G. Eiffel B.P. 49716, 37097 Tours CEDEX 2, France.

### RATIONALE

Consumers worldwide prefer Arabica coffee which represents the largest part of the coffee market. *Coffea arabica* which is the sole tetraploid of the *Coffea* genus, which encompasses 125 recognized species. *Coffea arabica* presents genetic and phenotypic differences with its both parents that can only be explained by a differential behavior of the two genomes in a tetraploid context. Knowing the genome sequence is a mandatory step for understanding the structural and functional particularities of this genome. It is also a prerequisite for developing performing breeding programs using Genomic Selection (GS) and/or Genome Wide Association Studies (GWAS).

### METHODS

A dihaploid was used to sequence the *C. arabica* genome, a doubled haploid to improve that of *C. canephora* (Denoëud et al., 2014) and a wild heterozygous diploid for *C. eugenioides*. A combination of genomics technologies were used to sequence, assemble and anchor the three genomes. Assemblies were produced in up to three steps: sequence assemblies from long reads sequencing, optical mapping and chromosomal conformation capture (Hi-C). They were then anchored using ultra high-density genetic maps obtained from resequencing of segregating populations of *C. arabica* and *C. canephora*. Gene discovery and annotation were performed thanks to high coverage RNASeq using long and short reads. Transposable elements and non coding RNAs were identified using dedicated softwares. In addition, 36 genotypes of wild and cultivated genotypes of *C. arabica* were also resequenced using short reads technology.

### RESULTS

High percentage of the three genomes were assembled: 85%, 94% and 98% for *C. arabica*, *C. canephora* and *C. eugenioides* respectively. After assigning contigs to the respective sub genomes for *C. arabica* anchoring was performed for the three genomes. No major chromosomal rearrangements in the *C. arabica* sub genomes compared to the parental ones were observed nor major disruption in gene composition of the parental genomes vs. the polyploid hybrid. The polyploidization did not provoke either a burst in transposable elements activity.

### CONCLUSIONS & PERSPECTIVES

High quality reference genomes were produced for three *Coffea* species. Genetic diversity of *C. arabica* was assessed. Ultra high density genetic maps were build. Evolutionary history of *C. arabica* was precised. We report here performing tools now available for breeders to conduct efficient breeding programs for key agronomic issues (yield, tolerance to pests, resilience to climate change, quality of the coffee beverage...).

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## Genome-wide association study identify SNPs and genomic regions for lipids and diterpenes contents in *Coffea arabica* related to its domestication

PEREIRA, Luiz Filipe P. \*, SANT'ANA, Gustavo C. \*, FERREIRA, Rafaelle V. \*\*, SILVA, Bruna S. R. \*\*, NOGUEIRA, Livia M. \*\*, PADILHA, Lilian \*, KITZBERGER, Cintia S. \*\*, SCHOLZ, Maria B. \*\*, SERA, Gustavo H. \*\*, DOMINGUES, Douglas \*\*\*, GUYOT, Romain \*\*\*\*, POT, David \*\*\*\*\*, CHARMETANT, Pierre \*\*\*\*\*, LEROY, Thierry \*\*\*\*\*

\*Embrapa Café, Brasília, DF, Brazil; \*\*IAPAR, Londrina, PR, Brazil; \*\*\*UNESP Rio Claro, SP, Brazil, \*\*\*\* IRD, Montpellier France, \*\*\*\*\*CIRAD, Montpellier, France.

### RATIONALE

Genome-wide association studies (GWAS) are an efficient approach to dissect the genetic architecture of complex traits. Lipids, including the diterpenes cafestol and kahweol, are key compounds that contribute to the quality of coffee beverages. A genome-wide association study was performed to identify genomic regions associated with lipid, cafestol and kahweol.

### METHODS

Using GBS, we genotyped 107 *Coffea arabica* accessions, including wild genotypes from the historical FAO collection from Ethiopia and cultivars. Lipids, cafestol and kahweol contents in green beans were determined by NIRS. For full description of genetic analysis and the four association methods: mrMLM, FASTmrEMMA, ISIS EM-BLASSO, pLARM EB see Sant'Ana et al. 2018.

### RESULTS

Using the diploid *Coffea canephora* genome as a reference for GBS data, we identified 6,696 SNPs. Population structure analyses suggested the presence of two to three groups ( $K = 2$  and  $K = 3$ ) corresponding to the east and west sides of the Great Rift Valley and an additional group formed by wild accessions collected in western forests. We identified 5 SNPs associated with lipid content, 4 with cafestol, 3 with kahweol and 9 with cafestol/kahweol ratio. Most of these SNPs are near by genes related to metabolic pathways of those compounds. Among all trait-associated SNPs detected by GWAS, three showed strong signals of directional selection between genetic groups identified using STRUCTURE ( $K = 3$ ). The group with most wild accessions presented very low frequencies of the reference alleles compared with the other two groups. This indicates that domestication and/or breeding process of *C. arabica* may have changed allelic frequencies of these loci in order to modulate lipids and diterpenes content.

### CONCLUSIONS & PERSPECTIVES

The genetic analysis helped to define which accessions are more important to preserve in order to have a good genetic representation of the FAO collection and provided insights on *C. arabica* domestication. The GWAS approach was efficient to identify markers and genomic regions associated to lipids and diterpenes in *Coffea arabica* that can be used into breeding programs.

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CRISPR/Cas9-mediated efficient targeted mutagenesis<sup>[1]</sup> has the potential to accelerate the domestication of *Coffea canephora*

Jean-Christophe Breitler, Eveline Dechamp, Claudine Campa, Leonardo Augusto Zebral Rodrigues, Romain Guyot, Pierre Marraccini, Hervé Etienne

## Abstract

Genome editing, which is an unprecedented technological breakthrough, has provided a valuable means of creating targeted mutations in plant genomes. In this study, we developed a genomic web tool to identify all gRNA target sequences in the coffee genome, along with potential off-targets. In all, 8,145,748 CRISPR guides were identified in the draft genome of *Coffea canephora* corresponding to 5,338,568 different sequences and, of these, 4,655,458 were single, and 514,591 were covering exons. The proof of concept was established by targeting the phytoene desaturase gene (*CcPDS*) using the *Agro-bacterium tumefaciens* transformation technique and somatic embryogenesis as the plant regeneration method. An analysis of the RNA-guided genome-editing events showed that 22.8% of the regenerated plants were heterozygous mutants and 7.6% were homozygous mutants. Mutation efficiency at the target site was estimated to be 30.4%. We demonstrated that genome editing by the CRISPR/Cas9 method is an efficient and reliable way of knocking out genes of agronomic interest in the coffee tree, opening up the way for coffee molecular breeding. Our results also showed that the use of somatic embryogenesis, as the method for regenerating genome-edited plants, could restrict the choice of targeted genes to those that are not essential to the embryo development and germination steps.

**Keywords** CRISPR/Cas9 · *Coffea canephora* · Phytoene desaturase · Somatic embryogenesis · Targeted mutagenesis



## WIDE *C. ARABICA* GENETIC STUDY BRINGS NEW INSIGHTS ON MOVEMENTS AND BREEDING HISTORY OF THE SPECIES.

Pruvot-Woehl S.<sup>1</sup>, Toniutti L.<sup>1</sup>, Al Hakimi A.<sup>2</sup>, Krishnan S.<sup>3</sup>, Klein P.E.<sup>4</sup>, Murray S.<sup>5</sup>, Solano W.<sup>6</sup>, Schilling T.<sup>1</sup>, Adugna G.<sup>7</sup>, Bertrand B.<sup>1,8</sup>, and Montagnon C.<sup>1</sup>

<sup>1</sup>World Coffee Research, 10940 SW Barnes Rd #334, Portland, OR 97225, USA, <sup>2</sup>Faculty of Agriculture, Sana'a University, Sana'a, Yemen, <sup>3</sup>Denver Botanic Gardens 909 York Street, Denver, CO 80206, USA, <sup>4</sup>Department of Horticultural Sciences, Texas A&M University, College Station, TX, USA, <sup>5</sup>Department of Soil and Crop Sciences, Texas A&M University, College Station, TX, USA, <sup>6</sup>CATIE, Turrialba, Costa Rica, <sup>7</sup>Department of Horticulture and Plant Sciences, Jimma University, Jimma, Ethiopia, <sup>8</sup>CIRAD UMR IPME Montpellier, France

### RATIONALE

While several studies have described the genetic diversity of *C. arabica*, none has included the whole range of Ethiopian accessions together with a large representation of the varieties cultivated worldwide. None included recently surveyed South Sudanese populations.

Over the last years, WCR has built a large database that actually includes all the dimensions of genetic diversity of the species: from Ethiopian, Yemeni and South Sudanese accessions to a large representation of old and new cultivated varieties. The results of the detailed analysis of this database (SSR) are presented here.

### METHODS

The data base represents i) One core collection established in 2014 including mainly Ethiopian accessions (FAO and ORSTOM surveys), ii) Populations of wild arabicas surveyed in South Sudan in 2014 and iii) a large representation of cultivated varieties worldwide.

More than 2000 entries of this database were genotyped using a set of 9 SSR markers. Multivariate analysis (PCoA) were run in order to decipher the underlying genetic diversity.

### RESULTS

For the first time, wild *C. arabica* populations of South Sudan are shown to bring new genetic diversity as compared to Ethiopian wild arabicas. A structuration of the Ethiopian accessions surveyed in the 60's (FAO and Orstom) is unraveled. The traditional Bourbon/Typica varieties are genetically related to the Ethiopian cluster east of the Ethiopian coffee area. This study gives a new light on the history of *C. arabica* movement around the world. While the genetic diversity of cultivated varieties around the world is confirmed to be relatively low, it is still possible to authenticate them through fingerprinting. Most varieties show a residual segregation and not fully fixed homozygous lines. Consequences for varieties authentication are discussed.

### CONCLUSIONS & PERSPECTIVES

This study is the first of its kind with SSR on a wide range of Arabica accessions and varieties. It gives us a new vision of the genetic diversity of the species and history of its movements. As exemplified by the South Sudan Arabica populations, new genetic diversity is to be found in the vast Arabica center of origin covering mainly Ethiopia but also South Sudan. India has been a very important and often overlooked step for the dissemination of genetic diversity out of Ethiopia. As for practical application, opportunities and challenges of varieties authentication through DNA fingerprinting are discussed.

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## INTROGRESSION OF COFFEA CANEPHORA GENOME IN RUIRU 11 SIBS AND ITS EFFECT ON QUALITY AND CBD RESISTANCE

GICHIMU, Bernard M.\*, GICHURU, Elijah K.\*\*, NYENDE, Aggrey B.\*\*\*, MAMATI, George E.\*\*\*  
\*University of Embu, Embu, KENYA, \*\*Coffee Research Institute, Ruiru, KENYA, \*\*\*Jomo Kenyatta University of Agriculture and Technology, Juja, KENYA.

### RATIONALE

Robusta coffee (*Coffea canephora*), which is the major source of resistance to Coffee Berry Disease (CBD) in Ruiru 11 has relatively poor beverage quality and therefore its genome introgression is expected to affect beverage quality in Ruiru 11 sibs. This study attempted to gain insights into *C. canephora* genome introgression in Ruiru 11 cultivar and how it affects its quality and CBD resistance.

### METHODS

The plant materials were Robusta coffee (*C. canephora*), introgressed *Coffea arabica* var. Hibrido de Timor (HDT), non introgressed *C. arabica* var. Caturra, SL28 and K7 and 34 Ruiru 11 sibs. Their genomic DNA was amplified with thirteen microsatellite primer pairs twelve of which had been pre-selected for introgression studies in Arabica coffee. Any common alleles in Robusta, HDT and Ruiru 11 sibs that were found to be missing in non-introgressed *C. arabica* genotypes were considered as introgressed alleles. Similarly, any alleles that were present in non-introgressed Arabica genotypes but missing in Robusta and HDT but were either absent or present in Ruiru 11 sibs were also considered to result from introgression. Sensory evaluation of cup quality, assessment of biochemical composition and evaluation of CBD resistance were conducted and their data correlated to the molecular data on introgression.

### RESULTS

The study established that the amount of canephora-genome introgressed in Ruiru 11 ranges from 8.7 to 24.14%. Although alien genome introgression is likely to include not only desirable target genes but also undesirable linkages, this study did not obtain any significant correlations between levels of introgression and either cup quality or biochemical composition of Ruiru 11.

### CONCLUSIONS & PERSPECTIVES

The study concluded that the beverage quality of some introgressed Ruiru 11 sibs is similar to that of non-introgressed varieties. The study also demonstrated that transfer of desirable genes into *C. arabica* from *C. canephora* is possible without adversely affecting the recipient variety and is not limited by differences in ploidy levels or suppression of recombination.

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## NEW ASSESSMENTS OF THE *COFFEA CANEPHORA* GENETIC DIVERSITY AND STRUCTURE ON WILD AND CULTIVATED ACCESSIONS USING SSR AND SNP MARKERS.

CROUZILLAT, Dominique<sup>1</sup>, MEROT, Virginie<sup>1</sup>, DARRACQ, Olivier<sup>1</sup>, BELLANGER, Laurence<sup>1</sup>, DE KOCHKO, Alexandre<sup>2</sup>, KIWUKA, Catherine<sup>3</sup>, UCU, Sumirat<sup>4</sup>, REVEL, Clara<sup>5</sup>, FERREIRA, DA COSTA NETO, João<sup>6</sup>, LEGNATE, Hyacinthe<sup>7</sup>, STOFFELEN, Piet<sup>8</sup>, KAMBALE, Bienfait<sup>9</sup>, PONCET, Valérie<sup>2</sup>  
1:Nestlé R&D Centre Tours, Plant Science Research Unit, France. 2:UMR DIADE, IRD, Montpellier, France. 3:NARO, Kampala, Uganda. 4:ICCRI, Jember, Indonesia, 5: ClassM, Marseille, France, 6 :INCA, Luanda, Angola, 7: CNRA, Divo, Ivory Coast, 8: Plantentuin Meise, Meise, Belgium, 9: Université Kisangani, RDC

### RATIONALE

Due to the increasing interest in *Coffea canephora* breeding for both climate changes and production of new hybrid clones with higher yield and disease tolerance, there is an important need to establish a compilation of the existing genetic diversity of this coffee species among the various existing collections. Previous studies based on microsatellite markers identified five diversity groups within the Guinea-Congo area (Gomez et al., 2009). In parallel, the development of genomic tools including SNP markers issued from the recent Robusta genome sequencing (Denoeud et al., 2014) and other ongoing resequencing programs allow us to refine the Robusta genetic diversity.

### METHODS

Based on a set of 19 SSR markers a total of 760 Robusta accessions, from different collections, IRD, CNRA, INCA, NARO, Meise Botanical garden and ICCRI, were analyzed and a core subset genotypes was selected for further genotyping using a Coffee8.5K SNP array encompassing 5575 polymorphic SNPs.

### RESULTS

The SSR markers used in this study confirmed the discrimination of five genetic groups (D (Guinean ), A (Conilon), B, C & E (Central Africa)) already identified in previous analyses. We could also characterize three other genetic origins: R originating from the Democratic Republic of Congo (DRC), O from Uganda and G from Angola.

The SNP survey also confirmed the global genetic structure established by the SSR markers. In addition it highlighted two major genetic clusters related to the geographical distribution. One in the Western areas of Africa (Guinea, West Congo, Cameroon and Angola) versus the other in Central and Esatern areas of the continent (Central Congol, DRC and Uganda).

### CONCLUSIONS & PERSPECTIVES

The global genetic diversity assessment of the *C. canephora* species was established on a large number of accessions issued from a wide range of collections representing one of the largest study performed so far. The genetic parameters of the Robusta groups characterized in this analysis are a valuable and powerful tool for further performing breeding programs.

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## Coffee somatic embryogenesis, a model to decipher fundamental mechanisms associated to totipotency, somaclonal variation and photo-autotrophy acquisition

ETIENNE, Hervé<sup>1,2</sup>, BREITLER, Jean-Christophe<sup>1,2</sup>, BERTRAND, Benoît<sup>1,2</sup>, DECHAMP, Eveline<sup>1,2</sup>, MARRACCINI, Pierre<sup>1,2</sup>, LERAN, Sophie<sup>1,2</sup>, CAMPA, Claudine<sup>2,4</sup>, DUANGSODSRI, Teerarat<sup>2,4</sup>, GEORGET, Frédéric<sup>1,2</sup>, AWADA, Rayan<sup>1,2</sup>

<sup>1</sup> CIRAD, UMR IPME, F-34398 Montpellier, France; <sup>2</sup> IPME, Univ Montpellier, IRD, CIRAD, Montpellier, France; <sup>4</sup> IRD, CIRAD, Univ Montpellier, IPME, Montpellier, France.

Nowadays coffee somatic embryogenesis (SE) can be considered as a model for woody perennial crops as its industrial application already allowed the dissemination of 50 millions of elite plants in the two cultivated species. The process works with high biological efficiency is observed at all developmental stages and somaclonal variation is mastered.

Current research beneficiates from this regeneration model as well as from the progress of coffee genomics and biotechnologies, focuses on two distinct objectives. The recent advances of this research and their respective outputs are reviewed and discussed in this presentation.

The first objective is to develop basic research based on –omics methodologies to decipher the fundamental mechanisms associated to cell reprogramming by focusing on two examples:

i) Taking advantage of optimized SE protocols, we will unravel totipotency mechanisms and molecular events involved in the key developmental switches occurring during cell dedifferentiation/redifferentiation. A multi approach characterization through cell imaging, massive transcriptomic, epigenetic and metabolomic analyzes is applied at all the key developmental stages from the leaf explant to torpedo-shaped embryos (7 well characterized developmental stages). Big data integration and a system biology approach will provide a thorough knowledge of the expression of totipotency.

ii) Coffee embryos acquire the capacity to photosynthesize very early in their developmental process (at the cotyledonary stage). Understanding the events leading to the acquisition of photo-autotrophy is also being studied by ecophysiological and molecular approaches applied to torpedo and cotyledonary-shaped embryo cultures under contrasted light, CO<sub>2</sub> and sugar conditions.

Otherwise, the second objective is to provide - through technological approaches - powerful tools to accelerate the optimization of coffee breeding:

i) The high-throughput molecular screening method has been recently developed by Nestlé that allows to miniaturize the SE process and opens the way to study the biological effect of new active molecules on particular developmental switches' efficiency. It will also allow the development of miniaturized EMS (Ethyl Methyl Sulphate) mutant banks by simplifying their setting up and further management.

ii) The genome editing of coffee genome by the Crispr/Cas9 technology recently described by using embryogenic tissues transformation gives us the possibility to rapidly and precisely manipulate plant genomes to introduce a trait of agronomic interest.



KRISHNAN, Sarada\*, BRAMEL, Paula\*\*, MONTAGNON, Christophe\*\*\*, SCHILLING, Timothy.\*\*\*  
\*Denver Botanic Gardens, Denver, CO, USA, \*\*Crop Trust, Bonn, Germany, \*\*\*World Coffee  
Research, College Station, TX, USA.

#### RATIONALE

Coffee plays a significant economic role globally and serves as a major source of foreign earnings in many producing countries. Despite challenges, world coffee production has grown steadily over the past 50 years, though it will be difficult to maintain this trend due to continued rise in production costs, as well as problems related to negative impacts of climate change and higher incidence of pests and diseases. The key to ensuring the sustainability of coffee will lie in utilizing the varied genetic resources to develop varieties with drought stress tolerances, pest and disease resistances, high cup quality, and increased production. In 2016, World Coffee Research and the Global Crop Diversity Trust spearheaded the development of the Global Conservation Strategy for Coffee Genetic Resources.

#### METHODS

A background study was done on the vulnerability of coffee genetic resources conserved ex situ and in situ, as well as on the main constraints to the use of these genetic resources. A survey of the status of major coffee collections was done, site visits were made to seven of these collections, and a study of the cost of conservation of the Centro Agronomico Tropical de Investigacion y Ensenanza's (CATIE) coffee collection was done. The main objective of these assessments was to assess the security of the current conservation system, its significant gaps, its resource requirements, and its significant constraints as well as opportunities from use.

#### RESULTS

The survey and site visits indicate that the current “system” is not sustainable, secure, cost effective, or rational. What is needed is a global system that will secure unique accessions as a global resource for use by future generations to ensure the sustainability of coffee production now and in the future.

#### CONCLUSIONS & PERSPECTIVES

Through engagement of multi-national stakeholders from various aspects of coffee production, processing, breeding, conservation and research, the goal of this Global Strategy is to ensure the conservation and use of coffee genetic resources for a positive, sustainable future of the crop and for those dependent on coffee for a livelihood. Key elements of the Coffee Conservation Strategy will be discussed.

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(Only 2 to 3 key references)



## Is coffee flowering the bottleneck in climate change adaptation?

RAHN, Eric<sup>1</sup>, LÄDERACH, Peter<sup>1</sup>, VAAST, Philippe<sup>2&3</sup>

<sup>1</sup>International Center for Tropical Agriculture (CIAT), Hanoi, Vietnam, <sup>2</sup> World Agroforestry Centre (ICRAF), Hanoi, Vietnam. <sup>3</sup> CIRAD, UMR Eco&Sols, Université Montpellier, France

### RATIONALE

Flowering is a particularly important trait in coffee (*Coffea arabica* L.). It determines the coffee yield, quality and production costs. It defines the critical period of highest demand for nutrients, water and pest and disease control. During this development phase, the plant is most susceptible to extreme climatic conditions. Factors such as temperature, light, soil and air water availability, carbon-to-nitrogen ratio, crop load and genotype can affect flowering. Recent advances in understanding the physiological mechanisms of the coffee plant, including the effects of elevated atmospheric [CO<sub>2</sub>], have greatly improved our abilities to predict possible impacts of climate change on coffee's photosynthetic capacity and allocation of photo-assimilates. Yet, we know very little regarding the effects of climate change on reproductive processes and hence final crop yield. The question arises thus, whether flowering might constitute the process most affected by increasing temperature and changes in rainfall pattern and intensity, even though the coffee's physiology might be able to partially adapt. This study reviews current knowledge on coffee flowering and its possible response to climate change. We use a simulation model to evaluate the impact of possible changes in climate on flowering success.

### METHODS

We use a process-based coffee model with a detailed flowering module representing different assumptions regarding flowering initiation, intensity, and success, accounting for multiple flowering events. The flowering module was implemented using an age-structured matrix population model that classifies the flowering process into five development stages: Nodes, floral initiation, ready to flower, pinhead stage, and bean maturation. The model is compared to observation data registered in Cajibío, Cauca, Colombia and climate change scenarios are explored using different assumptions.

### RESULTS

We highlight important knowledge gaps related to coffee flowering success. The key question will be whether floral initiation, abnormal flowering, and flower abortion are caused by heat and water stress independent of carbohydrate supply and hence unaffected by elevated atmospheric [CO<sub>2</sub>]. Heat sterility reduces bean yield independent of atmospheric [CO<sub>2</sub>], yet we know little about these temperature thresholds, nor whether differences exist among coffee varieties. We show how climate variability (e.g. El Niño-Southern Oscillation[ENSO]) affects flowering and biennial bearing in different climatic conditions, requiring careful adaptation of agronomic management depending on ENSO year.

### CONCLUSIONS & PERSPECTIVES

By combining field observations and simulation models we can test different assumptions on how climate affects coffee flowering (number of events, intensity and survival) and hence agronomic management needs, labour demand, bean yield, and profitability. Closing the knowledge gaps of the mechanisms of flowering success as affected by temperature, rainfall, and light will require detailed flowering data sets distributed across the coffee growing regions to test our current assumptions. Consequently, we would like to take this opportunity to develop a network to share data between institutions and researchers on this strategic issue.





## CONTRIBUTIONS TO THE CONSERVATION OF GENETIC RESOURCES OF COFFEE IN THE DEMOCRATIC REPUBLIC OF CONGO.

VANDELOOK, Filip\*, MWANGA MWANGA, Ithe\*\*, KAMBALE, Bienfait\*\*\*, ASIMONYIO, Justin\*\*\*, MASUMBUKO NDANGABA, Céphas\*\*\*\*, CHALUKOMA, Chantal\*\*\*\*\*, GODFROID, Ivan\*\*\*\*\*, MUMBERE, Léopold\*\*\*\*\*, LOMBOTO, Patrice\*\*\*\*\*, EBELE, Tchimi\*\*\*\*\*, KAMBALE VALIMUNZIGHA, Charles\*\*\*\*\*, NTORE, Salvator\*, LANATA, Francesca\*, STOFFELEN, Piet\*.

\*Meise Botanic Garden, Belgium, \*\*Centre de Recherche en Sciences Naturelles, Lwiro, D.R.Congo, \*\*\*Université de Kisangani, Kisangani, D.R.Congo, \*\*\*\*Université Officiel de Bukavu, Bukavu, D.R.Congo, \*\*\*\*\*Institut pour la Conservatoire de la Nature, Bukavu, D.R.Congo, \*\*\*\*\*Rikolto-RDC, Butembo, D.R.Congo, \*\*\*\*\*Université Catholique du Graben, Butembo, D.R.Congo, \*\*\*\*\*Institut National pour l'Etude et la Recherche Agronomique, Yangambi, D.R.Congo.

### RATIONALE

Robusta and Arabica are the far most important commercialized coffee species. Numerous publications and reports are warning for the impact of climate change on coffee production. In the context of the adaption of the coffee culture to climate change and the quest for resistance of coffee to emerging pests, crop wild coffee species are important. They can be a source of traits for breeding, or even as alternative species for production. *Coffea arabica* evolved in the Rift region through one hybridization event between *Coffea canephora* ('Robusta') and *Coffea eugenioides* (Lashermes 1999). Therefore West and Central Africa, including the (Albertine) Rift Region, are particularly interesting as they are the homeland of the ancestors of Arabica. The Congo basin and the Kivu are housing an important stock of genetic resources, but they are seriously underrepresented in coffee gene banks, both locally and globally (cf. Barmel 2017).

### METHODS

Recently the Garden started two projects aiming to better conserve the genetic diversity of *Coffea* in the Democratic Republic of Congo. In a first project, the Garden is assisting local partners to evaluate and rehabilitate the existing *Coffea canephora* (Robusta) collection of the INERA Yangambi. Local staff is trained and the local collection is enriched with 'new' genetic diversity collected in the wild and in backyards. In a second project, we will contribute to the ex-situ conservation of Coffee in the Kivu (with a focus on the high altitude forests and an endemic species from these forests). This project runs in collaboration with local universities, research institutes and an NGO supporting local coffee farmer cooperatives.

### RESULTS

These projects train local researchers and agronomists, establish a local network and contribute to the conservation of and the access to the genetic resources of coffee.

### CONCLUSIONS & PERSPECTIVES

The project makes important genetic resources of coffee available for breeding and mitigation to climate change, locally and globally.

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Association study of tree size and male sterility in a F2 *Coffea Arabica* population.

TONIUTTI, Lucile\*, BORDEAUX, Mélanie \*\*, KLEIN, Patricia \*\*\*, BERTRAND, Benoît\*\*\*\*, MONTAGNON, Christophe\*

\*World Coffee Research, Saint Mathieu de Trévières, FRANCE., \*\*Nicafrance, NICARAGUA,

\*\*\* TAMU A&M University (TAMU), TX, USA \*\*\*\* CIRAD, Montpellier, FRANCE

## RATIONALE

Male sterility and tree size are two major traits for breeders.

Male sterility enables to decrease the mass multiplication costs of F1 hybrids. Indeed, two types of Arabica varieties can be produced: fixed lines or F1 hybrids. F1 hybrids are more vigorous, resilient and less affected by diseases than fixed lines. However, it is more difficult to reproduce them making the cost of mass production higher. Producing F1 hybrid seems to be a good alternative to face the ongoing climate change and the rust crisis and using male sterility could enable limiting the costs.

Breeding for dwarf leads to production of coffee trees well adapted to mechanization. The objective of this study was to find markers associated to dwarf and male sterility in order to set up marker assisted breeding and to speed up varietal creation.

## METHODS

A F2 population has been created by crossing the dwarf sarchimor IAPAR59 and a tall male sterile coffee tree.

277 coffee progeny as well as the F1 and two parents were genotyped using GBS and the restriction enzyme PstI for complexity reduction. DNA from the coffee samples was restricted with PstI and a 12 bp unique barcode identifier was ligated to the cut-site prior to pooling for Illumina template preparation. 150 bp single end reads were collected on an Illumina HiSeq 2500. Seventy-two barcoded samples were pooled per lane on the Illumina flow cell and 150 bp single-end reads were collected. An average of 2.8M reads per sample was obtained for the 277 progenies. The two parents were blasted against the *C. canephora* genome to identify sequence polymorphisms between them. 871 markers were identified. The association between dwarf trait, male sterility and SNPs markers was tested by a Chi-squared test.

## RESULTS

A region containing 100 markers situated on the scaffold 612 on the chromosome 7 has been identified in association with male sterility. A PCoA performed on those markers showed a good discrimination between male sterile and male fertile genotypes. A selection based on those markers will enable to be more efficient through an early selection of male sterile plants in a segregating population.

A SNP marker of dwarfism has been identified on chromosome 3. One version of this SNP is totally absent in tall genotypes.

## CONCLUSIONS & PERSPECTIVES

An association has been found between a chromosome 7 region and male sterility. It could help breeder to enrich their working population with male sterile and then to reduce hybrid F1 production cost. A more precise study of this region has to be performed. A marker of dwarfism which could be useful to breed for mechanization adapted coffee trees has been discovered.

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MONCADA, Maria Del Pilar, TOVAR, Eduardo, MONTOYA, Juan Carlos, *et al.* A genetic linkage map of coffee (*Coffea arabica* L.) and QTL for yield, plant height, and bean size. *Tree genetics & genomes*, 2016, vol. 12, no 1, p. 5.



## DETERMINATION OF THE CHEMICAL COMPOSITION OF GREEN AND ROASTED COFFEE OF DIFFERENT COFFEE GENOTYPES AS A COMPLEMENTARY METHOD OF PHENOTYPING

ARRIETA, N.\*, GONZALES, R.\*, SALAS, D., UREÑA, K.\*

\*Costa Rican Coffee Institute, Coffee Research Center.

The prestige that has the coffee of Costa Rica has no comparison with any other origin. The coffee specialty production provides the main income for many families in the country. In the last years have been dramatically increase in the number of micro mills looking for offer better qualities to the international market. Nowadays, it is use besides sensorial or cupping tests complementary methods to elucidate why some coffees in some *terroirs* and some genotypes has a delightful quality (unique). Measurement of chlorogenic acids, oils, sugars and caffeine it can be done by different methodologies. The discussion is based on knowing if these genotypes may have different chemical compounds and the possible soil type, climate, altitude influence This study is intended to determine in two soil types (Andisol and Ultisol), three genetically groups by their origin (Catuaí x HdT, Villa Sarchí x HdT and pure arabics) and five different altitudes (800, 1070, 1180, 1280, 1317 and 1425 m.a.s.l.) chemical composition green coffee and roasted coffee. During the 2015/2016 harvest it was collected the cherries samples to fermentation process (full washed) on the Costa Rican Coffee Center Research mill. The coffee was storage during three months before the chemical assessment in the Chemical Laboratory. The caffeine and chlorogenic acids was doing by water extraction according the ICAFE procedures lab, and the reading was done by visible spectrophotometry.. The results indicate that there are conditions of climate, soil and altitude associated with genotype making to be able to get chemical different profiles. For *green coffee samples*, content of sugars and caffeine in the Ultisol soil type are higher than the Andisol soil type green coffee samples. The caffeine reading ranges ranged 1.25 in andisol-like and 1.45 in ultisol. sugars in Andisol soil was around 2.7 and ultisol soils 3.1. Likewise, the oils in Andisol soil were above 12.1 and Ultisol below 11.8. Meanwhile, for *roasted coffee samples*, the behavior was erratic with out any tendence. For *genetic group by their origin*, were the pure arabics which presented more oils in the Andisol soils type. The chemical composition of the soil together with the management of fertilizing the plants has been related to the possibility of obtaining outstanding coffee qualities. However, other studies on the influence of climate and the adaptability of genotypes to certain agro-ecological conditions can be deepened. We know that caffeine, for example, varies in quantity during fruit development and in different years. Due to the above, it should necessarily refer to other compounds that are not altered, such as oils. It is possible to use other methods complementary to the knowledge of different coffee genotypes. In breeding programs, there is currently more possibilities for phenotyping in a more integral way. The knowledge of other additional characteristics such as cup quality and deeper issues will allow to make better recommendations by the scientists. It is no longer enough to know only if a variety can have an intrinsically excellent cup quality, but also to deepen into why it has those attributes.



BERTRAND B\*, BARDIL A\*, ETIENNE H \*  
CIRAD, UMR IPME, F-34398 Montpellier, France

#### RATIONALE

Polyploidy impacts the diversity of plant species, giving rise to novel phenotypes and leading to ecological diversification. Many phenotypic changes with a high adaptive value, concerning vigor, biomass and adaptation to biotic and abiotic stress, would enable the polyploids to occupy new ecological niches and seem to be conducive to their use in agriculture.

#### METHODS

In order to observe adaptive and evolutionary capacities of polyploids, we compared the growth, primary metabolism and transcriptomic expression level in the leaves of the newly formed allotetraploid *Coffea arabica* species compared with its two diploid parental species (*Coffea eugenioides* and *Coffea canephora*), exposed to four thermal regimes (TRs; 18–14, 23–19, 28–24 and 33–29C).

#### RESULTS

The growth rate of the allopolyploid *C. arabica* was similar to that of *C. canephora* under the hottest TR and that of *C. eugenioides* under the coldest TR. For metabolite contents measured at the hottest TR, the allopolyploid showed similar behavior to *C. canephora*, the parent which tolerates higher growth temperatures in the natural environment. However, at the coldest TR, the allopolyploid displayed higher sucrose, raffinose and ABA contents than those of its two parents and similar linolenic acid leaf composition and Chl content to those of *C. eugenioides*. At the gene expression level, few differences between the allopolyploid and its parents were observed for studied genes linked to photosynthesis, respiration and the circadian clock, whereas genes linked to redox activity showed a greater capacity of the allopolyploid for homeostasis. Finally, we found that the overall transcriptional response to TRs of the allopolyploid was more homeostatic compared with its parents.

#### CONCLUSIONS & PERSPECTIVES

This better transcriptional homeostasis of the allopolyploid *C. arabica* afforded a greater phenotypic homeostasis when faced with environments that are unsuited to the diploid parental species.



## PHYSIOLOGICAL PLASTICITY: A KEY ELEMENT OF COFFEE HYBRIDS TO FACE LEAF RUST DISEASE ATTACK

Lucile TONIUTTI\*, Jean C BREITLER\*, Claudine CAMPA\*\*, Benoit BERTRAND\*, Herve ETIENNE\*, Charles LAMBOT\*\*\*, Juan Carlos HERRERA\*\*\*

\* CIRAD, UMR IPME “Coffee Adapt” Team, Montpellier, FRANCE

\*\* IRD, UMR IPME, Montpellier, FRANCE

\*\*\* Nestlé R&D Center, Plant Science Research Unit - NRC, Tours, FRANCE

### ABSTRACT

Arabica coffee is a main economic crop throughout Central and South American highlands, where millions of people get primary outcome. Last coffee leaf rust (CLR) epidemic event in 2011, which impacted 53% of the coffee region areas, provoking losses up to USD 500 million, remembered the high vulnerability of the coffee sector to the fungus *Hemileia vastatrix*, causal agent of this disease and considered as the main restrictive illness for all coffee regions around the world. The main problem remains that the current commercial varieties reputed as “resistant”, become sensitive to the CLR. During the last twenty years, coffee geneticist have created several intra-specific hybrids between commercial varieties and wild coffee accessions. Field observations confirmed that coffee hybrids are more productive and less impacted by the CLR even in regions where resistant materials become susceptible. Such field observations are in agreement with the general idea that hybrid plants present high homeostasis in terms of growth, yield and disease resistance. The aim of this research was to investigate the physiological response of coffee hybrids before and after inoculation with the CLR. Using controlled conditions, the metabolic response and physiological status of two hybrid genotypes were assessed under different stressful conditions involving three limiting factors: temperature, luminosity and nitrogen input. Hybrid behavior was compared to an inbred line tested under similar conditions. Results demonstrated that whatever the agronomic conditions and the temperature regimes, hybrids under test were less sensitive to CLR than the inbred line. Interestingly, the detailed transcriptomic analysis revealed that coffee hybrids seem to exhibit an altered circadian clock resulting in a higher photosynthetic efficiency together with an increased concentration of chlorophyll. Most of the over-expressed genes identified in the hybrids after CLR inoculation were associated to basal resistance mechanisms, while genes identified in the inbred line, were more related to abiotic stress responses. Overall our findings suggest that under stressful conditions, coffee hybrids are able to rapidly modify their energetic metabolism machinery leading to a more effective and rapid response to rust attack compared to the inbred line.

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## TOWARDS THE IDENTIFICATION OF CANDIDATE GENE NUCLEIC POLYMORPHISMS TO PREDICT THE ADAPTEDNESS OF UGANDENSE *C. CANEPHORA* POPULATIONS TO CLIMATE CHANGE.

De AQUINO, Sinara O.\*, TOURNEBIZE, Rémi\*\*, MARRACCINI, Pierre\*\*\*, MARIAC, Cédric\*\*, BETHUNE, Kevin\*\*, ANDRADE, Alan C.\*\*\*, KIWUKA, Catherine\*\*\*\*, CROUZILLAT, Dominique\*\*\*\*, ANTEN, Niels\*\*\*\*\*, DE KOCHKO, Alexandre\*\*, PONCET, Valérie\*\*

\*Federal Univ. Lavras, Lavras, MG, BR, \*\*IRD,UMR DIADE, Montpellier,FR, \*\*\*CIRAD, UMR IPME, Montpellier, FR, \*\*\*\*EMBRAPA Coffee-INOVACAFÉ, Lavras, MG, BR, \*\*\*\*\*NARO, Kampala, UG, \*\*\*\*\*Nestlé R&D, Tours, FR, \*\*\*\*\*Wageningen Univ., Wageningen, NL.

### RATIONALE

Testing whether and how natural populations are adapted to their local environment and predicting their response to future habitat alterations is of key importance in the face of future climate change. This is particularly the case for coffee trees for which the pace of climate change could be too fast and drastic for population adaptations. Using the geographic distribution of wild populations with contrasted habitats, the aim of the present study was to identify single-nucleotide polymorphisms (SNPs) in candidate genes (CGs) identified as being involved in the adaptation of *C. canephora* populations to their local environment. By identifying environmental factors driving these processes we would predict the adaptedness of the populations to their future local climate.

### METHODS

Based on the previous molecular studies (EMBRAPA/CIRAD/Nestlé studies) and using whole coffee genome sequence annotation (Denoeud *et al.* 2014, Dereeper *et al.* 2015), a set of 324 CGs was selected, such as those coding for dehydrins, heat shock proteins, enzymes of sugar metabolism, as well as transcription factors like DREB/CBF (dehydration responsive element binding/cold-binding factor). Wild accessions of *C. canephora* from Uganda with recorded position (geo-localized samples) were used to assess the relationship between climate variation ([www.worldclim.org/bioclim](http://www.worldclim.org/bioclim)) and CG nucleic diversity. We apply available statistical population genomic methods and model of allele distribution to detect CG-SNPs correlated with climate parameters. The LFMM (Latent Factor Mixed Models) R package (Frichot *et al.* 2013) was used for screening sequences for signatures of environmental adaptation in coffee genomes.

### RESULTS

The genotype-environment (GxE) association suggests regional adaptation with spatially varying environments. More specifically, we found selection signals tightly linked to several CGs involved in response to biotic and abiotic stress like *MYB20* (coding a transcription factor involved in the response to drought stress) and *DXMT* (enzyme involved in caffeine biosynthesis) genes.

### CONCLUSIONS & PERSPECTIVES

The selection signals detected support the hypothesis of present ecological gradient contributing to structure of the genetic diversity of Ugandense *C. canephora* populations. The amount and character of genetic variations observed in genomic regions associated with climatic variables will help us to predict whether *C. canephora* species will be able to adapt quickly enough to track global warming.

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MONTAGNON Christophe\*, LONZARICH Valentina\*\*, NEUSCHWANDER Hanna\*\*\*, SUGGI LIVERANI Furio\*\*, NAVARINI Luciano\*\*

\*World Coffee Research, 5 Avenue du Grand Chêne, 34270 Saint Mathieu de Tréviers France.

\*\* illycaffè SpA, via Flavia 110, I-34147 Trieste - Italy

\*\*\* World Coffee Research, 10940 SW Barnes Rd #334, Portland, OR 97225

## RATIONALE

Aromatic quality is a very important target for genetic improvement and breeding of *Coffea arabica*. For coffee geneticists, it is of paramount importance to set targets and breed for aromatic quality as expected by the coffee industry for the consumers. The objective of the study was to relate coffee industry quality demand to objective workable criteria for selection.

## METHODS

We presented 60 random commercial samples to a panel made of professional cuppers from the industry for a Signal Detection sensorial analysis. Based on the results, we selected 12 samples representative of the diversity of quality evaluated by the panel. On this sub-sample, we ran i) a Lexicon sensorial analysis and ii) a SPME analysis of Volatile Compounds (VOC) on both green and roasted coffee. We then implemented several statistical analysis to relate the appreciation of the quality from the professional industry coffee cuppers to some objective quality attributes and specific VOCs.

## RESULTS

After the Signal Detection sensorial Analysis, 3 consistent clusters were identified as : Super Specialty Quality Coffee, Specialty Coffee and Commercial Coffee. Lexicon analysis prove that Super Specialty had no negative flavor or aroma attributes and at least two favorable flavor or aroma attributes. Various VOCs appeared to be related to the Signal Detection Sensory Analysis. However, Limonene appeared to be the most discriminant one on green coffee.

## CONCLUSIONS & PERSPECTIVES

To our knowledge, it is the first public study searching for objective Coffee Aromatic Quality based on a panel made of coffee industry professional cuppers. Limonene appeared to be a good proxy for market coffee aromatic quality measured on green coffee. This opens a strategy for Coffee Aromatic Quality genetic improvement based on the screening of candidates for the limonene content of green coffee. Searching for molecular markers related to Limonene will further speed up the genetic progress.

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## ASIC Conference Abstract

While the global demand for coffee is steadily increasing, climate change and other threats are posing significant challenges to future coffee production. The specialty coffee industry is most vulnerable to climate change, due to it relying solely on the production of *Coffea arabica*, a coffee species that is highly dependent upon its ecosystem to provide stable and specific growing conditions for successful and profitable cultivation. The most effective strategy to ensure future arabica coffee production in the face of climate change is through the creation of new varieties, therefore requiring a greater understanding of the current genetic resources we have available to use for future breeding. In the present study, we utilized the next-generation sequencing technology ddRAD, to analyze the genetic diversity of a cultivated population of *C. arabica*, with focused analysis on the Panamanian Gesha varietal. Although the genetic diversity was detected, our analysis indicates high rates of similarity between the Gesha plants samples despite their morphological variation with our results highly suggesting these differences are due to phenotypic plasticity. We detected a total of 17 non-clonal unique multilocus genotypes which means the majority of our individuals sampled are not clones and therefore the study site has been mainly propagated through seedlings. Lastly, our results indicate one of the sampled Gesha Dwarf individuals is more genetically related to the Catuai varietals, than the Gesha population sampled. This finding infers the Gesha Dwarf may have experienced gene flow or hybridization with the Catuai population. With farmers already experiencing the effects of climate change, it is imperative to continue to investigate solutions to ensure the longevity of the coffee industry that contributes to the economic livelihoods for millions of individuals.





BATISTA, Dora\*\*\*, SILVA, Diogo N.\*\*\*, MODESTO, Inês\*\*\*, MIGUEL, Célia\*\*\*\*, PAULO, Octávio S.\*\*, VÁRZEA, Vitor\*, SILVA, Maria do Céu\*

\* Centro de Investigação das Ferrugens do Cafeeiro (CIFC), LEAF, ISA, University of Lisbon, Lisbon, Portugal; \*\*cE3c, FCUL University of Lisbon, Lisbon, Portugal; \*\*\*IBET/ITQB NOVA, Oeiras, Portugal; \*\*\*\*BioISI, FCUL University of Lisbon, Lisbon, Portugal.

**RATIONALE:** Recurrent epidemics of coffee leaf rust, caused by the fungal pathogen *Hemileia vastatrix* (*Hv*), have been constraining the sustainable production of Arabica coffee for more than one and a half centuries. Although the deployment of coffee resistant varieties has successfully contributed to control the disease, the highly adaptability of the fungus shaped by the dynamic system of host-pathogen co-evolution has been leading to the gradual breakdown of resistance in the field [1]. This extreme situation has triggered a deep sense of urgency to improve pathogen surveillance and control measures, and *Hv*'s research has gained considerable momentum. However, no direct link between such a high phenotypic diversity and molecular diversity has been found yet. In this sense, we are focused on understanding host adaptation and virulence evolution through pathogen population and evolutionary genomic studies.

**METHODS & RESULTS:** To achieve this goal, we have been applying different approaches, including phylogenomics, population genomics with RAD-sequencing data, detection of selection signatures and candidate gene analysis. Here we will present our latest progresses, results and limitations. *Hv* genetic structure and population dynamics were dissected through the analysis of 6783 phylogenetically informative SNPs, within a final matrix of 19 505 SNPs, from the RAD-Seq genotyping of 29 isolates. We unveiled three phylogenetic lineages highly structured according to coffee hosts (diploids vs tetraploids), that may actually represent a complex of cryptic species, as well as evidence of recombination and footprints of introgression [2]. Interestingly, we detected a reduced proportion of shared genetic variation among the *Hv* isolates from the tetraploid hosts-derived lineage. Moreover, an extremely low level of polymorphism was found in candidate genes of putative adaptive significance under a population frame work, but, in contrast, highly divergent multicopies of a retrotransposon were identified across different isolates.

**CONCLUSIONS & PERSPECTIVES:** Overall, our work provides evidences that coffee host is a major selective pressure and that genetic variation may be unevenly distributed along the genome. Our results also represent a paradigm shift in our current understanding of this pathogen's evolutionary history, challenging the current view of *Hv* as an unstructured and asexual species, and alerting to the possibility of virulence factors exchange between rust lineages. We will discuss the value of these studies to guide disease control strategies and how we intend to follow up for uncovering virulence-related loci and identifying the genomic variation that gives rise to different pathotypes, under our new project PATHOmics, and which challenges it entails.

**REFERENCES:** [1] Talhinas et al. (2017). *Mol Plant Pathol.*, 18:1039–1051; [2] Silva et al. (2018). *Mol Plant Pathol.*, 19: 1742–1753.



## EARLY ACTIVATION OF DEFENCE-RELATED GENES AND POD AND PPO ACTIVITIES ARE ASSOCIATED WITH COFFEE RESISTANCE TO *COLLETOTRICHUM KAHAWAE*

DINIZ, Inês<sup>\*\*</sup>, AZINHEIRA, Helena<sup>\*\*</sup>, FIGUEIREDO<sup>\*\*\*</sup>, Andreia, GICHURU, Elijah<sup>\*\*\*\*</sup>, BATISTA Dora<sup>\*,\*\*,\*\*\*\*</sup>, OLIVEIRA, Helena<sup>\*\*</sup>, GUERRA-GUIMARÃES, Leonor<sup>\*\*</sup>, SILVA, Maria do Céu<sup>\*\*,\*</sup>

<sup>\*</sup>CIFC, ISA, Universidade de Lisboa, Oeiras, Portugal, <sup>\*\*</sup>LEAF, ISA, Universidade de Lisboa, Lisboa, Portugal, <sup>\*\*\*</sup>BioISI, FCUL, Universidade de Lisboa, Lisboa, Portugal, <sup>\*\*\*\*</sup>KALRO, Ruiru, Kenya, <sup>\*\*\*\*\*</sup>cE3c, FCUL, Universidade de Lisboa, Lisboa, Portugal

**RATIONALE:** The hemibiotrophic fungus *Colletotrichum kahawae* (*Ck*), the causal agent of coffee berry disease (CBD), is the most devastating threat to *Coffea arabica* production in Africa at high altitude, and its dispersal to Latin America and Asia represents a serious concern. Understanding the basis of coffee resistance to *Ck* is of high priority to support breeding strategies for durable resistance against this pathogen. In this work, the coffee variety Catimor 88, exhibiting field resistance in Kenya, was used aiming to unveil the candidate genes and proteins putatively involved in the resistance response to *Ck*.

**METHODS:** Hypocotyls of two coffee varieties, resistant (Catimor 88) and susceptible (Caturra) were inoculated with a conidial suspension of *Ck* (isolate Que2, from Kenya), and samples were collected at several hours post inoculation. A set of genes putatively involved in resistance showing differential expression were retrieved from an Illumina RNA-seq dataset from the same coffee – *Ck* interactions. qPCR was used to evaluate the expression of 10 genes related to pathogen recognition and signaling (Receptor-like kinase - *RLK*, Leucine rich repeat receptor-like serine/threonine-protein kinase At2g16250 - *LRR-K*, Proline-rich receptor-like protein kinase - *PERK3*, Calmodulin-like protein - *CML*, Patatin-like phospholipase – *PTL*), cell wall modifications (UDP-arabinose 4-epimerase 1 - *MUR4*, Pectinesterase/pectinesterase inhibitor 41 - *PME41*) and peroxidases (Cationic peroxidase 2 - *PNC2*, Lignin-forming anionic peroxidase-like - *PER4*). Peroxidase (POD) and polyphenol oxidase (PPO) activities were evaluated by spectrophotometry and electrophoretic methods. Histochemical localization of POD and lignin detection were also performed.

**RESULTS:** Gene expression analysis revealed a stronger induction of recognition, signaling and cell wall modification genes (*RLK*, *LRR-K*, *CML*, *PTL*, *PME41*) in the resistant variety during fungal penetration. The differential expression of *PPO 10723* transcript isoform was detected together with a significant increase in total PPO activity. Moreover, the increase of total POD activity was also observed, and the induction of lignin-forming anionic peroxidase-like gene was supported by the increase of an anionic isoenzyme. Peroxidase was localized in the host cell walls at infection sites and could be related with lignification.

**CONCLUSIONS & PERSPECTIVES:** Our results showed that coffee resistance is associated with an induction of several defense responses, that all together and in different time points seem to contribute to restricting fungal growth. The new data obtained, in association with RNA-Seq data, will enable to identify potential biomarkers of disease resistance that, once validated, will be useful for marker-assisted selection in coffee breeding programmes.



ARIYOSHI, Caroline\*, SANT'ANA, Gustavo C.\*\*, SERA, Gustavo H.\*\*, NOGUEIRA, Livia M.\*, FERREIRA, Rafaelle V.\*, da SILVA, Bruna S. R.\*, FELÍCIO, Mariane S.\*\*\*, DOMINGUES, Douglas S.\*\*\*, RODRIGUES, Lucas M. R.\*\*\*\*, PEREIRA, Luiz F.P.\*\*\*\*\* \*Universidade Estadual de Londrina, Londrina, PR, BR, \*\*Instituto Agronômico do Paraná, Londrina, PR, BR, \*\*\*Universidade Estadual Paulista, SP, BR, \*\*\*\*Instituto Agronômico de Campinas, Campinas, SP, BR, \*\*\*\*\*Empresa Brasileira de Pesquisa Agropecuária, Brasília, DF, BR.

## RATIONALE

Coffee production is limited by a number of diseases, including bacterial halo blight (BHB) caused by *Pseudomonas syringae* pv. *garcae*. BHB is responsible for severe damages in nurseries and in new plantations. BHB control is done, mainly, with chemical compounds; however, this strategy has adverse environmental effects and it also has impacts in production costs. The development of varieties resistant to BHB is a sustainable alternative. For this purpose, Genome-Wide Association Study (GWAS) is a powerful tool for identifying SNPs that can be used to accelerate breeding programs. GWAS requires genetic diversity based in unrelated individuals. *C. arabica* accessions coming from the historical FAO collection from Ethiopia are a valuable source for GWAS. The aim of this work was to identify SNPs and candidate genes associated with *P. syringae* pv. *garcae* resistance in *C. arabica*.

## METHODS

In this work, we used phenotyping and genotyping-by-sequencing (GBS) of 122 *C. arabica* accessions (Ethiopia). The tags obtained by GBS were aligned with *C. canephora* and *C. eugenioides* genome references to SNP identification. The data were filtered with minor allele frequency (MAF) > 0.05, call rate > 0.8 and heterozygosity < 0.9 using TASSEL software version 5.2.37. Resistance to BHB was evaluated on a scale of 0 to 5, where 0 corresponds to complete immunity and 5 to high susceptible plants. The genotypic and phenotypic data were associated using the multi-locus random-SNP-effect mixed linear model (mrMLM) R package (WANG et al., 2016).

## RESULTS

We identified 10.805 SNPs after filtering and 27 SNPs were associated to resistance to BHB. Two SNPs with the highest LOD scores (15.6 and 13.6) are allocated on Chr. 2. Close to those SNPs, we found genes encoding a serine-threonine kinase protein, a serine-threonine phosphatase and a CC-NB-LRR protein. These three genes are widely known to be associated to disease resistance in plants (COLL et al., 2011).

## CONCLUSIONS & PERSPECTIVES

We intend to validate SNPs using allele-specific fluorescent probes, thus facilitating the application of marker-assisted selection for coffee breeding. We also intend to analyze transcriptional response of these genes during plant-pathogen interaction. These efforts may elucidate a cascade of coffee resistance to BHB.

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## **SECTION 4: Abstracts – Poster Contributions**



## AMELIORATION OF COLD INDUCED STRESS IN COFFEE (*Coffea arabica* L.) SEEDLINGS BY TiO<sub>2</sub> NANOPARTICLES

ACIDRI, Robert\*, ISHIGAKI, Tadashi\*, SASAGAWA, Daisuke\*, WACAL, Cosmas\*, BASALIRWA, Daniel\*, SAWAI, Yumiko\*\*, OHMI, Kotoku\*, NISHIHARA, Eiji.\*

\*Tottori University, Tottori, Japan, \*\*Sawai Coffee Limited, Tottori, Japan.

### RATIONALE

Generally, coffee production has expanded to many regions originally classified as unsuitable for coffee growing. In addition, the current trends in the climatic variability pose a threat to coffee cultivation due to the increasing extreme environmental conditions to which coffee is not adapted<sup>1</sup>. Abiotic stresses such as cold stress affect normal cell functioning leading to destruction of macromolecules resulting in mortality. However, the recent advancements in nanotechnology provides new possibilities in biotechnology as well as improving tolerance to both abiotic and biotic stresses by improving the physiological performance of many plants including coffee<sup>2</sup>. This study therefore evaluated the effects of TiO<sub>2</sub> nanoparticles (NP) on coffee seedlings under cold stress by assessing the physiological status of coffee plants in terms of membrane integrity, chlorophyll fluorescence, enzymes and a number of macromolecules involved ROI detoxification.

### METHODS

*Coffea arabica* seeds were pre-germinated and grown under natural greenhouse conditions for six months in 10 cm diameter pots with an irrigation regime of 5 ml half strength Hoagland solution. The seedlings were then transferred and acclimated in a growth chamber for one month at 25°/20°C cycle and 12h photoperiod with an irradiance of 250 μmol m<sup>-2</sup> s<sup>-1</sup>. Thermal treatments were initiated by lowering the temperature to 4°C. Six treatments including control were studied by varying the concentration of foliar-applied TiO<sub>2</sub> NP (7 – 40 nm) that is to say; Control – retained, 0, 5, 10, 15, 20 ppm. Fluoro-photosynthetic measurements were taken every other day whereas sampling for biochemical assays was done after 10 days.

### RESULTS

Generally, net carbon assimilation was reduced during the thermal treatments. Nevertheless, accumulation of TiO<sub>2</sub> NP in the leaf cells particularly the chloroplasts increased the net photosynthetic rates moreover maintaining positive values throughout the experiment. Coupled with protection against membrane damage, elevated activities of enzymatic antioxidants and non-enzymatic antioxidants, TiO<sub>2</sub> NP maintained higher rates of ribulose 1, 5 – bisphosphate enzyme a key enzyme in the carbon photosynthetic pathway.

### CONCLUSIONS & PERSPECTIVES

TiO<sub>2</sub> nanoparticles increased the tolerance of *Coffea arabica* seedlings to cold stress. TiO<sub>2</sub> NP and other nanoparticles can be used to alleviate other biotic and abiotic stresses<sup>3</sup> although field studies still need to be undertaken. Since nanoparticles are absorbed within the plant tissues and can be consumed, their effect on human health should as well be evaluated before utilization.

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## SEEKING TO GENERATE GENETIC DIVERSITY IN ARABICA COFFEA TO OBTAIN BETTER TRAITS OF RESISTANCE TO PESTS AND DISEASES THROUGH THE USE OF MUTATION INDUCTION

ARRIETA, N.\*, BARQUERO, M.\*, CESPEDES, R.\*, INGELBRETCH I.<sup>1</sup>, NIELEN, S.<sup>1</sup>, ROBLES, A.\*, UREÑA, K.\*

\*Costa Rican Coffee Institute, Coffee Research Center.

<sup>1</sup>International Atomic Energy Agency.

Coffee is one of the most important crops in Latin America and has a significant socio-economic impact for thousands of families. In Costa Rica it is the third most important product for agricultural sector exports and provides the main income for many families in the country. However, coffee is under threat due to the Coffee Leaf Rust disease (CLR). Genetic diversity in the specie *C. arabica* is limited. There is no knowledge about different sources of resistance to pests and diseases. It is imperative then to go to new genetics and explore their potential in breeding programs. The use of mutation induction techniques has been successfully implemented in certain crops to obtain desirable agronomic characteristics. Mutation breeding in coffee a promising approach to develop new varieties resistant to CLR. Mutation breeding in combination with *in vitro* techniques and other biotechnologies offers opportunities for the generation of new improved coffee varieties in a shorter period as compared to classical plant breeding. As the technology is new for coffee, basic tests related to mutation induction need to be done. Two materials were used to perform gamma ray treatments. *It irradiated seeds and embryos rescued from seeds.* Were *Coffea arabica* var. Venecia seeds, with a humidity of 27,3%. The applied irradiation doses were 0, 80, 100, 120, 140, 160 and 180 Gy. For each treatment, three replicates of 200 g were used, with a seed number range of 765-808 units per replicate. Eighty days after treatment the number of seedlings was quantified, the hypocotyl height and radicle length were measured, and the opening of cotyledons was determined for each dose. At the dose of 80 Gy the germination was reduced over the control in 9.65%, at 100 Gy in 34,06%, at 120 Gy in 52,76 %, at 140 Gy in 60,24%, at 160 Gy in 65,56% and at 180 Gy in 75,40. Seedling growth was affected, and a delay in opening of the cotyledons was observed at higher doses. This radiosensitivity test based on seed germination as compared to unirradiated control revealed that the LD50 for the used variety is in the range 100 – 120 Gy experimentally and according to the regression is 127,8 Gy. The results show the lethal dose (DL50) in 127,8 Gy, for the establishment parameters, being an advance to continue with measurements of genetic and phenotypical parameters to go forward on coffee breeding programs looking for new sources to resistance against CLR. For *in vitro* introduction of zygotic embryos, five treatments were performed 0, 10, 20 Gy and 0, 30 Gy. For the 0, 10 and 20 Gy treatments green fruits were used and for the 0, 30 Gy mature fruits were used, each had ten repetitions, with 10 embryos per repetition. Cotyledon, hypocotyl and root length and width were determined in the regenerated plantlets 30 days after irradiation. The results obtained show different effects of the irradiation treatments regarding the measured characteristics. It was observed that the length and width of the cotyledon decrease in size when the applied doses increased. As to the root and hypocotyl measurements in width and length there was no specific trend, however, some doses had a stimulating effect to growth in comparison with the non-irradiated treatment, this phenomenon of growth stimulation is known as hormesis and is characteristic of low doses of irradiation. The results show a growth reduction in the width and length of the cotyledons, also it was possible to demonstrate growth stimulation for the variables width and length of the hypocotyl and radicle, in response to the radiation dose.



SOUSA, Tiago Vieira<sup>1</sup>, ALKIMIM, Emily Ruas<sup>2</sup>, RESENDE, Marcos Deon Vilela<sup>3</sup>, OLIVEIRA, Antonio Carlos Baião de<sup>4</sup>, ZAMBOLIM, Laércio<sup>1</sup>, PEREIRA, Antonio Alves<sup>5</sup>, SAKIYAMA, Ney Sussumu<sup>1</sup>, CAIXETA, Eveline Teixeira<sup>4\*</sup>

<sup>1</sup>Universidade Federal de Viçosa, <sup>2</sup>Universidade Federal do Triângulo Mineiro, <sup>3</sup>Empresa Brasileira de Pesquisa Agropecuária - Embrapa Florestas, <sup>4</sup>Empresa Brasileira de Pesquisa Agropecuária - Embrapa Café, <sup>5</sup>Empresa de Pesquisa Agropecuária de Minas Gerais - Epamig. \*Corresponding author – eveline.caixeta@embrapa.br

Genomic Selection (GS) have made possible to maximize the genetic gains per unit of time in various annual and perennial plant species. However, in *Coffea arabica*, the species with the highest economic relevance in the genus *Coffea*, no study of GS has been published. Thus, this work had the purpose of (i) evaluating the applicability and accuracy of GS in predicting the genomic genetic value; (ii) estimating genetic parameters; and (iii) evaluating time reduction of the selective cycle, by the use of GS in breeding populations of the arabica coffee. Total of 195 individuals of arabica coffee comprising 13 families from a F<sub>2</sub> generation, susceptible backcross and resistant backcross to the coffee rust were phenotyped for 18 agronomic traits (yield; incidence of rust; incidence of cercospora; infestation of leaf-miner; vegetative vigor; fruit maturation cycle; uniformity of fruit maturation; size of ripe fruits; leaf length; leaf width; length of a plagiotropic branch; number of reproductive knots; number of vegetative knots; number of fruits per plagiotropic branch; volume of fruits per plagiotropic branch; plant height; diameter of the plant canopy; diameter of the plant stem) in the years 2014, 2015 and 2016. These individuals were also evaluated by 21,211 SNPs molecular markers. Experiments with perennial species such as *C. arabica*, usually show unbalanced data due to the adversities found in the experimental field along time. Considering the unbalanced data, phenotypic data needed to be adjusted and analyzed by the approach of mixed models (REML/BLUP – Residual or Restricted Maximum Likelihood/Best Linear Unbiased Prediction) (Henderson, 1975). GS analysis was performed using the method G-BLUP and the procedure RKHS (Reproducing Kernel Hilbert Spaces) with the Bayesian algorithm. Heritability and the selective accuracy were estimated, evidencing a moderate to high magnitude, for the majority of the evaluated traits. The results from GS analysis showed the possibility of reducing time in the selective cycle, which maximized the genetic gains per unit of time when performing selection. The density effect of molecular markers in the GS analysis was evaluated and showed, in general, an increase in selective accuracy with an increment in the density of molecular makers. Genomic selection in *Coffea arabica*, as well as in other species, showed potential as an auxiliary and complementary tool to the selection methods applied in genetic breeding of this species. The agronomic traits demonstrated a high complexity, influenced by several QTLs and low genomic heritability, evidencing the importance to incorporate methodologies of genomic selection in Breeding Programs for *C. arabica*.

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## ARABICA GENOME MANUAL ANNOTATION USING A COLLABORATIVE PLATFORM : WEBAPOLLO

AUSSEL, Rudy\*, CROUZILLAT Dominique\*, DE KOCHKO, Alexandre\*\*, PONCET, Valérie\*\*, ROMBAUTS, Stephane\*\*\*, MUELLER, Lukas\*\*\*\*, RAMBANI, Aditi\*\*\*\*, LEPELLEY, Maud\* on behalf of the Arabica Coffee Genome Consortium (ACGC).

\*Nestlé R&D Centre, Plant Science Research Unit, 101 Av. Gustave Eiffel, 37097 Tours, France \*\*

UMR DIADE, IRD, BP 64501, 34394 Montpellier CEDEX 5, France. \*\*\*\*VIB Center for Plant Systems Biology, Ghent, Belgium. \*\*\*\*\* BTI, Cornell University, US.

### RATIONALE

*Coffea arabica* accounts for 70% of world coffee production. It is an allotetraploid species with a genome size of approximately 1.3 Gb, derived from the hybridization of two diploid progenitors, *Coffea canephora* and *Coffea eugenioides*. An international consortium (ACGC) coordinated by IRD and Nestlé undertook the genome sequencing of these three coffee species. A robust annotation is important to obtain a high quality *Coffea arabica* genome and to characterize the gene content of this coffee species. These data will ultimately serve to understand coffee genomes evolution and to decipher key metabolic pathways.

### METHODS

WebApollo genome annotation platform (Lee et al., 2013) was implemented by ACGC. Reference genome sequence, automatic annotation and functional data (RNA-seq and Iso-Seq) were loaded in the browser. Published gene sequences, functional evidences, and gene-models generated by automatic pipelines were used to manually correct sequencing errors, restore incorrectly predicted splicing sites and improve the genomic organization of gene-models.

### RESULTS

To date, more than 300 genes out of the 56,668 encoding genes detected in *Coffea arabica* genome were manually annotated including those involved in the well-known caffeine, sucrose and chlorogenic acids metabolic pathways. Thanks to this work, we successfully identified the homeologous genes on both *Coffea canephora* and *Coffea eugenioides* sub-genomes, defined their genomic organisation, and even discovered new genes involved in the investigated pathways.

### CONCLUSIONS & PERSPECTIVES

This annotation effort is a key requirement to build *Coffea arabica* gene-models reference database that can ultimately be used to conduct transcriptomic studies, analyze gene structures, assess transposable element composition and perform phylogenetic analysis. The ultimate goal is to release this reference database to the public domain and continuously improve and enrich it thanks to a shared effort with the entire coffee scientific community. In the future, coffee breeders would undoubtedly benefit from these data, particularly useful for coffee breeding programs.

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GIOMO, Gerson S.\*, ROMANO, Lucicléia S.\*

\*Institute Agronomic of Campinas (IAC), Campinas, SP, BRAZIL

## RATIONALE

Brazil is the biggest world coffee producer but mostly Brazilian varieties are descendant of few genotypes, it is being supposed that exist some genetic restriction for the production of high beverage quality. To increase the genetic variability for cup quality it was introduced in Brazil, a long time ago, several coffee varieties from Congo, Ethiopia, Kenya and Tanzania [1]. The sensory evaluation is essential to get detail information about each coffee genotype for use as criteria of selection in coffee breeding program. In this research were evaluated the beverage quality and described the sensory profile of several non-commercial coffee varieties and hybrids aiming to identify promising genotypes for coffee quality improvement in Brazil.

## METHODS

This study was carried out in 2017 crop season at Instituto Agronômico de Campinas, São Paulo State, Brazil. It was evaluated 31 genotypes of *Coffea arabica* from Brazil, Congo, Ethiopia, Kenya and Tanzania. Samples of ripe fruits from each individual genotype were prepared by the dry and wet process and the parchment coffees were sun dried until to reach 12% of water content. Samples of coffee beans were submitted to descriptive analysis of cup quality according to the Specialty Coffee Association (SCA) procedures [2].

## RESULTS

The results showed that one variety and two hybrids were Outstanding Specialty Coffee, which the highest cumulative score was 93 points for one Tanzanian variety with flavor characteristics that reminder watermelon, jelly fruits and citrusy, 91 points for H12366 Brazilian hybrid described with pineapple and marmalade and 90 points for H12123 Brazilian hybrid highlighted by caramelly, tutti-frutti and winey taste. The others 28 coffee varieties, including 14 Brazilian hybrids and 14 foreign varieties were Excellent Coffees, showing cumulative SCA score between 86 and 89 points.

## CONCLUSIONS & PERSPECTIVES

Considering that all coffee samples were obtained in the same environmental conditions, under rigorous quality control in the post harvest procedures, it is supposed that the beverage quality differences, emphasizing the flavor, can be attributed to genetic effects. Some non-commercial varieties and hybrids presented real potential to increment the coffee cup quality in Brazil and could be used in the future to specialty coffee production, genetic recombination and selection in breeding programs.

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## IDENTIFICATION OF PHYSIOLOGICAL TRAITS AND EQUIPMENTS TO SELECT COFFEA CANEPHORA FOR DROUGHT TOLERANCE

Sara OUZZANI\*\*\*, Rémi BLANCHARD-GROS\*, Assane NDONG \*\*, Nathaly VIAL\*\*\*, Charles LAMBOT\*\*\*, Jérôme SPIRAL\*\*\*

\* present address : , Gautier semences, Eyragues, FRANCE

\*\* Nestlé R&D Center, Abidjan, COTE d'IVOIRE

\*\*\* Nestlé R&D Center, Plant Science Research Unit - NRC, Tours, FRANCE

### ABSTRACT

Increase of global warming and severe dry periods already started to negatively affect coffee cultivation areas and national productions. Coffee tree is able to support moderate drought period, but severe water stress leads to large leaf loss and physiological disorders impacting green coffee yield. To maintain coffee production in extreme climatic conditions, a priority is to select coffee varieties performing better under limited water availability. Plant adaptation to drought is a complex phenomenon and follows a sequence of physiological reaction induced by water scarcity. Based on literature survey (DaMatta *et al.*, 2002; Barlett *et al.*, 2016) and experiments developed under greenhouse conditions, we identified physiological traits and equipments able to differentiate coffee trees under water stress. Regulation of stomatal closure is the first differentiation indicator of water stress between clones. Then, the leaf water potential decreases and the photosynthetic apparatus is degraded. With coffee trees, chlorophyll degradation occurs in the final stages of the plant desiccation making it difficult to be used as a sensitive indicator. Trials under greenhouse controlled conditions and field, allowed to select one clonal hybrid having fine stomatal regulation, moderate leaf water potential reduction and better growth recovery after water stress. Biochemical compounds identified as potential drought markers by Dos Santos *et al.* (2015) and expressed during water stress were analysed (mannitol, galactinol, raffinose, stachyose and proline). Current observation indicates that raffinose and proline contents seem to be related to drought tolerance and is under validation. Further investigations on xylem conductivity are organised as a tentative to elucidate the better growth recovery observed after severe water stress in the tolerant clonal hybrid.

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Evaluation of genotypes of *Coffea arabica* L. from the germplasm active bank of Minas Gerais to produce specialty coffees<sup>1</sup>

<sup>1</sup>Support: Fapemig and Consórcio Pesquisa Café

MALTA, Marcelo R.\*, ALCÂNTARA, Elifas N.\*, FASSIO, Larissa de O.\*, CARVALHO, Gladyston R.\*, PEREIRA, Antônio A.\*, SILVA, Ackson D.\*\*, MOTA, Diego M.\*\*, ALVARO, Laisa, N.\*\*  
\*Empresa de Pesquisa Agropecuária de Minas Gerais, Lavras, Minas Gerais, Brazil, \*\*Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil.

## RATIONALE

In recent years, due to the growing market for better quality coffees, we have been looking for cultivars with characteristics that meet the expected quality standard. The challenge of breeders and researchers is to develop coffee cultivars with desired agronomic traits and high beverage quality. The genetic component has a marked effect on coffee quality. For that reason, coffee genetic breeding programs have investigated ways of combining the vegetative characteristics, yield potential, and resistance to pests and diseases with the beverage quality characteristics of coffee. This work aimed to evaluate the potential of access of *Coffea arabica* L. from the Germplasm Active Bank of Minas Gerais to produce specialty coffees.

## METHODS

To achieve that goal the quality of 56 genotypes of *Coffea arabica* L. from the Germplasm Active Bank of Minas Gerais, Brazil, was evaluated, in an experiment installed in the experimental field of EPAMIG in Patrocínio, Minas Gerais, Brazil. Ripe coffee beans were collected by manual picking on cloth and forwarded for processing dry obtaining the natural coffee. After processing the coffees were dried in screened bottom sieves, being revolved periodically until they reached 11-12% water content (b.u.). After drying, the samples were stored for 60 days in cold chamber to 18°C. After the storage period the samples were then prepared to be subjected to sensory analysis according to the methodology of the Specialty Coffee Association of America (SCAA) to assess the quality of the coffee (Lingle, 2011).

## RESULTS

According to preliminary results there are differences in the various sensory notes of the different coffee accesses evaluated, where it was observed scores ranging from 80.37 to 86.33 points. In general, all accesses of *Coffea arabica* analyzed were considered specialty coffees, once presented score equal or superior to 80 points, and characterizing them as specialty coffees according to the Protocol of the SCAA. However, still according to the SCAA Protocol, according to the score display, the coffee will fit in a particular category. Scores between 80 to 84 points are classified as special coffees. Already scores between 85 to 89 points are classified as excellent coffees. Thus, the genotypes MG0224 (Pacamara Paraná), MG0036 (Bourbon Amarelo), MG0133 (Sumatirão Ponta Roxa), MG0043 (Bourbon Amarelo), MG0277 (Híbrido de Timor), MG 0625 (BE5 Wush-Wush), MG0233 (Obatã Amarelo), and MG0016 (Bourbon Vermelho) fall into the category of excellent coffees/specialty, since they feature sensory end note equal to or greater than 85 points.

## CONCLUSIONS & PERSPECTIVES

The results of this study demonstrate that the Active Germplasm Collection of Minas Gerais represents an important collection of genotypes with high potential for beverage quality that can be used in breeding programs aiming the production of plant varieties to the market of specialty coffees.

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evaluation of elite genotypes of *Coffea arabica* L. from the breeding program of EPAMIG aiming to obtain specialty coffees<sup>1</sup>

<sup>1</sup>Support: Fapemig and Consórcio Pesquisa Café

MALTA, Marcelo R.\*, ALCÂNTARA, Elifas N.\*, OLIVEIRA, Antônio C.B.\*\*\*, PEREIRA, Antônio A.\*, SILVA, Ackson D.\*\*\*, MOTA, Diego M.\*\*\*, ALVARO, Laisa, N.\*\*\*

\*Empresa de Pesquisa Agropecuária de Minas Gerais, Lavras, Minas Gerais, Brazil, \*\*Empresa Brasileira de Pesquisa Agropecuária, Brasília, DF, Brazil, \*\*\*Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil.

## RATIONALE

The search for excellent quality coffee has grown on a large scale, justifying the investment in research in this area. It is known that the coffee beverage quality is evaluated by sensory attributes that the beans present after the roasting process, and they are directly influenced by the genetic component and the cultivation environment. In order to check the potential of different elite genotypes of coffee genetic improvement program of EPAMIG to produce specialty coffees this work has been developed.

## METHODS

To achieve that goal the quality of 30 elite genotypes of *Coffea arabica* L. was evaluated in an experiment installed at the Experimental Field of EPAMIG in Patrocínio, Minas Gerais, Brazil. The coffee harvest was performed when most fruits reached the ideal maturation stage, that is, cherry fruit, being collected by manual picking on cloth and forwarded to the wet processing with the pulped coffee being obtained. After processing the coffees were dried in screened bottom sieves, into masonry yard, being revolved periodically until they reach 11-12% water content (b.u.). After reaching the pre-set water content, the samples were stored for 60 days in cold chamber to 18°C. After this storage period samples were then prepared to be subjected to sensory analysis according to the protocol of the Specialty Coffee Association of America (SCAA) to assess the quality of the coffee.

## RESULTS

According to preliminary results, the majority of elite genotypes of *Coffea arabica* L. assessed present potential for the production of specialty coffees, with elite genotypes of coffee producers of excellent quality, especially for the genotypes Paraíso 2, H493-1-2-10 (Catuai Vermelho IAC 144 x Híbrido de Timor) and UFV-7158 (Caturra Vermelho x Híbrido de Timor), which reached sensory notes equal to or greater than 90 points.

Coffees obtained from the “Híbrido de Timor” derived cultivars (*C. arabica* x *C. canephora*) have similar or better cup quality (Pereira et al., 2010) than the best cultivars (Bourbon or Caturra). The Híbrido de Timor germplasm and its derivatives show potential to be used in coffee breeding programs which seek quality for the specialty coffee market (Sobreira et al, 2015).

## CONCLUSIONS & PERSPECTIVES

The majority of elite genotypes of *Coffea arabica* L. assessed present potential for the production of specialty coffees. The Híbrido de Timor germplasm and its derivatives show potential to be used in coffee breeding programs which seek quality for the specialty coffee market.

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## **Analysis of genetic diversity and population structure of Arabica coffee (*Coffea arabica* L.) germplasm available in Indian gene bank using SRAP markers**

Mishra, Manoj Kumar., Jingade, Pavankumar., Huded, Arun K., Kosaraju, Bharathi., Muniswamy, Bychappa., Devasia, Jeena., Padmajyothi, Dandamudi., Suryaprakash, Nayani., Raghuramulu, Yenugula

Plant Biotechnology Division, Unit of Central Coffee Research Institute,  
Coffee Board, Manasagangothri, Mysore, Karnataka, India  
Corresponding author contact: manojmishra.m@gmail.com

### **RATIONALE**

Genetic diversity forms the foundation of a breeding program. The identification and mobilization of useful genetic variation are critical for future genetic gain and protection of crop plants against climate change. The genetic diversity of arabica coffee (*Coffea arabica*) has been substantially eroded during the process of domestication and selective breeding processes. However, there was evidence that barring a few, the arabica germplasm collection available in different gene bank worldwide are woefully underutilized. This is largely due to the absence of comprehensive genetic information of the accessions maintained in the gene bank. Herein we report the genetic diversity and population structure of arabica germplasm accessions available in Indian gene bank.

### **METHODS**

Ninety arabica germplasm accessions comprising USDA world collections obtained during the 1950s and Ethiopian collections obtained under FAO expeditions during 1960 were screened separately for genetic diversity using Sequence related amplified polymorphism (SRAP) markers. Electrophoretic data analysis was carried out using NTSYS-PC 2.10e software. The Bayesian bar plot was constructed using STRUCTURE program ver.2.2. to delineate the clusters of genetically similar accessions.

### **RESULTS**

The SRAP molecular marker analysis revealed 67.25 % polymorphism among USDA world arabica germplasm collection compared to 89.65% of polymorphism among Ethiopian arabica germplasm collection under FAO expedition. The average polymorphism information content of Ethiopian Arabica germplasm was 0.65 which was significantly higher than the average PIC values (0.28) obtained in USDA world arabica germplasm collection. Among Ethiopian Arabica collections, maximum diversity was observed in the accessions collected from Shoa and Kaffa provinces. Based on the frequency of rare alleles and population structure analysis several individual accessions of USDA world collections and Ethiopian germplasm collections were identified as potential parental material for exploitation in the breeding programs.

### **CONCLUSIONS & PERSPECTIVES**

The present study demonstrated the effectiveness of SRAP marker in unraveling the genetic diversity in arabica germplasm collections which could be useful in the breeding program.

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## GENETIC PERFORMANCE OF ROBUST COFFEE PROGENIES RESULTING FROM PHENOTYPIC SELECTION

MISTRO, Júlio C.\*, MANTOVANI, Elaine S.\* and FAZUOLI, Luiz C.\*

\*Centro de Café - Instituto Agronômico (IAC/APTA), Campinas, SP, Brazil,

### RATIONALE

The objective of this research was to estimate genetic parameters to quantify the variability of a progenies of robusta coffee (*Coffea canephora* Pierre) in order to verify the efficiency of the genetic selection.

### METHODS

An experiment was carried out in Parapuã (state of São Paulo, Brazil), using a randomized block design with 25 half-sib progenies, three replicates and ten plants per plot. These progenies are in the second selective cycle, and in the previous cycle their selection was made phenotypically. For the statistical and biometric analyzes, performed by the Selegen software, the mixed linear models (REML/BLUP procedure) were used considering all harvests (five) and years with high yields (three). Genetic additive, permanent and interaction genetic variances and the following genetic parameters were estimated: individual and average heritabilities, coefficients of genetic, environmental and relative variation besides the genetic gain with the selection.

### RESULTS

The additive genetic variance estimated for the years of high yields was higher than the total number of years, but this did not reflect a high individual heritability, and both situations presented this coefficient practically the same, same fact verified for the coefficients of genetic variation, environmental and relative. For the years of high yields there was a high estimate for the variance of the interaction genotypes x environments. The relative coefficient of variation, one of the main parameters for the selection, was well below the ideal value. This reflected in the low gains with the selection so much for the years of high productions as for all the years. Probably these results reflect the low number of replicates used in the experiment.

### CONCLUSIONS & PERSPECTIVES

Due to the high genotype x environment interaction and/or a phenotypic selection performed in the previous cycle showed that the selection in this cycle will not be efficient. In order for the selection to be efficient it will be necessary to carry out more harvests so that the genetic effect prevails over the environmental one; in experimental planning the number of repetitions should not be low; phenotypic selections should be avoided at any stage of breeding programs.

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MISTRO, Júlio C.\*, MANTOVANI, Elaine S.\*, FAZUOLI, Luiz C.\* and SILVA-FILHO, Marcelo J.\*\*  
\*Centro de Café - Instituto Agronômico (IAC/APTA), Campinas, SP, Brazil; \*\*Fundação Procafé, Franca, SP, Brazil.

### RATIONALE

In recent years an Arabica coffee (*Coffea arabica*) Bourbon has been sought after by the specialty coffee market. Despite having an unquestionable sensorial quality, the cultivar does not present high yields, which leads to it being more used in commercial cultivation. The objective of this study was to evaluate a Bourbon coffee in order to select genotypes with high productivity.

### METHODS

An experiment was carried out with 28 treatments: 27 Bourbon coffee genotypes, 13 with red fruits and 14 with yellow fruits, and one control cultivar (Mundo Novo) in randomized blocks with three replicates and ten plants per plots without irrigation in the city of Franca (SP - Brazil). Three harvests were performed after pruning of the plants.

### RESULTS

The average productivity of the experiment was 40.6 scs/ha and 15 Bourbon genotypes were above this average; the Red Bourbons (RB) had an average productivity of 39.2 scs/ha and the Yellow Bourbons (YB) 41.9 scs/ha. The control cultivar Mundo Novo presented 51.9 scs/ha, the most productive. The highest productivity was obtained by genotype 25 (YB with 51.6 scs/ha). We performed a second average taking into account only the genotypes that were above 40.6 scs/ha, resulting in a new average of 45.0 scs/ha. In this sense only seven Bourbons were above this second average: treatment 1 - RB (45.0 scs/ha), 2 - RB (50.4 scs/ha), 12 - YB (47.5 scs/ha), 15 - YB ), 22 - RB (45.8 scs/ha), 25 - YB (51.6 scs/ha) and 26 - YB (45.1 scs/ha). In the first year after pruning, some Bourbons had high yields (treatments 25 with 88.9 scs/ha, 2 with 75.1 scs/ha and treatment 1 with 72.5 scs/ha), as well as Mundo Novo (80.3 scs/ha). Genotypes 14, 15 and 20 were highlighted by the smaller variations of yields among the three fact crops of extreme importance and were above the general average of the experiment.

### CONCLUSIONS & PERSPECTIVES

It is concluded that there are several lines of Bourbon coffee, both red and yellow, with high yields, as good as the New World; there are genotypes of Bourbon that have smaller oscillations between the productions. The selected genotypes will be evaluated in other regions and then commercially recommended. They may also be used in hybridizations with leaf rust resistant cultivars.

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## *Coffea arabica* var. *laurina*: *in vivo* VOLATILE ORGANIC COMPOUNDS (VOCs) RELEASE UNDER WATER DEFICIT STRESS

KHOMENKO, Iuliia\*, CRISAFULLI, Paola\*\*, BIASIOLI, Franco\*, TURELLO, Luca\*\*, MALVICINI, Gian Luca\*\*, NAVARINI, Luciano\*\*

\*Dept. Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM), Via E. Mach 1 - 38010 San Michele all'Adige, Italy, \*\*illycaffè S.p.A., Via Flavia 110 – 34147 Trieste, Italy.

It is known that plants synthesize a wide variety of volatile organic compounds (VOCs) to facilitate interaction with the environment that surrounds them in order to attract pollinators and possible seed dispersants as well as to protect from biotic and abiotic stresses. Climate change is leading to increasingly extreme temperatures and drought periods, which are major abiotic factors affecting coffee production. So far, no attention has been paid in investigating the *in vivo* VOCs release of *C. arabica* plants as a consequence of abiotic stresses and this largely stimulated the present investigation. In particular, *in vivo* VOCs release has been monitored up to incipient water deficit stress in order to compare, under controlled conditions, two groups of *C. arabica* var. *laurina* plants kept at two different soil water regimes. As far as we know, it is the first time *in vivo* VOCs emission from coffee plants has been investigated.

### RATIONALE

One group of coffee plants (wet) was regularly hydrated, another group (dry) was kept under “no soil water” regime and the *in vivo* VOCs release has been monitored for about 3 months in order to study the chemicals emission profile and to follow both formation and evolution of possible molecular signaling of an incipient state of water deficit stress. Different moisture/temperature conditions have been tested.

### METHODS

*C. arabica* var. *laurina* healthy plants were obtained from seeds (origin El Salvador). The plants, grown in a greenhouse and 1 year's old, were put in a climate cabinet and fully enclosed with a teflon bags properly connected to PTR-ToF-MS 8000 instrument (Ionicon Analytik, Austria). Air of each bag was sampled for 2 min in series by an automated inlet switching system and the sampling was performed to cover the time course of the experiment. Leaf tissues and stomata were preliminary observed in wet and dry plants by both optical and electron microscopy.

### RESULTS

Independently on hydration regime, several VOCs, mostly produced during day time, have been detected including: methanol, acetaldehyde, ethanol, acetone, acetic acid, di-methyl-sulfide, isoprene, monoterpenes, benzene, phenol, hexenal, hexanal, xylene. No significant differences in both VOCs chemical identity and release have been observed in dry and wet plants kept under different conditions. No headspace molecular signaling arises from the different tested abiotic stresses.

### CONCLUSIONS & PERSPECTIVES

Circadian cycle is observed for most compounds and VOCs are produced mostly during day time. Clear emission of several compounds mostly from *de novo* biosynthesis was detected. No obvious differences between dry and wet plants VOCs emission were observed up to incipient water deficit stress and no molecular markers highlighted such condition. Dry plants are very resistant until they stay in the climate cabinet putting in evidence the drought-tolerant character of *C. arabica* var. *laurina*. Further studies are necessary to provide knowledge in this promising area not yet fully exploited in coffee research.





## GENETIC EXPRESSION OF DEFENSE-RELATED SEQUENCES IN TOLERANT AND SUSCEPTIBLE PLANTS TO *Ceratocystis fimbriata*.

NARANJO, Nazareth\*, ROBLES, Alejandra\*\*, BARQUERO, Miguel.\*\*

\*Tecnológico de Costa Rica- Degree student.

\*\*Instituto del café de Costa Rica-ICAFE.

### RATIONALE

*Ceratocystis fimbriata* cause the disease known as "steam canker" in coffee, which obstructs the vascular system and causes the death of the plant in any of the stages of development, causing economic losses to coffee farmers. Identification of plants with resistance to this disease is an alternative to this problem, nevertheless, the possible mechanisms involved on the defense are not known.

### METHODS

We evaluated variations on the expression of six defense-related sequences by qPCR during the firsts 24 hours of *Ceratocystis fimbriate* infection on susceptible and resistance plants.

### RESULTS

Resistant plants shown an increase on the expression of sequences related with PR-10 protein and chlorogenic acids synthesis. Major increase were quantified 11 hours after the inoculation with *Ceratocystis fimbriata* in the tolerant plants.

### CONCLUSIONS & PERSPECTIVES

Chlorogenic acid related sequence and PR-10 protein seen to have an important role in the coffee defense reaction against *Ceratocystis fimbriata*, mainly by the rapid synthesis response during the first stages of the infection. We are currently evaluating heredability of this characteristics on resistant plants segregation.



ROMANO, Lucicléia S.\*, GIOMO, Gerson S.\*

\* Instituto Agronômico de Campinas (IAC), P.O. Box 28, 13001-970, Campinas, SP, Brazil

## RATIONALE

Previous coffee quality evaluation performed in Brazil have indicated that Yellow Bourbon variety frequently present high beverage quality with outstanding sensory attributes [1, 2]. This research was carried out to evaluate the green bean physical characteristics and beverage quality of fifteen *Coffea arabica* genotypes in high altitude environmental and the objective was to compare the quality performance of Yellow Bourbon progenies. The differences observed on physical and sensorial quality among the genotypes indicate that there are a real possibility to select new Yellow Bourbon variety to improve the specialty coffee production in Brazil.

## METHODS

The experiment was carried out in 2017 crop season in Northwest São Paulo State, Brazil. It was evaluated 14 Yellow Bourbon and one Yellow Mundo Novo progenies. Ripe fruits from each of the experimental plot were prepared by the wet process. The parchment coffees were sun dried and samples from up to 16/64 inch sieve size without defective beans were submitted to sensory evaluation by certified cup tasters. The beverage quality assessment was done by descriptive analysis of cup quality according to the Specialty Coffee Association (SCA).

## RESULTS

The results showed that there are important differences both on green bean physical characteristics and beverage quality among Yellow Bourbon and Yellow Mundo Novo progenies. Concerning beverage quality it was noted that Yellow Bourbon B.A CJ 04.10, B.A CJ 30.20 and Mundo Novo IAC 4266 reached the highest SCA score in this experiment, respectively 89.9, 87.5 and 87.0. The highest SCA scores were observed in Yellow Bourbon progenies emphasize its excellent intrinsic cup quality, while the good green bean physical characteristics (bean size) do not necessarily correspond to the better beverage quality.

## CONCLUSIONS & PERSPECTIVES

Considering that all varieties were from the same environmental and prepared by the same postharvest processing procedures, it is supposed that the coffee quality differences could have occurred due genetic effects, indicating the possibility of variety selection to attend the specialty coffee market demand. The highest overall score presented by some Yellow Bourbon progenies confirm the high beverage quality potential of this variety.

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## CURRENT SPATIO-TEMPORAL DYNAMICS OF COFFEE PESTS IN KENYA

ALWORA, Getrude\*, GICHURU, Elijah, KATHURIMA, Cecilia.

Kenya Agricultural and Livestock Research Organization- Coffee Research Institute, Ruiru Kenya

\*gedohal@yahoo.com

Distribution of coffee pests in time and space is a key aspect of sustainable and effective management. The aim of this study was to determine the current prevalence, incidence and severity of coffee pests in the main coffee growing counties in Kenya.

Data was collected through survey was done by administering structured questionnaires at house hold level in the 18 counties and random sampling of farms. Coffee Berry Disease(CBD) and Coffee Leaf Rust(CLR) occurrence and severity was recorded by monthly scoring of data in farms located in high disease zones (Kiambu and Nyeri for CBD and Juja-Azania for CLR). Survey data was summarized and analysed using SPSS statistical package while disease data was analysed using COSTAT version 6.45.

Coffee Leaf Rust was the most prevalent disease with a prevalence level of 40%, CBD at 29%, BBC at 3% and root diseases at 6%. Green scales were the most prevalent insect pests with a prevalence level of 16% and leaf miners at 13%. Black jack, oxalis and wondering Jew were the most prevalent weeds at 20%, 18% and 16% respectively. Coffee Berry Disease had different progress curves in Kiambu County (Coffee Research Institute, Yara and Ndumberi) and in Nyeri County. Coffee Leaf Rust infections in Azania started in April and increased to attain a peak of 70.1% severity in July. Further differences in the levels of diseases in different areas were observed during surveys done in May 2015.

The different disease patterns in the three areas will be important in planning or developing spray programmes. Farmers in Kiambu will apply the first protective spray in February and apply through to July while those in Nyeri and CRI will apply the first spray in March. However, in Nyeri the last spraying would be in June while at CRI will be July.

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## BACCHARIS GLUTINOSA (CHILCA) ROOTS EXTRACT. EFFECT ON HEMILEIA VASTATRIX.

LAM-GUTIERREZ Anayancy,<sup>1</sup> SILVA Maria do Céu,<sup>2,3</sup> RUÍZ-VALDIVIEZO Víctor Manuel,<sup>1</sup> GARRIDO-RAMÍREZ Eduardo Raymundo,<sup>1</sup> AZINHEIRA Helena Gil,<sup>2,3</sup>

<sup>1</sup>Instituto Tecnológico de Tuxtla Gutiérrez, Tecnológico Nacional de México, Tuxtla Gutiérrez, Chiapas INIFAP, Campo Experimental Ctr Chiapas, México. <sup>2</sup>LEAF-Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal. <sup>3</sup>Centro de Investigação das Ferrugens do Cafeeiro, Instituto Superior de Agronomia, Universidade de Lisboa. Oeiras, Portugal. Centro de Investigação das Ferrugens do Cafeeiro, Linking Landscape, Environment, Agriculture And Food (LEAF), Instituto Superior de Agronomia (ISA), Unversidade de Lisboa

### RATIONALE

Coffee (*Coffea arabica* L.), one of the key export and cash crops in tropical and subtropical countries, suffers severe losses from the rust fungus *Hemileia vastatrix* Berkeley and Broome, the causal agent of coffee leaf rust.

In Mexico, in last years the coffee leaf rust has been a terrible problem, specially in Chiapas, where the production of coffee has been reduced in 50%.

The *Baccharis glutinosa* is used in traditional medicine by the native people of Mexico, and their antifungal activity has been observed on phytopathogenic fungi; the aerial parts have been tested producing a fungistatic effect against *Aspergillus flavus*, *A. parasiticus* and a fungicidal effect on *F. verticillioides*.

In this study, we investigated the inhibitory effect of methanol extract obtained from the crude extract of roots of *B. glutinosa* on *H. vastatrix*, that could be used as natural alternative to biological control of coffee leaf rust.

### METHODS

Different concentrations of methanolic extracts from *B. glutinosa* roots were used to investigate the percentage of germination and appressoria formation of *H. vastatrix* on detached leaves of *Coffea arabica* var. Caturra.

Extracts of *B. glutinosa* roots were also used to study the esterase activity of spores and gene expression, by RT-qPCR of chitin deacetylases, MAPKinases and other signaling molecules during the infection process of *H. vastatrix*.

### RESULTS

Concentrations of *B. glutinosa* above 1 mg/ml cause significant reduction on the germination, appressoria formation and esterase activity. The results of RT-qPCR would give some light on the effect of *B. glutinosa* roots extracts on the infection process of *H. vastatrix*

### CONCLUSIONS & PERSPECTIVES

This study reporting the biological activity of *B. glutinosa* roots that could be used as natural alternative to control of *H. vastatrix*

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## CYTOGENETIC AND TRANSCRIPTOMIC APPROACHES TO UNDERSTAND THE NUCLEAR CYCLE OF HEMILEIA VASTATRIX.

RIBEIRO Teresa,<sup>2</sup> AZINHEIRA Helena Gil,<sup>1,2</sup> CARVALHO Rita,<sup>1</sup> TAVARES Sílvia<sup>1,2,3</sup>  
MONTEIRO Marta,<sup>4</sup> COELHO Marco,<sup>5</sup> SILVA Maria do Céu,<sup>1,2</sup> LOUREIRO João,<sup>6</sup> TALHINHAS  
Pedro,<sup>1,2</sup> MORAIS-CECÍLIO Leonor,<sup>2</sup>

<sup>1</sup>Centro de Investigação das Ferrugens do Cafeeiro, Instituto Superior de Agronomia, Universidade de Lisboa. Oeiras, Portugal <sup>2</sup>LEAF-Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa. Lisboa, Portugal. <sup>3</sup>Section for Plant and Soil Science, Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen. Frederiksberg Copenhagen, Denmark. <sup>4</sup>Instituto Gulbenkian de Ciência. Oeiras, Portugal. <sup>5</sup>UCIBIO-REQUIMTE, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa. Caparica, Portugal. <sup>6</sup>CFE, Centro de Ecologia Funcional, Departamento de Ciências da Vida, Universidade de Coimbra. Coimbra, Portugal

**RATIONALE:** *Hemileia vastatrix* (*Hv*), the causal agent of coffee leaf rust is the most important disease of Arabica coffee worldwide. *Hv* is a hemicyclic fungus with the urediniosporic life cycle as it is most important (if not only) source of inoculum. Urediniospores are dikaryotic and represent the asexual cycle. Teliospores occur very rarely and germinate *in situ*, producing a promycelium from which four basidiospores are formed. Basidiospores cannot infect coffee, but no other host plant has been identified.

Flow cytometric studies to quantify rusts genome size showed the presence of 1C, 2C and a low proportion of 4C nuclei in different stages of the urediniosporic cycle of *Hv* and other rust fungi. These results suggest the presence of diploid nuclei that supposedly only occur in teliospores, and are compatible with the occurrence of karyogamy and meiosis before sporulation.

**METHODS:** Flow cytometry analysis along the infection process and FISH with 45S rDNA probe in sorted 1C, 2C and 4C nuclei separated from infected leaves and infected leaf structures nuclei was used to confirm the occurrence of diploid nuclei. In parallel a transcriptomic approach along the infection cycle of *Hv* to study the expression profiles of genes related with karyogamy and/or meiosis was done aiming to study gene regulation during life cycle of rusts.

**RESULTS:** The 1C nuclei population, which becomes undetectable in matured and germinating urediniospores, is composed by unreplicated haploid nuclei. The analysis of the 2C population from infected leaves revealed the presence of replicated haploid nuclei as well as unreplicated diploid nuclei. The population 4C is composed by replicated diploid nuclei. Most karyogamy-related genes [1] and some meiosis-related genes [2] are present in *Hv* genome, but only some of the karyogamy-required genes were identified in *Hv* EST libraries [3]. The expression profiles obtained are related the nuclear cycle dynamic.

**CONCLUSIONS & PERSPECTIVES:** The genes expression and its relation with the nuclei populations obtained along the infection process will help to clarify the occurrence of karyogamy and meiosis or other parasexual phenomenon.

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VIEIRA, Ana<sup>\*, \*\* , \*\*\*</sup>, SILVA, Diogo N.<sup>\*, \*\* , \*\*\*</sup>, VÁRZEA, Victor<sup>\*, \*\* , \*\*\*</sup>, PAULO, Octávio P.<sup>\*\*</sup>, BATISTA, Dora<sup>\*, \*\* , \*\*\*</sup>

\* Centro de Investigação das Ferrugens do Cafeeiro, Instituto Superior de Agronomia, Universidade de Lisboa, Oeiras, Portugal, \*\* Computational Biology and Population Genomics group, cE3c - Centre for Ecology, Evolution and Environmental Changes, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal, \*\*\* Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal

## RATIONALE

Pathogenic fungi are emerging at an increasing rate on a wide range of host plants, leading to tremendous threats to global economy and food safety. Several plant pathogens have been considered invasive species, rendering large-scale population genomic analyses crucial to better understand their demographic history and evolutionary potential. *Colletotrichum kahawae* (*Ck*) is a highly aggressive and specialized pathogen, causing Coffee Berry Disease in Arabica coffee in Africa. Given the devastating impact of this disease, *Ck* potential dissemination to out of Africa is greatly feared. In this work we applied a population genomics approach to generate thousands of SNPs spaced throughout the genome for a broad range of *Ck* isolates to better understand its demographic history and evolutionary potential.

## METHODS

In this work 30 *Ck* isolates, collected from almost all coffee regions where CBD occurs, and five isolates from the ancestral lineage were sequenced using RAD sequencing. The best *de novo* assembly strategy yielded 27099 SNPs across 15007 loci. The phylogenetic relationships was inferred with a Maximum Likelihood and Bayesian analyses from a single concatenated alignment. The population structure was evaluated by two individual centered approaches, PCA and DAPC. The genetic differentiation among the populations was assessed by calculating the overall and distribution of SNP  $F_{ST}$  values for each population pair following using VCFTOOLS and Arlequin. Finally, to explore the type of expansion and reproduction system of *Ck* we used Poppr v2.4.0..

## RESULTS

Our results strongly supports that *Ck* is a true clonal pathogen, perfectly adapted to green coffee berries, completely differentiated from the ancestral lineage, with three completely differentiated populations (Angola, Cameroon and East African). Two independent clonal lineages were found within Angola as opposed to the remaining single-clonal populations. The most probable colonization scenario suggests that this pathogen has emerged in Angola and immediately dispersed to East Africa, where these two populations began to differentiate, followed by the introduction in Cameroon from an Angola population. However, the differentiation between the two Angola clonal lineages masks the mechanism for the emergence of the Cameroon population.

## CONCLUSIONS & PERSPECTIVES

Our results suggests that *Ck* seems to have a low evolutionary potential and dispersion ability, being human transport the mostly likely scenario for its potential dispersion. Therefore, if the quarantine practices already in place are correctly followed, such as special care when importing plants from Africa, using only dry seeds to circulate plant material and complying with phytosanitary measures, the dissemination of *Ck* outside Africa may most likely be prevented.



## **COFFEE LEAF RUST (*Hemileia vastatrix* Berk. & Br) ON ARABICA COFFEE (*Coffea arabica* L.) AT ITS CENTRE OF ORIGIN, ETHIOPIA**

Kifle Belachew\*, Demelash Teferi, Gabisa Gidisa and Nagasa Dechasa

\*Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Centre, Plant Pathology Research Section, Jimma, Ethiopia

### **RATIONALE**

Coffee leaf rust caused by biotrophic fungus *Hemileia vastatrix* Berk. & Br., is worldwide the most important disease in Arabica coffee, causing annually considerable economic damage (>\$ 2 billion). In Ethiopia it has been considered as less damaging and low land coffee disease, however, the disease has reproduced to unusually high levels in many Arabica coffee growing areas in Ethiopia. Thus, this study aims to evaluate the reaction of released coffee varieties and to analyse the temporal and spatial distribution of coffee leaf rust at its centre of origin.

### **METHODS**

A study was conducted on twenty six (26) released Arabica coffee varieties under a range of environmental conditions at on-station and sub stations of Jimma National Coffee Research Center in Ethiopia. The treatments were outlined using two stage nested design in which coffee trees were nested under locations. Coffee leaf rust incidence, severity and number of sporulating lesion density (SLD) were quantified from five selected coffee plants per variety per season over three consecutive years (2015, 2016 and 2017). The climate variables (temperature, rain fall and relative humidity) of each station were recorded and correlated.

### **RESULTS**

The results showed that CLR was prevalent with significantly ( $p < 0.001$ ) varied intensity of damage among coffee varieties, seasons and locations. The initial incidence and SLD were significantly varied from 17.6-73.8% and 3.0-23.7, respectively. The average highest disease incidence of 60.7, 45.3 and 39.3% was recorded at Tepi, Metu and Agaro, with correspondingly severity of 32.7, 26.7 and 22.0%, respectively. Coffee variety 74110, 741, 744, 7487 and 75227 scored the highest incidence of CLR with 67.75, 52.61, 51.21, 51.1 and 50.32 % respectively. CLR incidence was low in July (13.9%) and high in January (59.6%). The increased intensity of CLR was strongly associated with coffee cultivars ( $r = 0.63$ ), altitude ( $r = 0.52$ ), season ( $r = 0.51$ ) and shade level ( $r = 0.48$ ).

### **CONCLUSION & PERSPECTIVE**

From the present study, the magnitude of coffee leaf rust disease differ based on coffee varieties, seasons and locations. Moreover, the prevalence of CLR becoming high and these situations will result in significant yield loss thereby impacting the country's economy. Therefore, host response reaction can be used as criteria for varietal selection and breeding program should focus on CLR resistant varieties before the disease causes severe crop loss.

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## **RESISTANCE SCREENING OF RELEASED COFFEE VARIETIES AGAINST COFFEE THREAD BLIGHT (*Corticium koleroga*) AT SOUTHWEST ETHIOPIA**

Kifle Belachew\* and Nagasa Dechasa

\*Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Centre, Plant Pathology Research Section, Jimma, Ethiopia

### **RATIONALE**

Coffee thread blight (*Corticium koleroga*) is an emerging coffee disease with damaging potential in Ethiopian coffee production due to climate change. Rapid spreading nature of the disease and perennial character of coffee have made management of coffee thread blight difficult through the conventional control, like use of fungicides. While use of genetically resistant coffee varieties, particularly specific to certain agro-ecologies can be an essential approach to reduce coffee yield losses associated with coffee thread blight in Ethiopia. Therefore, long-term prospects of successful management of coffee thread blight depend principally upon employing resistant coffee varieties. This study aims at to screen resistant/tolerant released coffee varieties from Southwest part of the country against coffee thread blight in laboratory and greenhouse conditions.

### **METHODS**

The study was conducted using 37 coffee varieties at Jimma Agricultural Research Centre, South-Western Ethiopia in 2016 and 2017. The laboratory and greenhouse studies were conducted using complete randomized design (CRD) with four replications. Inoculation in green house started when the seedlings attained the full expanded cotyledon stage (seventy days after sowing) using a representative isolate of *Corticium koleroga*, the causative agent of coffee thread blight.

### **RESULTS**

The results revealed that there was highly significant difference among coffee varieties tested for coffee thread blight in laboratory and greenhouse. The diseases severity varies among different coffee varieties and ranged from 0.00 to 70.96 %. Coffee varieties 74110, 7487, 7440, 754 and 74112, exhibited above 50 % diseases severity, indicating highly susceptible reactions to coffee thread blight. Similarly, coffee varieties 744 and 74140 express moderately susceptible reaction as exhibited 25.15 and 30.45 % respectively. Coffee varieties expressed resistant reactions against the disease were F-59 and F-35 without disease expression (0.00%). Moreover, incubation period exhibited highly significant ( $P < 0.01$ ) variation among treatments. The mean incubation period among the tested varieties in days ranged from 0 to 77. The results suggest that coffee varieties with low disease severity, long incubation period and high survival rate conferred resistant against coffee thread blight disease.

### **CONCLUSION & PERSPECTIVE**

Overall, this study implied that the potential of obtaining coffee thread blight disease resistant coffee variety from Southwest Ethiopia. The study also revealed potential alleles found in the coffee gene pool of Ethiopia may hold the key to the species long-term survival providing the traits needed to cope with new diseases and climate change.

### **REFERENCE**

Kifle Belachew, Demelash Teferi and Leggese Hagos. 2015. Coffee Thread Blight (*Corticium koleroga*): a Coming Threat for Ethiopian Coffee Production. Journal of Plant Pathology and Microbiology. 6(9): 1- 6





## CHARACTERIZATION OF PHYSIOLOGICAL RACES AND ANALYSIS OF EFFECTIVE CANDIDATE PROTEINS IN POPULATION OF *HEMILEIA VASTATRIX* IN BRAZIL.

SILVA, Rosemeire Alves\*, ZAMBOLIM, Laercio\*, CAIXETA, Eveline Teixeira\*\*

\* Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, MG, Brasil.

\*\* Embrapa Café, Universidade Federal de Viçosa, Viçosa, MG, Brasil.

New physiological races of *Hemileia vastatrix* have emerged infecting most of the rust resistant cultivars of coffee worldwide. The most efficient strategy to control the disease is the use of resistant varieties. However, obtaining resistant genotypes has been a challenge due to the high adaptive potential of the fungus, and it is increasingly difficult to characterize it through the collection of a race differentiating series. Our objective was to characterize the *H. vastatrix* races of the main coffee producing regions of Brazil and to study the effector candidate proteins predicted for *H. vastatrix* that may contribute to pathogenicity with different races. Monopustular isolates (56) were collected from *Coffea canephora*, *Coffea arabica* and Hibrido de Timor, and multiplied in Caturra. The DNA of the isolates was extracted for genomic studies, and 47 primer combinations, designed from effector candidate proteins, were used to amplify the genes of interest. Sequences were aligned for the 46 isolates by the DNA Baser program; conserved domain study were done by the Pfam program, and the protein categorization by Blast2GO. Alignment of the nucleotide sequences, proteins and clustering of the isolates was done by the clustalw program. The nucleotide difference was analyzed by the weblog program and phylogeny study was used to separate the isolates. Seven races were identified, with five previously described in Brazil and two for the first time, race XXIX and XXX. It was also found 15 combinations of new virulent genes (pathotypes). The 47 genes studied were conserved in all isolates, linked to 15 biological processes of fungus development and 14 domains. It was found difference in nucleotide sequence with the presence of heterozygous and homozygous individuals. Phylogeny analysis showed different individuals with the Hv-09 isolate being the most divergent. The information generated in this research may help to better understand the interaction between *H. vastatrix* and the coffee plants. The knowledge of the genes that are involved in the infectious process can help in the understanding of the molecular mechanisms that lead to the supplanting of the resistance by new races of *H. vastatrix*.

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## PARTIAL RESISTANCE TO NEMATODE *Meloidogyne paranaensis* IN HÍBRIDO DE TIMOR COFFEE GENOTYPES

ITO, Dhalton S.\*; FERNANDES, Lucas E.\*\*; SHIGUEOKA, Luciana H.\*, SILVA, Santino A.\*, FONSECA, Ines C.B.\*\*; PEREIRA, Antonio A.\*\*\*, SERA, Gustavo H.\*

\*Instituto Agronômico do Paraná - IAPAR, Londrina, PR, Brazil. \*\*Universidade Estadual de Londrina – UEL, Londrina, PR, Brazil, \*\*\*Empresa de Pesquisa Agropecuária de Minas Gerais – EPAMIG, Belo Horizonte, MG, Brazil.

### RATIONALE

The Brazilian coffee crop has suffered considerable productivity losses due to occurrence of nematode *Meloidogyne paranaensis*. This specie is very harmful to coffee plants, inducing several problems related to nutrients absorption such as growth reducing, leaf drop and general plant decline, which even can cause plant death. Nowadays, *M. paranaensis* is highly widespread in Brazil. In Minas Gerais State, the Brazilian's largest coffee producer, this nematode is disseminated among important coffee regions, becoming a serious problem. This specie is also present in another coffee producing countries like Guatemala and Mexico. There are few source of resistance to *M. paranaensis* in coffee, one of them was found in *C. canephora*. The Híbrido de Timor genotype is an Arabica coffee resistant to leaf rust, carrying *C. canephora* genes. The aim of this study was to evaluate the resistance to *M. paranaensis* in Híbrido de Timor coffees.

### METHODS

10 Híbrido de Timor accessions from EPAMIG/UFV's germplasm bank were evaluated. The cultivar Mundo Novo IAC 376-4 was used as a susceptible control. Seedlings with three to four pairs of leaves were transplanted into plastic cups with a capacity of 700 mL and 1,200 eggs and juveniles J2 of *M. paranaensis* (IP) were inoculated after one month. The evaluations were performed 134 days after inoculation, when the data of the number of eggs and juveniles J2 per gram of roots and the final population of nematodes (FP) were obtained. The reproduction factor (RF) was calculated using the formula:  $RF = IP / FP$ . To classify the resistance levels of the genotypes the reduction of the reproduction factor (RRF) was used, being classified from highly resistant to highly susceptible.

### RESULTS

Different levels of partial resistance were observed among the HT genotypes, especially HT UFV 408-28, which presented moderate resistance.

### CONCLUSIONS & PERSPECTIVES

The best genotype can be used to develop new coffee cultivars with simultaneous resistance to nematode *M. paranaensis* and leaf rust caused by *Hemileia vastatrix*.

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## THE CURRENT COFFEE WILT DISEASE UPSURGE AND OCCURANCE IN HOTSPOTS IN UGANDA IS DUE TO SEXUAL STAGE OF THE PATHOGEN, *Gibberella xylarioides*



OLAL Sammy\*, MUTAI fred\*\*, OLANGO Nicholas\*., KAGEZI Godfrey\*., KUCEL Patric\*, MUSOLI Pascal\*, WAGOIRE Wamala William\*.

\*National Agricultural Research Organization (NARO), P.O. Box 295, Entebbe, UGANDA

\*\*Makerere University, P.O Box 7062, kampala, Uganda

### RATIONALE

The life cycle of *Gibberella xylarioides* involves both asexual and sexual stages and occur in freshly infected and dying trees respectively. The sexual stage is controlled by a 'Male' mating type known as (MAT-1) and 'Female' mating type known as (MAT-2). Sexual stage creates genetic variability in the pathogen and may increase virulence and adaptation to environmental changes posing threats even to resistant materials (Geiser, 2005). Early diagnosis and elimination of infected trees is therefore paramount in the control and management of coffee wilt disease .

### METHODS

A survey was done in three regions in Uganda comprising Buganda, Busoga and Bunyoro noting the disease incidences, mode of the disease spread, farmers' practice in the recommended uprooting and burning of infected trees and collecting specimens for laboratory analysis. Identification of each mating type was done both biotypically and by molecular method.

### RESULTS

The ratio of MAT-1 to MAT-2 was found to be 1:9. In fields where both types were found, the disease incidence ranged from 10 to 50% spreading in hotspot fashion. Most Farmers were found to cut infected trees about 300cm above the ground level allowing the sexual stage to set in around the collar region.

### CONCLUSIONS & PERSPECTIVES

Coffee wilt disease is still a major challenge in coffee production in Uganda and the problem is aggravated by the product of the pathogen's sexual stage; currently causing annual financial loss of up to \$17 million. Coupling with poor farmer practice that allows sexual stage to set in, collective effort by countries where the disease exist if required to control and manage the disease. With the improvement in the diagnostics of each mating type using molecular method (PCR based) for well equipped laboratories and documented pictorial cultural and morphological methods for diagnostic laboratories lacking PCR facilities (Olal et al. 2018 – In press), the prevalence of the two mating type can now be determined with high efficiency impacting on increase coffee production through more efficient CWD management.

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## ARABICA COFFEES FROM ETHIOPIA WITH RESISTANCE TO NEMATODE *Meloidogyne paranaensis*

SERA, Gustavo H.\* , SERA, Tumoru\* , HOLDERBAUM, Melina M.\*\* , SHIGUEOKA, Luciana H.\* , SANTIAGO, Débora C.\*\* , ITO, Dhalton S.\*

\*Instituto Agronômico do Paraná (IAPAR), Londrina, PR, Brazil \*\*Universidade Estadual de Londrina, Londrina, PR, Brazil.

### RATIONALE

The nematode *Meloidogyne paranaensis* causes great economic losses for Brazilian coffee. Plant resistance has been considered one of the main nematode management strategies. Resistance to *M. paranaensis* has been found in *Coffea canephora*, *C. congensis* and wild *C. arabica* L. accessions from Ethiopia. Although there are resistance sources to nematodes, few ungrafted Arabica coffee cultivars present resistance. Actually, *C. arabica* cv. IPR 100 is the only one cultivar that it has been widely grown in infested areas in Brazil. The aim of this study was to evaluate the resistance to *M. paranaensis* in Ethiopian *C. arabica* accessions from the IAPAR's germplasm bank.

### METHODS

The assessment of resistance to *M. paranaensis* was carried out in 12 Ethiopian *C. arabica* accessions from FAO collection and an access from Ethiopia called Geisha, using open pollinated seeds. *C. arabica* cv. Mundo Novo IAC 376-4 was used as susceptible check. Two experiments (Exp. 1 and Exp. 2) were carried out in a greenhouse at the IAPAR (Londrina, PR, Brazil). The experiments were installed in a completely randomized design with 15 treatments, 8 replications, and one plant per plot. Seedlings with three to four pair of leaves were transplanted into 700ml plastic cups. After one month, 1200 eggs and J2 juveniles of *M. paranaensis* (Population 98.1, Apucarana, PR, Brazil) were inoculated. The assessments were carried out 130 days after inoculation by the nematodes per gram of roots (Nema.g<sup>-1</sup>) and reproduction factor (RF). The reduction in the reproduction factor (RRF) was used to classify the resistance levels of the genotypes. For all variables were performed analysis of variance and Tukey's test ( $\alpha = 5\%$ ).

### RESULTS

In both experiments, it was observed that all genotypes presented lower RF and Nema.g<sup>-1</sup> and they differed from Mundo Novo IAC 376-4, except for E302 that did not differ. The most resistant resistant accessions were E228, E209, E464, E123, E333 and E546, as they presented RFs lower than 1.0 in both experiments and they were classified as highly resistant. Accessions E428 and E298 also had low values of RFs, but these were higher than 1.0 in at least one of the experiments. The E131, Geisha and E279 genotypes were highly resistant only in Exp. 1 and have heterozygous resistance, but also have the potential to be highly resistant genotypes in homozygous if advanced individual plants for the next generation of self-pollination.

### CONCLUSIONS & PERSPECTIVES

Ethiopian wild accessions identified as highly resistant may be used in breeding programs to obtain new cultivars with resistance to *M. paranaensis*.



## RESISTANCE TO RED MITE IN *Coffea arabica* GENOTYPE INTROGRESSED WITH *Coffea racemosa* GENES

SERA, Tumoru\*, CARDUCCI, Fernando C.\*\*, FONSECA, Inês C. de B.\*\*, SANTOS, Willian G. dos\*\*, PEREIRA, Carlos T. M.\*, SERA, Gustavo H.\*

\*Instituto Agronômico do Paraná (IAPAR), Londrina, PR, Brazil \*\*Universidade Estadual de Londrina, Londrina, PR, Brazil.

### RATIONALE

The red mite (*Oligonychus ilicis*) is considered an important pest for *C. canephora*, which causes economic losses more frequently in hotter and drier regions. This pest also occurs in *C. arabica* and it can cause losses of up to 50 % in productivity in areas with high infestation and without chemical control. The use of genetic resistance to mites would be important in the management of this pest, but so far there are few studies on the resistance of coffee plants. In the IAPAR's breeding program, genotype IAC 1195-5-6-2, which is an Arabica coffee with introgression of *C. racemosa* genes, has been used aiming to transfer resistance to leaf miner (*Leucoptera coffeella*) and drought. In addition, IAPAR developed cultivars of Arabica coffee with introgression of genes of different species such as *C. canephora* and *C. liberica*, but it is not yet known about the resistance to red mite of these cultivars and genotypes. The aim of this study was to evaluate the resistance to red mite in *Coffea arabica* genotypes with introgression of genes of different species.

### METHODS

The experiment was conducted in a greenhouse at the IAPAR, in Londrina, PR, Brazil, between January 2016 and January 2017. Seedlings of five coffee genotypes with six pairs of leaves were transplanted into PVC tubes of 0.30 m in diameter x 1.2 m in height, with a total volume of 0.2826 m<sup>3</sup> of substrate in a 1:1 ratio of soil and sand. The experiment was set up in a completely randomized design with five treatments and 12 replications of one plant per plot. The cultivars of *C. arabica* IPR 100, IPR 99 and IPR 103 were evaluated, the first with introgression of *C. liberica* genes and the last two of *C. canephora*. An Arabica F<sub>4</sub> line named IAPAR H0113-40-26-10 with introgression of *C. racemosa* also was evaluated. The cultivar Catuaí Vermelho IAC 99, which is a pure *C. arabica*, was used as susceptible control. The resistance of genotypes to red mite was evaluated by a visual evaluation in January 2017. This evaluation was based on the percentage of leaf area with the typical symptoms caused by this mite, represented by the tanning of the upper face of the leaves. The data of the percentage of the leaf area with symptoms were submitted to analysis of variance and the Tukey's test at 1% of significance.

### RESULTS

By the means test it was possible to observe that the cultivars IPR 99, IPR 100 and IPR 103 did not differ from the susceptible control Catuaí Vermelho IAC 99. Only the line IAPAR H0113-40-26-10 differed from this control and presented resistance to the red mite. IAPAR H0113-40-26-10 presented 21.52% of leaf area with symptoms, while Catuaí presented 54.76%. It is probable that IAC 1195-5-6-2 be the resistance source to red mite of this F<sub>4</sub> line.

### CONCLUSIONS & PERSPECTIVES

The F<sub>4</sub> line IAPAR H0113-40-26-10 presented partial resistance to red mite and will be advanced for next self pollination generation to develop a new cultivar.



## Assessing the disease resistance performance of compact coffee selections in Tanzania

URASA, Josephine M., KILAMBO, Deusdedit L., MTENGA, Damian J., MONYO, Grace K  
Tanzania Coffee Research Institute P. O. Box 3004 Moshi, Tanzania  
Email [Josephine.urasa@tacri.org](mailto:Josephine.urasa@tacri.org)

### ABSTRACT

Bourbon (N39) and Kent (KP 423), the major commercial Arabica Coffee varieties, grown in Tanzania, have fine liquor quality but succumb to coffee berry disease (CBD) caused by *Colletotrichum kahawae* and leaf rust incited by *Hemileia vastatrix*. Yield losses of 30–90% are commonly observed even in well fungicides protected crops. Research programme to develop resistant cultivars has been quite successful. In this work, 39 coffee hybrids of compact stature were selected and established in four (4) on-station evaluation trials and 28 on-farms in different agro-ecological areas to assess their adaptation and resistance to CBD & coffee leaf rust. The locations consisted of low coffee growing areas (< 1200 m a.s.l.), highly favorable for leaf rust; medium (1200–1400 m a.s.l) favorable for both CBD and leaf rust, and high (> 1400 to 1800m a.s.l) highly favorable for CBD. For on-station trials, the 39 compact hybrid breeding lines were assigned in a RCBD with 3 replicates: CVT1 with 11 breeding lines, N39-6 and PNI088 as controls; CVT2 13 breeding lines, N39-6 and PRO 127 as controls and CVT4 with 11 breeding lines and N39, PNI088 and N39-6 as controls. Multiple pruning system was adopted, other management practice were performed as recommended for coffee growing, except no fungicide application. CBD and leaf rust assessment was done using a rating scale of 1–6; where 1 no disease and 6 severe infection. This was followed by computation of Disease Reaction Classification (DRC) whereby 1 & 2 Resistant, 3 Moderately Resistant, 4 Moderately Susceptible, 5 Susceptible, 6 Severe. The level of CBD and leaf rust was recorded high in the commercial variety N 39. But most of the tested coffee lines fall in the MR and R, Disease Reaction Category (DRC). Since all the 39 compact hybrid breeding lines passed the test of CBD and CLR resistance, an indication that they possess a wide genetic base, evaluation of their yield potential and cup taste is underway in order for them to qualify for commercial use.

Key words: *Disease resistance, performance, Tanzania*



## CHARACTERIZATION OF PHYSIOLOGICAL RACES AND ANALYSIS OF EFFECTIVE CANDIDATE PROTEINS IN POPULATION OF *HEMILEIA VASTATRIX* IN BRAZIL.

SILVA, Rosemeire Alves\*, ZAMBOLIM, Laercio\*, CAIXETA, Eveline Teixeira\*\*

\* Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, MG, Brasil.

\*\* Embrapa Café, Universidade Federal de Viçosa, Viçosa, MG, Brasil.

New physiological races of *Hemileia vastatrix* have emerged infecting most of the rust resistant cultivars of coffee worldwide. The most efficient strategy to control the disease is the use of resistant varieties. However, obtaining resistant genotypes has been a challenge due to the high adaptive potential of the fungus, and it is increasingly difficult to characterize it through the collection of a race differentiating series. Our objective was to characterize the *H. vastatrix* races of the main coffee producing regions of Brazil and to study the effector candidate proteins predicted for *H. vastatrix* that may contribute to pathogenicity with different races. Monopustular isolates (56) were collected from *Coffea canephora*, *Coffea arabica* and Hibrido de Timor, and multiplied in Caturra. The DNA of the isolates was extracted for genomic studies, and 47 primer combinations, designed from effector candidate proteins, were used to amplify the genes of interest. Sequences were aligned for the 46 isolates by the DNA Baser program; conserved domain study were done by the Pfam program, and the protein categorization by Blast2GO. Alignment of the nucleotide sequences, proteins and clustering of the isolates was done by the clustalw program. The nucleotide difference was analyzed by the weblog program and phylogeny study was used to separate the isolates. Seven races were identified, with five previously described in Brazil and two for the first time, race XXIX and XXX. It was also found 15 combinations of new virulent genes (pathotypes). The 47 genes studied were conserved in all isolates, linked to 15 biological processes of fungus development and 14 domains. It was found difference in nucleotide sequence with the presence of heterozygous and homozygous individuals. Phylogeny analysis showed different individuals with the Hv-09 isolate being the most divergent. The information generated in this research may help to better understand the interaction between *H. vastatrix* and the coffee plants. The knowledge of the genes that are involved in the infectious process can help in the understanding of the molecular mechanisms that lead to the supplanting of the resistance by new races of *H. vastatrix*.

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## DIVERSITY AND GENE PYRAMIDING FOR RESISTANCE TO COFFEE BERRY DISEASE AND COFFEE LEAF RUST IN HÍBRIDO DE TIMOR GENOTYPES

SILVA, Rosemeire Alves<sup>\*</sup>, ZAMBOLIM, Laercio<sup>\*</sup>, CAIXETA, Eveline Teixeira<sup>\*\*</sup>

<sup>\*</sup> Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, MG, Brasil.

<sup>\*\*</sup> Embrapa Café, Universidade Federal de Viçosa, Viçosa, MG, Brasil.

The main phytosanitary problems affecting global coffee production are the fungal diseases known as rust, caused by *Hemileia vastatrix*, and coffee berry disease (CBD), induced by *Colletotrichum kahawae*. The main disease control strategy is the use of resistant coffee cultivars. Híbrido de Timor is the most important source of resistant used in breeding programs worldwide. The objective of this work was to characterise the diversity and disease resistance of 152 Híbrido de Timor genotypes from the germplasm collection of the Universidade Federal de Viçosa. Accessions were phenotyped with *H. vastatrix* races II and XXXIII. Molecular analysis was carried out with 29 random microsatellite markers (SSR) and two SSR associated with the CBD resistance gene *Ck-1*. All accessions in the germplasm collection were resistant to *H. vastatrix* race II, and 141 were resistant to *H. vastatrix* race XXXIII. Based on the presence of markers, there were 106 CBD-resistant genotypes, and the 152 accessions clustered in 21 different groups. A unique molecular profile (fingerprinting) was determined for each individual using 52 alleles from 22 SSR markers. The Híbrido de Timor germplasm was highly diverse and included 99 accessions with pyramided multiples genes conferring resistance to *H. vastatrix* races II and XXXIII and CBD. This study showed that the Híbrido de Timor germplasm collection includes diverse genetic material resistant to both *H. vastatrix* races and *C. kahawae*. Detailed analyses will enable the effective selection of sources of durable resistance to the major coffee diseases.

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## ESTABLISHING AGROFORESTRY SYSTEMS IN HAWAI'I USING ENDMEIC HARDWOOD SPECIES AND SELECTED VARIETIES OF COMMERCIAL COFFEE

DUDLEY, Nicklos\*, JONES, Tyler\*\*, BURDEN, Juli\*\*

\*Hawai'i Agriculture Research Center, HI, USA, US Department of Agriculture, NRCS  
Conservation Innovation Grant

### RATIONALE

The project's primary purpose was to further the development of a high value, specialty agroforestry crop system for Hawaii and the Pacific. The project achieved this goal through the implementation, evaluation and monitoring of an ecologically stable, agroforestry system on abandoned agricultural lands. *Acacia koa* is an endemic hardwood tree with high economic value, ecological value, and cultural value. *Acacia koa* is also an endemic legume that regulates water and sunlight to the plants around it making it an ideal candidate for a commercial agroforestry system.

### METHODS

The Hawaii Agriculture Research Center (HARC) used in-house screening methods for *Fusarium* resistant *Acacia koa*. The project was set up to serve as a viable commercial scale demonstration project. HARC hired consultants and certified q-graders to harvested and analyze 7 different varieties of coffee and decided based on cupping scores to plant Pink, Yellow, and Red Bourbon varieties. Intercropped with the coffee was a selection of *Fusarium* resistant *Acacia koa* from 200 different families. The project was finalized by planting and design of orchard, measuring growth performance, project outreach, and the taking the coffee to market as a final product.

### RESULTS

The orchard was planted in Spring of 2014. During the first two years of vegetative growth it became clear that the Red and Yellow bourbon had the most vigorous growth. The first commercial harvest was fall of 2017. The yield was lowest on the pink bourbon. The *Acacia koa* branches were pruned so the branches would not obstruct the coffee productions area.

### CONCLUSIONS & PERSPECTIVES

The project is progressing well and ongoing. Several other landowners have bought koa and coffee to replicate the other farm. In Spring of 2017 the coffee pest Coffee Berry Borer (CBB) was discovered at the site. Challenges of growing coffee are the management of CBB, organization and cost of labor and management. With the organization of more labor in an area a commercial agroforestry Koa and Coffee systems could be a good tool for farmers in Hawai'i.

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BLISKA, Flavia M. M.\*, BLISKA JUNIOR, Antonio.\*\*, PARTELLI, Fábio L. \*\*\*, FAZUOLI, Luiz C.\*

\* Agronomic Institute, Campinas, SP, Brazil, \*\* Faculty of Agricultural Engineering, Campinas, SP, Brazil, \*\*\*Universidade Federal do Espírito Santo, São Mateus, ES, Brazil.

#### **RATIONALE**

The varieties or cultivars of coffee available for commercial cultivation differ in agronomic, phytotechnical and qualitative characteristics. Consequently, they are indicated for specific regions, cropping systems and market niches. For the intrinsic potential characteristics of each variety to be obtained, it is essential that the health and quality of the seeds, plant tissues and seedlings be guaranteed through the certification of companies producing seeds and seedlings. The exploitation of varieties with different characteristics, aiming at the use of improved genetic material, is important in the elaboration of the business plan and in the strategic planning of the farms and can be determinant for its competitiveness and sustainability. Therefore, this study analyzes the management of genetic resources in the main Brazilian coffee regions, and aims to provide subsidies for technical assistance, rural extension and the formulation of public policies.

#### **METHODS**

This study consists of the evaluation of the use of genetic material in Brazilian coffee growing, based on the Identification Method of Management Degree - MIGG Café (BLISKA JÚNIOR *et al*, 2015), through the evaluation of 1136 coffee farms from 2014 to 2017.

#### **RESULTS**

The management of genetic resources among Brazilian coffee companies is positively correlated to their respective levels of business management, indicating that those best structured in the planning of activities, rational use of financial and human resources, agricultural systematization and post-harvest processing processes, as well as market and labor, environmental and tax legislation, generally understand better the importance of the improved genetic material, adapting them to the edaphoclimatic characteristics of the regions where the farms are located and to the different production systems. Effective genetic resource management actions in the Robust/Conilon coffee are actually greater than in Arabica. The requirement for certification of seedlings and seeds is higher among companies with some kind of agricultural certification.

#### **CONCLUSIONS & PERSPECTIVES**

Despite the existence of varieties with different characteristics of resistance to pests, diseases and nematodes, adapted to specific climatic conditions or adverse soil conditions, an opportunity that must be explored by companies, this does not always occur. Among the possible reasons are: insufficient regional experimentation of varieties in rural coffee companies, low availability of seeds, lack of access to information, lack of strategic planning and the conservatism of rural entrepreneurs in the coffee segment, especially in relation to Arabica coffee. Consequently, the potential of new genetic materials is still under-explored, especially among family businesses.

#### **REFERENCE**

BLISKA JÚNIOR, A. *et al*. Validating the management degree identification method in the coffee production using focus group. *Revista de Economia Agrícola*, São Paulo, v. 62, n. 1, p. 41-54, 2015.



## MANAGEMENT OF INPUTS IN THE AGRICULTURAL COFFEE PRODUCTION IN BRAZIL

BLISKA, Flavia M. M.\*, BLISKA JUNIOR, Antonio.\*\*, SILVEIRA, Jane M. C.\*\*\*, TURCO, Patrícia H. N.\*\*\*\* BLISKA, Adriano A.\*\*\*\*\*.

\* Agronomic Institute, Campinas, Brazil, \*\* Faculty of Agricultural Engineering/Unicamp, Campinas, Brazil, \*\*\*APTA Regional, Mococa, Brazil; \*\*\*\*APTA Department of Strategic Management, Campinas, Brazil; \*\*\*\*\* Institute of Economics/Unicamp, Campinas, Brazil.

### RATIONALE

To meet the increasing global demand for food and energy in a sustainable way, agricultural production will need to be linked to efficient production systems for water, energy and nutrient use. The use of these inputs below ideal levels can result in less productivity than potential and favor the incidence of pests and diseases, resulting in lower grain quality. Despite advances in the use of those inputs, the degradation of natural resources continues to grow. Due to the importance of the rational and precise use of water, fertilizers and agricultural pesticides for the preservation of the environment, aiming at the sustainability of the coffee segment, increasing its competitiveness and the rural development, this study evaluates the management level of inputs in the agricultural production of coffee, in the main Brazilian coffee regions.

### METHODS

This study consists of the evaluation of management indicators of water, fertilizer and pesticide use in coffee growing, based on the Identification Method of Management Degree - MIGG Café (BLISKA JÚNIOR *et al.*, 2015), through the evaluation of 1136 coffee farms from 2014 to 2017.

### RESULTS

The survey identified that 89% of coffee rural companies regularly use chemical analyzes in the nutritional control of their crops and apply the recommendations of laboratories or specialized professionals. However, only 37% of them use precision equipment fields to measure the electrical conductivity, pH or soil moisture. In coffee companies with some kind of agricultural certification, the inputs management is significantly higher than in non-certified companies, as well as higher than the respective Brazilian averages. The average values of adoption of the management indicators are directly proportional to the sizes of the farms, so the lowest level of use of nutrition and irrigation control equipment in coffee plantations occurs among smallholders. Among the companies producing Arabica coffee, the adoption of those equipment is superior to that identified in the Robusta/Conilon coffee.

### CONCLUSIONS & PERSPECTIVES

Nutrition and irrigation control in Brazilian coffee farms is very low. It is important to make entrepreneurs aware of the importance of using precision equipment in coffee production systems. The management of the use of chemical nutrients and the benefits that can be obtained through the implantation of intelligent systems of irrigation, both in the productivity of the crops as in the quality of the grains and the coffee beverage, still have great space for growth.

### REFERENCE

BLISKA JÚNIOR, A. *et al.* Validating the management degree identification method in the coffee production using focus group. *Revista de Economia Agrícola*, São Paulo, v. 62, n. 1, p. 41-54, 2015.



## F1 HIBRIDOS RESPONSE TO FERTILIZER APLICACION IN THE CENTREAL VALLEY OF COSTA RICA

CHAVES, Víctor\*; DELGADO, Carlos\*,  
\*Instituto del Café de Costa Rica (ICAPE).

### RATIONALE:

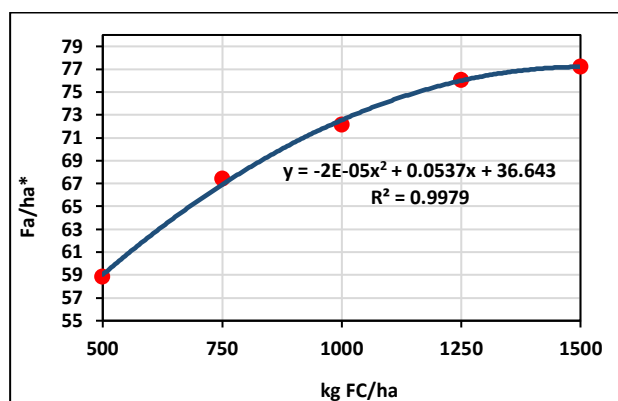
Considering the high productivity of the F1 hybrids reproduced by somatic embryogenesis, it is of special interest to evaluate their fertilization requirements.

### METHODS:

The trial was established at the experimental station ICAPE (CICAFE) in Barva, Heredia at 1180 masl, in a soil Andisol. F1 hybrids (T-5296 x Rume Sudan) were planted in 2008, in full sunlight exposure, one plant per hole and at a distance of 2,10 x 1,10 m. The treatments were implemented since 2010, and evaluation consisting of 5 levels of fertilization, corresponding to 500, 750, 1000, 1250 and 1500 kg/ha of Complete Formula 18-5-15-6-0,2 (N, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O, MgO, B), respectively supplemented with extra Nitrogen of 45, 68, 90, 113 and 135 kg/N ha of Nitrogen, based on ammonium Nitrate. Complete Formula partitioned into two applications (May, July), while extra Nitrogen was applied in October. Treatments were placed under a randomized complete block design, with 6 replicates.

### RESULTS:

In the average of 8 harvests, a quadratic response is presented with the maximum production at the highest evaluated dose (1500 FC + 135 N); however, the economic optimum is around 1250 kg FC + 113 kg N, from which the increase in production is moderate and which would be supplying annually the equivalent per hectare of 338 kg N, 63 kg P<sub>2</sub>O<sub>5</sub>, 188 kg K<sub>2</sub>O, 75 kg MgO and 2.5 kg B.



**Figure 1. Productive response curve. Average 8 harvest.**

\*Fa: 258 kg cherry fruit

### CONCLUSIONS & PERSPECTIVES:

Under an adequate fertilization program F1 hybrids have shown high productivity during 8 years of harvests.



## EXPLORING THE INTEGRATED SOIL FERTILITY MANAGEMENT APPROACH OF SAFERNAC MODEL WITH SELECTED ORGANIC ADDITIVES IN A TANZANIAN ECOSYSTEM

**MARO, GodSteven P.,\* JANSSEN, Bert H.,\*\* MREMA, Jerome P.\*\*\* MSANYA, Balthazar M.,\*\*\* MBWAMBO, Suzana G.\***

\*Tanzania Coffee Research Institute P. O. Box 3004 Moshi, Tanzania. \*\* Plant Production Systems, Wageningen University, the Netherlands. \*\*\*Sokoine University of Agriculture, Morogoro, Tanzania

Corresponding author: Email [godsteven.maro@tacri.org](mailto:godsteven.maro@tacri.org)

### **Abstract**

The new model SAFERNAC can follow either a baseline approach (SOIL and PLANT alone) or an integrated soil fertility management (ISFM) approach (SOIL, PLANT and INPUT together). The aim of this study was to explore the latter approach in a Tanzanian coffee ecosystem. Cattle manure, selected coffee ecosystem residues and green manures were mixed with two soil types – Haplic Nitisol and Cutanic Acrisol, at 5% organic to soil ratio; moistened to field capacity and incubated in 10 litre plastic containers at room temperature. Duplicate soil samples were taken at intervals up to 180 days and analyzed for  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3\text{-N}$ , available P and exchangeable K. The cumulative  $\text{N}_{\text{min}}$ , P and K values were used to test the model under different nutrient management options (1, 5 and 10 tons organics per ha alone on one hand, and supplemented with 160 kg N, 60 kg P and 160 kg K on the other). SAFERNAC demonstrated that the Acrisols are less fertile, and therefore more responsive, than Nitisols. The model demonstrated the behavior of different organics when applied to the soil alone and in combination with mineral fertilizers, coming up with suggestions to prospective organic and conventional farmers. The green manure plants (*Albizia*, *Mucuna*, *Lupine*, *Canavalia* and *Crotalaria*) performed significantly better than the rest of the test organics (manure, leaves, pulp and husks) and were picked as the best bets for coffee ISFM in Tanzania.

*Keywords:* Coffee ecosystem, ISFM, Organic additives, SAFERNAC, Tanzania



## SOIL FERTILITY EVALUATION FOR THE POTENTIAL COFFEE AREAS OF EASTERN TANZANIA – MOROGORO AND MVOMERO DISTRICTS

**Godsteven Maro\***, Suzana Mbwambo, Harrison Monyo and Epafra Mosi  
*Tanzania Coffee Research Institute, P.O. Box 3004, Moshi, TANZANIA*

\*Corresponding author, Email [godsteven.maro@tacri.org](mailto:godsteven.maro@tacri.org)

### **Abstract**

Despite its history as the area where Arabica coffee first landed in Tanzania, the Eastern Coffee Zone is still regarded as a minor coffee producer. In an effort to provide soil related information that will contribute towards transforming this area into a major coffee area, soil fertility evaluation for Arabica and Robusta coffee was done in two districts of Morogoro and Mvomero, which involved soil survey in 18 and 21 villages respectively. Field characteristics were recorded and soil samples collected from depths of 0-30 cm, 30-60 cm and 60-90 cm. A total of 75 and 107 soil samples from respective districts were analyzed for soil texture, pH-water, organic carbon, total nitrogen, available phosphorus, cation exchange capacity, exchangeable bases and extractable micronutrients (Cu, Fe, Mn and Zn). Several approaches were attempted including qualitative soil fertility assessment against the requirements of the two coffee species, quantitative evaluation of soil fertility in terms of the supply potential of N, P and K in kg nutrient equivalent (kE) per ha, spatial and multivariate statistical analysis. Most of the soils (> 70%) are moderately fertile, thus needing moderate ISFM efforts to produce coffee economically. A north-south soil fertility trend was noted, with the northernmost wards of Kanga and Maskati (Mvomero) having less than 200 kE ha<sup>-1</sup> of available NPK. At the other end, the southernmost wards of Bwakila, Mvoha and Mtombozi (Morogoro) excelled the list with over 500 kE ha<sup>-1</sup>. The principal component analysis showed pH, OC, available P, Ca/Mg, Fe, Cu and Zn accounting for 30.33% of the total variability, and CEC, base saturation and exchangeable sodium percentage accounting for 19.16%. Six ward clusters with related fertility parameters were identified, with clusters best expressed in terms of CEC, followed by pH, OC and available P. Soil fertility limitations are low pH, low nutrient cation level (particularly Ca and K), low OC, low N and very low micronutrient levels. Priority intervention strategies in the study districts were suggested, including liming, manuring, composting, green manuring, recycling of crop residues, application of Minjingu Rock Phosphate and supplementation of micronutrients.

**Keywords:** *Soil fertility, Potential coffee areas, Eastern Tanzania.*



## Behaviour of phosphorus in relation to its absorption, translocation and use efficiency in four varieties of *Coffea canephora*

HAMADI, Almas\*, Nyambilila, Amuri\*\*.

\* Tanzania Coffee Research Institute Box 3004 Moshi, \*\*Sokoine University of Agriculture, Department of Soil and Geological Sciences, Box 3008, Morogoro.

Corresponding author: Email [almasi.hamad@tacri.org](mailto:almasi.hamad@tacri.org)

### ABSTRACT

In recent years, the consequences of the depletion of P resources and P-fixation in acidic soils have aroused scientists' interest to explore further on its behaviour (absorption, translocation and use by the plants) in different varieties of Robusta coffee so as to manage its use efficiency. This study aimed at characterizing the four varieties of *Coffea canephora* regarding their capacities of phosphorus absorption, translocation and usage, an important concept to the investigation of varieties with potential to adapt to limited soil P. A screen house pot experiment was conducted in a 3x4 factorial Completely Randomized Design with three rates of P as TSP fertilizer. The pots were filled by soil medium with analyzed pH of 4.55 and extractable P of 35.42 mg/kg. Factors were three rates of P (0, 37.5, 75g/pot) and four varieties of *Coffea canephora* (Bukoba 1, Maruku 10, Muleba 2, Maruku 1). Phosphorus efficiency indices (absorption efficiency, translocation efficiency and use efficiency) were calculated on Excel spreadsheet and the data were analyzed by using Genstat software 14<sup>th</sup> edition. Pairwise means separation of the efficiency indices was done by using Fisher's protected method and the results revealed the differential behaviour of Phosphorus in terms of translocation efficiency and use efficiency ( $p < 0.05$ ) in different varieties at different rates. Under low P (0 g/pot) two varieties (Maruku 1 and Bukoba 1) appeared to respond well. But for the absorption efficiency no statistical significance was observed and the probable reason could be low pH of the soil medium (4.55). This study has therefore proved that varieties differ on their efficiencies for P depending on the distribution and availability of P within the soil. Farmers growing coffee in areas with low P are advised to opt for varieties such as Maruku 1 and Bukoba 1 which could utilize the limited resources found in the area with optimum yield.

**Key words:** *Phosphorus behaviour, Varieties, Coffea canephora*



## EFFECTS OF SEEDLING MULTIPLICATION METHODS ON GROWTH AND YIELDS OF NEW TALL AND COMPACT *Coffea arabica* VARIETIES IN TANZANIA

**MBWAMBO, Suzana G., MARO, GodstevanP., NKYA, Emmanuel O.**  
Tanzania Coffee Research Institute P. O. Box 3004 Moshi, Tanzania.

Corresponding author: Email [suzana.mbwambo@tacri.org](mailto:suzana.mbwambo@tacri.org)

### ABSTRACT

Tanzania coffee research institute (TaCRI) has developed and released 19 hybrid Arabica varieties with high yields, good cup quality and resistance to coffee berry disease and leaf rust. Seedling multiplication methods include clonal propagation, grafting, hybrid seeds and tissue culture. The aim of this study was to compare field performance among tall and compact *Coffea arabica* seedlings raised through the four methods. Two side-by-side experiments, one using second generation tall variety and the other one using compact variety were established at Lyamungu in September, 2014. A randomized complete block design with three replications was used to compare the four methods. Data collected included vigour, number of bearing primaries, bearing nodes per primary, plant height, berry clusters, root: shoot ratios and yields. The collected data were subjected to ANOVA using COSTAT software. Then the means were separated using Tukey's HSD method at 0.05 significance level. Plant characteristics were affected by different methods at varying levels of significance. For the first year, the ranking for bearing primaries, internodes, canopy width and stem girth was cuttings > seeds > grafts > tissue culture. The first two maintained prominence in the second year, though seeds were higher in canopy width, internodes and stem girth. Root-shoot ratios were not significantly ( $p > 0.05$ ) affected in both years. Only varietal difference was observed in the second year, with compact variety having higher ratios. Yield was significantly ( $p < 0.01$ ) affected by methods and the ranking was seeds > cuttings > grafts > tissue culture. From the two year data collected so far, it is tentatively recommended that the presence of tap-root (seeds) or lack thereof (cuttings) has little effect if any on growth and yield of Arabica coffee.

*Keywords:* Seedling multiplication, techniques, *Coffea arabica*, Tanzania





## RESPONSE FUNCTIONS OF COMPACT *Coffea arabica* VARIETIES TO N, P AND K NUTRIENTS AT HIGH PLANTING DENSITY IN TANZANIA

**MBWAMBO, Suzana G.\*, MARO, Godsteven P., NKYA, Emmanuel O.**  
Tanzania Coffee Research Institute P. O. Box 3004 Moshi, Tanzania.

\* Corresponding author: Email [suzana.mbwambo@tacri.org](mailto:suzana.mbwambo@tacri.org)

### ABSTRACT

In the past years the Tanzanian coffee farmers used to grow traditional tall coffee varieties which are susceptible to coffee berry disease (CBD) and coffee leaf rust (CLR). As an effort to overcome these two major diseases, TaCRI has developed tall and compact *Coffea arabica* with durable resistance and better yields than the traditional varieties. The availability of those varieties allows closer spacing which entails also better uptake of available soil nutrients due to denser rooting. The aim of this study was to assess the response of compact *Coffea arabica* to Nitrogen, Phosphorus and Potassium nutrients (NPK) at high planting density. The experiment was established in December, 2013 at Lyamungu, following a randomized complete block design with split-split plots and three replications. Three spaces were treated as the main factor (2\*1.5m, 2\*1.25m and 2\*1m), four coffee breeding lines as sub factor (CVT2, CVT1, PNI 088\*F23/7/Tree 60 and PNI 088\*F23/8/Tree152) and 3 fertilizer rates as sub-sub factor (75g, 150g and 225g of NPK 20:10:10), each rate applied three times per year. Data collected included stem girth, number of bearing primaries, bearing nodes per primary, plant height, berry clusters and yields. The accruing data were processed on Excel Spreadsheet and later exposed to ANOVA using STATISTICA V7 software. The means were separated using Tukey's HSD method at 0.05 significance level. CVT2, CVT1 and PNI 088\*F23/7/Tree60 lines resulted into significantly ( $p < 0.05$ ) higher canopy width and strong stem girth than PNI 088\*F23/8/Tree152. There was no significant difference ( $p > 0.05$ ) between the lines in terms of number of berry cluster, bearing nodes per primary and number of bearing primaries. 2m\*1.5m and 2m\*1.25m spaces resulted into significantly ( $p < 0.05$ ) higher number of bearing nodes per primary, number of bearing primaries and stem girth as compared to 2m\*1m space. CVT1 and CVT2 resulted into higher yield (1190 and 1300kg clean coffee ha<sup>-1</sup> respectively) which was significantly different ( $p < 0.05$ ) from PNI 088\*F23/7/Tree152 (1000kg clean coffee ha<sup>-1</sup>). Significantly ( $p < 0.05$ ) higher yield (1272kg clean coffee ha<sup>-1</sup>) was also obtained at the space of 2m\*1.25m as compared to the space of 2m\*1m (1067kg clean coffee ha<sup>-1</sup>). Different fertilizer rates used (75g, 150g and 225g) had no significant effect on the tested parameters. It is tentatively recommended that 75g of NPK (20:10:10) if applied three times per year at the space of 2m\*1.5m or 2m\*1.25m is enough for CVT1 and CVT2 lines under the age of four years.

*keywords:* Planting density, NPK nutrients, *Coffea arabica*, Tanzania



## Effects of cutting position along mother plants on rooting of hybrid coffee varieties

Jeremiah M. Magesa<sup>1</sup>, Theodosy J. Msogoya<sup>2</sup> & Cornel L. Rweyemamu<sup>2</sup>

<sup>1</sup>Tanzania Coffee Research Institute, P.O Box 3004, Moshi, Tanzania

<sup>2</sup>Department of crop Science and Horticulture, P.O Box 3005, Chuo Kikuu Morogoro, Tanzania

\*Corresponding author: E-mail: [jeremiah.magesa@tacri.org](mailto:jeremiah.magesa@tacri.org)

### Abstract

The study was conducted on-station at Tanzania Coffee Research Institute (TaCRI) from December 2013 to March 2014 to evaluate the effect of stem cuttings position along the mother plants on rooting of hybrid coffee varieties. Stem cuttings of coffee varieties were assessed in a rooting medium of forest soil and sand at a ratio of 2:1 by volume under semi-controlled environment. A split-plot experiment in a randomized complete block design (RCBD) with four replications was used. The main factor was five improved hybrid Arabica coffee varieties (N39-1, N39-2, N39-4, KP423-1 and KP423-2) and the sub-factor consisted of four types of positions (stem cuttings collected from the base, middle, apex and conventional treatment used was the mixture of the above cuttings applied as the control). Four months after planting, stem cuttings were evaluated for root growth characteristics. Data collected were subjected to analysis of variance (ANOVA) using CoStat software version 6.311 and treatment means were separated based on Tukey's test at ( $P \leq 0.05$ ). Results obtained indicated that the positions of stem cuttings along the mother plant had a significant effect ( $P = 0.04$ ) on rooting of coffee varieties whereas rooting was highly significant ( $P = 0.00$ ) affected by varieties. Further, interaction between varieties and position of stem cuttings significantly ( $P = 0.04$ ) affected the rooting percentage and number of lateral roots at ( $P = 0.01$ ). This study also indicated that clonal multiplication of coffee stems cuttings differed with varieties and position along the mother plant with stem cuttings taken from basal and middle positions having the highest rooting percentage. It is recommended that, stem cuttings from basal and middle position of mother plants be selected for massive production of varieties N39-1, KP423-1 and KP423-2.

**Key words:** Basal cuttings, Clonal propagation, Multiplication, Stem cuttings, Vegetative



## RESPONSE OF DIFFERENT APPLICATION RATE OF DOLOMITIC LIME ON COFFEE SEEDLING BRANCHES INCREASE IN ACID SOILS OF MBOZI DISTRICT, TANZANIA.

PANGALAS, Dismas\*, HAMAD Almas, MAGESA, Jeremiah

Tanzania Coffee Research Institute (TaCRI), P.O Box 3004, Moshi, Tanzania.

\* Corresponding author: Email [dismas.pangalas@tacri.org](mailto:dismas.pangalas@tacri.org)

### Abstract

Soil acidity is among the major constraints to coffee productivity in Mbozi District, Tanzania, limiting nutrients availability to coffee plants. An experiment was conducted in the 2017/18 season to assess the response of different dosages of dolomitic lime as a soil amendment. We used a completely randomized block design with lime treatment at four levels (0 gm, 400 gm, 800 gm and 1000 gm) per plot of 4 trees, replicated three times. These lime rates were based on a standard rate of 2.6 ton ha<sup>-1</sup> for raising pH from 4.85 to 6.5, estimated by Adam and Evans buffer solution method. NPK fertilizer was applied as control. Eighteen months after planting TaCRI 1F seedlings were evaluated for vegetative growth, namely number of primary branches, length of primaries branches and plant height. The collected data were subjected to analysis of variance (ANOVA) using GenStat 15<sup>th</sup> Edition and treatment means were separated by Duncan Multiple Range Test at 0.05 level of significance. There was significant difference in number of branches between coffee seedlings that received 400gms or more of lime treatments and the control. The highest number of branches were recorded in seedlings treated with 400 and 800 gms of lime. As there was no significant difference between the two application rates, farmers in the acid soils of Mbozi are advised to use 400 gms per 4 trees (= 133 kg ha<sup>-1</sup> at standard plant density of 1330 trees per ha) to enhance fast vegetative growth, hence early crop establishment which could influence yield.

**Key words:** Acid soil, coffee branches, dolomitic lime



PAPPO, Emily E., FLORY, S. Luke.  
University of Florida, Gainesville, FL, USA

## RATIONALE

Arabica coffee (*Coffea arabica*) production is highly sensitive to changes in the quantity and timing of precipitation, thus shifts in precipitation patterns that are predicted under future climate change may become a major challenge for producers. Selection of cultivars for water stress tolerance could be critical for producers to maintain high-quality production under changing environmental conditions. Our research uses experimental rainout shelters to evaluate variation in water stress tolerance among *C. arabica* cultivars in Costa Rica.

## METHODS

To help inform planting decisions for producers faced with changing precipitation patterns, we evaluated responses to water stress among five *C. arabica* cultivars (H1, H10, Catuai, Catuai 44, and Villa Sarchi) in Tarrazú, Costa Rica. We experimentally reduced precipitation using replicated rainout shelters and accounted for potential shelter effects by constructing control structures over paired plots (8 replicates). We then measured soil moisture, humidity, and temperature in rainout and control treated plots over time, and evaluated *C. arabica* seedling relative growth rate of height and stem diameter as an indication of plant performance.

## RESULTS

The rainout treatment resulted in 9% lower soil moisture during the wet season and almost 19% less soil moisture during the dry season, but there were no measurable differences in light, temperature, or humidity. There was unusually high rainfall during the wet season such that seedlings under the rainout treatment exhibited significantly greater growth rates than those in the control plots. However, the magnitude of the response varied by cultivar. The F1 hybrids (H1 and H10) showed high performance under both treatment and control conditions while Villa Sarchi displayed a pronounced increase in growth rate under rainout, suggesting low tolerance to the high soil moisture conditions during the wet season.

## CONCLUSIONS & PERSPECTIVES

Understanding differences in performance among cultivars under extreme climate conditions such as unusually high precipitation may be critical for maintaining coffee production under climate change. Our results suggest that H1 and H10 are relatively more resilient than the more commonly planted Catuai, Catuai 44, and Villa Sarchi cultivars when exposed to the stress of high soil moisture. Despite the relatively high cost of hybrid seedlings, their faster growth rate and tolerance of variable environmental conditions may make these cultivars worth the investment.



## KNOWLEDGE REPRESENTATION OF THE SPECIALTY COFFEE AGRIBUSINESS SYSTEM AND ITS IMPACTS ON THE IMPROVEMENT OF THE FINAL PRODUCT QUALITY

TRAUER, Eduardo\*, VALDATI, Aline de B.\*\*\*, TODESCO, José L.\*\*\*, COSTA, Eduardo M.\*\*  
\*State University of Santa Catarina (Esag/Udesc) and Federal University of Santa Catarina –  
Engineering and Knowledge Management Program (EGC/UFSC) \*\* (EGC/UFSC), Florianópolis,  
SC, Brazil.

**RATIONALE:** As one of the most consumed beverages in the world, surpassing 3 billion cups daily, coffee is of huge importance in the world economy (Illy, 2016). Coffee is an organic product with a complex Agribusiness System (AS) and involves knowledge-intensive processes beginning with genetics and seed firms followed by agricultural input suppliers, producers, merchandisers, processors, retailers and consumers. Many interests and much knowledge are involved in this AS but not all of them are explicit. This gap of knowledge available may impair the quality of coffee as a finished product. Added to this are the different interests of research in the areas of Agriculture, Medicine and Business (AMB) that involve studies with coffee. In a recent systematic review of the key words used in scientific publications that address the topic of coffee, the non-transdisciplinarity amongst the areas of AMB was evident. As a way to address the lack of transdisciplinarity, we propose the representation of knowledge through a Coffee Ontology.

**METHODS:** First we conducted a literature review at the scientific base Scopus in 2018 on the themes *agribusiness system*, *specialty coffee* and *knowledge representation through ontology*. The next step was a systematic search in order to find the main interests of the areas AMB related to Coffee. The field research collected the primary data through semi-structured interviews with professional experts in different areas of the Specialty Coffee Agribusiness System. The last step was organisation of the knowledge to develop a proposal for a Specialty Coffee Ontology.

**RESULTS:** "The roles of the individual compounds on the physiologic effects of coffee are not well-characterized", emphasises Hatzold (2012, p.10). The ontologies allow the reuse and transmission of omit knowledge and their implementation allows us to create shared information spaces that support a decision based on the integration of the organisational knowledge and the individuals who are part of a production network (Kudryavtsev et al. 2017). Knowledge representation through the Coffee Ontology allowed us to clearly identify the importance of the explanation of transdisciplinary knowledge to improve the quality of coffee as a finished product with high added value. The quality can positively impact on better market positions highlighting its positive influences on human health.

**CONCLUSIONS & PERSPECTIVES:** The main contribution of this work is to highlight the need for a relationship between the areas of Agriculture, Medicine and Business in their coffee research and professional decisions and could provide a common vocabulary about the main properties and influencers during the Specialty Coffee Agribusiness System from seed to cup.

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SNOECK, Didier\*, LAURENT, Jean-Baptiste\*\*, DURINI, Nicolas\*, DROMARD, Marine\*\*\*

\* Cirad, UR Systèmes de Pérennes ; \*\* Cirad, UR AIDA ; \*\*\* Cirad, Dgdrs-valo – Avenue Agropolis – 34394, Montpellier (France)

## RATIONALE

"Fertexpert" is a set of tools that calculates the right dose of the right fertilizer formula for each coffee plantation. Unlike current methods that require taking a soil sample per surveyed plot to determine the fertilizer formula, our system makes use of the achievements of coffee nutrition research. Therefore, our tool is particularly suitable for companies / NGOs that support small coffee planters and have to provide adapted fertilizer recommendations to a large number of planters (for up to 20,000) at an easily affordable cost.

## METHODS

The model combines fertilizer formulas obtained from thematic maps (based on soil and climate maps) with easily measurable field descriptors using IOT, making it quick and inexpensive. The tool set uses survey forms (built with Open Data Kit) and a GIS (QGis). The geolocalized data are then processed using a spatial query followed by statistical queries to select the available commercial fertilizer formulas and doses that are best adapted to the surveyed coffee plantations. It has been tested in Côte d'Ivoire and Burundi. The results were compared to those given by the soil diagnosis method using a PCA statistical analysis to assess the relevance of the descriptors.

## RESULTS

The tool set was developed on a CIRAD web portal. The online system only uses open source tools with a free license. The fertilizer recommendations per coffee plot are displayed on both a table and a digital map. The comparison of recommendations given by the Fertexpert tool and the traditional recommendations from soil diagnosis showed non significant differences.

## CONCLUSIONS & PERSPECTIVES

Fertexpert allows providing each coffee farmer with a fertilizer formula and dose that corresponds to the real context of their plots (right formula at the right place). A fully usable version of the tool on tablet and smartphone (in offline mode) is also under study.

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RIGAL, Clément<sup>1,2</sup>, VAAST Philippe<sup>3,4</sup>, XU, Jianchu<sup>1,2</sup>

<sup>1</sup> Kunming Institute of Botany, Chinese Academy of Sciences, China. <sup>2</sup> World Agroforestry Centre (ICRAF), Kunming, China. <sup>3</sup> World Agroforestry Centre (ICRAF), Hanoi, Vietnam. <sup>4</sup> CIRAD, UMR Eco&Sols, Université Montpellier, France

## RATIONALE

The rapid expansion of intensive monoculture in coffee farms, from the 1980's onward, resulted in coffee becoming an important commodity crop in Yunnan Province, China. This expansion was followed by a large-scale transition towards shaded coffee, starting in 2013 with the free distribution of shade tree seedlings by local governments. The current study initiates an early on follow up of this unprecedented large scale conversion by focusing on impacts of shade tree species on soil fertility at a temporally and spatially fine scale.

## METHODS

124 soil samples were collected in 2017-2018, both in the dry (winter) and rainy (summer) seasons, within and outside of coffee rows, below and outside of the canopy of 3 commonly found shade tree species (*Bischofia javanica*, *Cinnamomum camphora* and *Jacaranda mimosifolia*). Analysis were run for chemical composition (pH, OM, N, P, K, Ca, Mg), biological communities (nematodes abundance, PLFA) and enzymatic activities ( $\beta$ -glucosidase, N-acetylglucosaminidase and acid phosphatase).

## RESULTS

- 1) There was a **marked seasonal effect** on soil communities (higher nematodes, bacterial and fungi communities during the rainy season). P cycling also increased during the rainy season, but C and N cycling slowed down.
- 2) **Soils below coffee plants** had higher chemical fertility (OM, Ca and Mg) than paired samples outside of coffee rows. During the rainy season, soils below coffee also had more abundant soil communities (nematode, bacteria and arbuscular mycorrhiza fungi) and nutrient cycling rates (C, N and P).
- 3) **Shade trees had a positive impact** on soil chemical fertility below coffee plants (pH, OM, N, P and Ca) and a buffering effect on soil communities during the dry season (bacteria and fungi).

## CONCLUSIONS & PERSPECTIVES

This study sets up the baseline (open coffee) and first follow up after the transition to agroforestry (5 years) on soil fertility. Results testify of the high heterogeneity of soil fertility within coffee farms, at a fine temporal and spatial scale. Importantly, shade trees were shown to have a locally positive impact on soil chemical and biological fertility after as little as 5 years, although no significant differences could be found between the 3 shade tree species. Future studies should keep monitoring changes as shade trees grow older.

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## **Quantitative Ochratoxin Detection in Green Coffee in 10 Minutes Without Using An Organic Solvent Extraction**

DAVENPORT, John\*,

\*VICAM, A Waters Business, Milford, MA, USA

Vicam's Ochra-V AQUA lateral flow test kit is designed to provide quantitative results of ochratoxin contamination in green coffee samples without the need for organic solvents such as methanol or acetonitrile. This allows for a fast, safe and cost effective way of screening green coffee samples for further production.

Ochra-V AQUA has a limit of detection in green coffee of 2ppb and a detection range of up to 30ppb. The linearity of Ochra-V AQUA across the detection range is 0.998 (R-squared) on spiked green coffee samples. Ochra-V AQUA has also been shown to correlate well with HPLC testing on naturally contaminated green coffee samples.

It should be noted that uncertainty introduced between extractions increases the difficulty in comparing the two methods directly. In a study using multiple naturally contaminated green coffee samples the extract to extract variation of Ochra-V AQUA can be shown to be similar to the extract to extract variation achieved with HPLC testing. Due to this variation between extractions it can be difficult to determine a true contamination level making comparisons of a single sample difficult. When multiple extracts of the same sample are tested using HPLC and Ochra-V AQUA the average ochratoxin contamination level detected is comparable between both testing methods.

The take away from this study is that Ochra-V AQUA is able to quantitatively determine ochratoxin contamination levels in green coffee and can be used as an effective test for screening green coffee samples.



## **Impact of agro-forestry systems on coffee yield, coffee plant morphology, physical and chemical attributes of green coffee beans and aroma generation of roasted coffee beans**

Su Xu\*, Elias de Melo Virginio Filho\*\*, Robert Linforth\* and Ian Fisk.\*

\*Food Science, Biosciences, University of Nottingham, LE12 5RD, UK; \*\*Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), 7170 Turrialba, Costa Rica.

A long experiment has been established since 2000 at CATIE (Tropical Agricultural Research and Higher Education Center), Turrialba, Costa Rica. Twenty agro-forestry systems with different shade types and managements (organic and non-organic) consisting of an incomplete randomized block-design with shade tree as main effect and subplots represented by management were set up. The mean 13-year yield, bienniality index (BI) of coffee yield, pruning, shade cover and morphology (height, diameter, total branches and productive branches) of coffee plants in these agro-forestry systems were measured. The effects of different managements and shade types on the physical and chemical attributes of green coffee beans and aroma and colour generation of roasted coffee beans were investigated. The organic management always showed the more stable production, while the coffee yield and morphology were always worse under the intensive organic (IO) management than under the intensive conventional (IC) management. Comparing with the moderate conventional (MC) management, the intensive organic (IO) management did not only have a similar or even higher productivity, but it also had higher total lipid and protein in the green beans and a stronger ability to generate more flavour and colour. The full sun system had a higher total coffee yield and bienniality index (BI) of coffee yield, green bean density and total protein content and greater flavour generation and colour after roasting. Comparing with the timber system, the service system did not only have the higher coffee yield and better coffee plant morphology, but it also produced green beans with higher total protein and roasted beans with the more flavour and colour. Comparing with the non-legume shade tree, the legume shade tree only influenced the total protein content of green coffee beans and further improved the performance of flavour and colour in the roasted coffee beans.

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## IMPACT OF STORAGE IN MODIFIED ATMOSPHERES OF GREEN COFFEE BEANS (*Coffea arabica* L.) ON THE QUALITY MARKERS

TRUJILLO-CARRETERO, Carolina\*, GONZÁLEZ-RIOS, Oscar\*, and SUÁREZ-QUIROZ, Mirna L.\*

\*Tecnológico Nacional de México/Instituto Tecnológico de Veracruz, Unidad de Investigación y Desarrollo en Alimentos, MÉXICO

**RATIONALE:** The storage plays an important role in the quality evolution during the handling of the green coffee beans (GCB). A previous study demonstrated that GCB stored in jute sacks after 6 months of storage lead to fungal population dynamics, decreased chromaticity in GCB by bleaching and, then, affected overall quality. The objective of this work was to evaluate the physicochemical, microbiological and sensory characteristics during the storage of coffee in modified atmospheres (vacuum and nitrogen) and determine its effect on quality markers.

**METHODS:** Lots of 3 kg of green coffee (*C. arabica*) were stored in modified atmospheres: vacuum and N<sub>2</sub> at two temperatures, 18 ° C and room temperature. A monthly sampling was carried out during 6 months to evaluate chromaticity (colour), water activity, moisture content, granulometric analysis, physical defects, viability in green coffee, fungal infection and ochratoxin A production. Finally, the drink was sensorially evaluated through a panel trained in discriminatory (2AFC) and descriptive techniques.

**RESULTS:** The characteristic green colour of the beans was measured with the chromaticity and this decreased from 11.8 to 11.0 and L\* increased of 62.9 to 63.3 in the GCB stored in ambient local conditions. The physico-chemical parameters such as moisture content, Aw, granulometric analysis and physical defects, do not show significant changes in the conditions of stored studied. The viability of the GCB decreased from 43 to 16% and the per cent of fungal infection increased of 17 to 34%, not observing significant difference between treatments. The trained panel did not detect sensory differences of the drink in the discriminatory tests

**CONCLUSIONS & PERSPECTIVES:** The use of modified atmospheres (N<sub>2</sub> and Vacuum) allowed to conserve the physical-chemical, microbiological and sensorial quality of the GCB during 6 months, compared with conventional methods such as the use of jute and polypropylene sacks.

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## SENSORY AND BIOCHEMICAL FINGERPRINTING OF COMMERCIAL COFFEE VARIETIES UNDER DIFFERENT GEOGRAPHICAL CONDITIONS IN KENYA

KATHURIMA, Cecilia\*, GICHURU, Elijah\*, ALWORA, Getrude,\* NJOROGE, Ezekiel\*

\*Kenya Agricultural & Livestock Research Organization, Research Institute,  
PO BOX 4 (00232), Ruiru-Kenya

Kenya coffee is known for its quality and appreciated in the world market. The varieties K7, SL34, and SL28 are the recommended traditional coffee varieties in Kenya (Mwangi, 1983) while cultivars Ruiru 11 and Batian are recommended as improved varieties to alleviate the problem of coffee berry disease (CBD), coffee leaf rust (CLR). The objective of this study was to evaluate the sensory and biochemical profiles of Batian, Ruiru 11 and SL 28 coffee varieties grown under different geographical conditions at Nyeri, Embu and Bungoma Counties in Kenya. Ripe coffee berries were harvested during the peak period in 2012/2013 coffee year and wet processed using standard recommended procedures. The sensory attributes fragrance/aroma, flavor, aftertaste, acidity, body, balance and overall were assessed by a panel of seven trained judges and scored on a scale of 10 points. The attributes clean cup, uniformity and sweetness were added to the scores of fragrance, flavour, aftertaste, acidity, body, balance and overall to give the total score which is an indicator of the sensory performance. Caffeine, trigonelline, total chlorogenic acids and oil were determined using specific methodologies and quantified on dry weight basis. The sensory profiles presented in this study showed that Batian, Ruiru 11 and SL 28 coffee varieties have the potential of attaining specialty grade. The levels of caffeine, trigonelline, oil and total chlorogenic acids were within those reported for arabica coffee and did not show any significant differences ( $P > 0.05$ ) among the varieties analysed. The environment was significant on the sensory and biochemical profiles. In order to reap maximum benefits, farmers must plant the recommended coffee varieties and adopt best agricultural practices (BAPS).



## EFFECT OF VARIETY, SHADE AND ALTITUDE ON SENSORY COMPONENTS OF COFFEE: MURANGA COUNTY, KENYA

Kathurima C.W.\*, GO Alwora\*, EK Gichuru\*, Njoroge EK\*, Boulanger R.\*\*

\*Kenya Agricultural & Livestock Research Organization, Research Institute,  
PO BOX 4 (00232), Ruiru-Kenya

\*\*Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD),  
TA 80/16, 34398 Montpellier Cedex 5, France

In Kenya coffee quality is determined by the grade proportions and beverage quality which are influenced by various factors including variety, shade and altitude. The objective was to characterize the quality of coffee produced by farmers producing traditional varieties and Ruiru 11 under shade or under full sun in Muranga County. One hundred and five farmers from 13 farmers' Cooperative societies were sampled from an altitude of 1300 to 1900MASL. The type of shade was characterized and it was found that in some cases trees along the hedge were observed to cast shade on coffee. In other farms, big trees in the coffee fields provided shade to the coffee below. Cherry was harvested and wet processed. Green coffee was roasted to medium roast, weighed out as whole beans (five cups of each sample). A panel of five judges evaluated the sensory attributes of the samples. Ten sensory attributes assessed for each coffee on a ten point scale: Fragrance/Aroma, flavour, aftertaste, acidity, body, balance, uniformity, clean cup, sweetness and overall perception.

Generally, traditional varieties had significantly better fragrance, flavour, acidity and overall perception as rated by the panel. Altitude and shade did not seem to have any significant effect on the sensory characteristics. In terms of defects, coffee berry borer attack was significantly observed irrespective of the variety or whether coffee was under some shade or in full sun. The results are discussed in relation to possible impact of variety, altitude, and shade on coffee quality observing that shade/agro-forestry is one of the upcoming changes of coffee production systems to mitigate against climate change.



KITZBERGER, Cintia S.G.\* , GOMES-SANTIS, Valéria B.\* , MORAIS, Heverly\* , ROSISCA Juliandra R.\* , SCHOLZ, Maria Brígida S\* , SANTORO, Patrícia H.\*

\* Instituto Agronômico do Paraná, Londrina, IAPAR, BR.

## RATIONALE

In order to obtain high specialty coffees, various cultivation methods are under study. Among these systems, shade-grown coffee has provided a good environment for the sustainable production of specialty coffees. This system meets the producer demands, mainly the use of native Brazil trees. The shade cultivation reduces temperature stress and slows down coffee maturation modifying flavor and aroma precursors contents. The objective of this work is to evaluate the chemical composition of unroasted coffee, produced in agroforestry systems and in full sun, for chlorogenic acids, sucrose, lipids, proteins and caffeine.

## METHODS

The experiment was conducted at the Agronomic Institute of Paraná, Londrina, Paraná, Southern Brazil. The evaluated cultivar was IPR 98 developed by this institute. The treatments were shaded trees with the native *Trema micranta* (stm) and *Heliocarpus popayanensis* (shp) trees and coffee trees conducted in full sun. Coffee cherry fruits were harvested from May to July at three consecutive harvests 2015, 2016 and 2017. The chlorogenic acid isomers (GCA) evaluated were: caffeoylquinic (3-CQA, 5-CQA and 4-CQA), feruloylquinic (FQA) and dicaffeoylquinic (3,5, 3,4-diCQA and 4,5-diCQA). These acids were measured by HPLC-RP [1]. Sucrose, lipids, proteins and caffeine were also analyzed by means of near-infrared spectroscopy (NIRS) and using prediction models [2]. Principal component analysis (PCA) was used to analyze the data by XLSTAT statistical software program.

## RESULTS

The harvests of 2015 and 2017 presented lower production due to the biennial cycle and also differentiated from 2016 for presenting higher levels of lipids and lower levels of caffeine, sucrose, 3-CQA and 4-CQA. It was verified that in the harvest of 2015 the coffees were different from the other harvests due to the higher levels of 5-CQA. In 2015 harvest was observed a lower rainfall index and lower radiation level in March indicating an unfavorable macroclimate. In this harvest, the CQA/DCQA ratio, indicating the maturation level, was similar between treatments: 3.96 (full sun), 3.86 (shp) and 3.76 (stm). The stm and shp systems presented higher protein content, 5-FQA, 3,4-DCQA and 3,5-DCQA in 2016 and 2017, the latter two compounds are associated with high-quality beverage. A differentiation between the shaded microclimates in 2016 and 2017 was observed. The 2016 harvest had a higher caffeine, sucrose, 3-CQA and 4-CQA contents, and this year was marked by the higher rainfall index in May, the lower average temperature in May and June and the constant radiation index from March to June. The CQA/DCQA ratio was higher for this harvest presenting values of 3.55 in the full sun, 4.49 in shp and 4.05 in stm. High values of this ratio together with high levels of 3-CQA and 4-CQA indicate mature beans. In contrast, 2017 presented high levels of 5-FQA, correlated with lower quality of the beverage and the lower ratio of CQA/DCQA observed in all systems evaluated, with values of 3.13 full sun, 2.61 shp and 2.65 stm.

## CONCLUSIONS & PERSPECTIVES

The microclimate generated by the agroforestry systems favors the composition of coffees with high-quality beverage, however the annual macroclimatic conditions and the biennial production of the coffee also influence and must be considered.

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## INFLUENCE OF THE LEVEL OF SHADING ON THE PROFILE OF CHLOROGENIC ACID ISOMERS AND PHYSICOCHEMICAL COMPOSITION OF COFFEE CONSORTIUM WITH *HAVEA BRASILIENSIS*

GOMES-SANTIS, Valéria B.\*, ROSISCA Juliandra R.\*, SCHOLZ, Maria Brígida S.\*, KITZBERGER, Cintia S.G.\*, MORAIS, Heverly\*.

\* Instituto Agrônômico do Paraná, Londrina, IAPAR, BR.

### RATIONALE

Coffee agroforestry systems play an important economic and environmental role, allowing diversification of incomes for producers and greater preservation of natural resources. Several studies demonstrate the effect of shading on the sensory quality beverage, it is believed that the late coffee bean maturation increase the accumulations of aroma and flavor precursor compounds. Besides the sensory quality is correlated to physicochemical composition of coffee beans that is affected by these factors. The present study evaluates the effect of different levels of shading on coffees consorted with trees of *Havea brasiliensis*.

### METHODS

The experiment was conducted at the experimental station of the Agronomic Institute of Paraná - Southern Brazil. Arabica coffee IAPAR 59 was cultivated consortium with trees of *Havea brasiliensis* in five levels of shading: full sun (sun), and three distances of double lines of *H. brasiliensis* spaced between them (13 meters (13s), 16 meters (16s) and 22 meters (22s)) and full shade (shade). Which received 0%, 45%, 23%, 9% and 85% of shading, respectively. The harvest period occurred according to the visual beans maturation and was simultaneous in all the treatments, from May to July at 2016. Proteins, caffeine, sucrose, lipids and reducing sugar was performed by near infrared spectroscopy (NIRS) using developed prediction models [2]. The chlorogenics acids isomers (ACG): caffeoylquinic (3-CQA, 5-CQA, and 4-CQA), feruloylquinic (5-FQA), and dicaffeoylquinic (3,5; 3,4-diCQA and 4,5-diCQA) acids and citric, malic and quinic acids were determined by HPLC-RP [1,2]. Principal component analysis (PCA) was used to analyze the data by XLSTAT statistical software program.

### RESULTS

The coffee grown in full shade and 13s, which received higher shading indices, were characterized by high concentrations of 5-CQA, 5-FQA, 3,5 diCQA, sucrose, caffeine and quinic acid. Previous studies have indicated that the 5-CQA, 5-FQA, 3,5 diCQA isomers accumulate in immature grains and are associated with lower quality beverages. Thus, such treatments showed a slower maturation and, therefore, their harvests should occur later than treatments with less shading. The higher content of caffeine, sucrose and quinic acid in these treatments also indicated incomplete maturation. The coffees in full sun, 22s and 16s showed high levels of 3-CQA, 4-CQA, 3,4 di-CQA and reducing sugar. High concentrations of 3-CQA and 4-CQA isomers indicate complete maturation of coffee beans and high 3,4-diCQA values are correlated with higher-quality beverage and are also indicative of maturation [3]. The coffee trees cultivated at full sun, 22s, 13s and full shade showed higher levels of malic and citric acids. However, 13s and full shade had high concentrations of malic acid, indicating incomplete maturation, in contrast to 22s and full sun, which presented higher content of citric acid, that accumulates in mature beans. The 16s treatment was characterized by the high concentration of 4,5-diCQA.

### CONCLUSIONS & PERSPECTIVES

Cafés grown in systems with high levels of shading have a composition profile indicative of immature beans, although presenting visual maturity of the beans, therefore, an ideal harvesting point should be established according to shade level.

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NAKENDO, Saleh\*, \*\*, MUSOLI, Pascal, P\*\*, LUMU, Moses\*\*, NALUKENGE, Ann\*\*, ALUKA, Pauline\*\*, SEBUGENYI, Isreal\*\*, BAKOMEZA, Fidel\*\*\*, RWEIHANGWE, Doreen\*\*\*, KANANURA, Edmund\*\*\*, KANGIRE, Africano\*\* and WAGOIRE, William\*\*

\*Africa Coffee Bureau (ACB), 13803 Castle Boulevard, STE 33, Silver Spring, MD 20904, USA,

\*\*National Coffee Research Institute (NaCORI), P.O Box 185, Mukono, Uganda, \*\*\*Uganda Coffee Development Authority (UCDA), P.O Box 7084, Kampala, Uganda

**RATIONALE:** Quality drives demand and pricing at the international coffee trade, and consumers continue to demand for it (Nakendo *et. al.*, 2013). A study was done in Uganda to evaluate different processing methods on bean physical qualities and premised on reports elsewhere, that mode of processing plays a big role in the quality of the resultant green bean (Knopp *et. al.*, 2006).

**METHODS:** Fresh cherry composite sample was portioned into 3 sub-samples subjected to three processing methods thus, natural, pulped natural and fully washed and replicated thrice. Sample dried to 12% moisture and stored for 6 months, hulled and green bean physical qualities thus, insect borer damage and moisture content after 6 months storage determined. Bean borer damage was determined in 100 bean count by gravimetry and moisture content determined using a calibrated moisture meter. Statistics was done using Minitab software version 16.2.

**RESULTS:** A negative correlation between insect damage and moisture drop in the bean was noted ( $r^2 = -0.99$ , p-Value = 0.002). Bean moisture was statistically significant (p-value = 0.001) in the treatments. Insect insurgence in the bean was statistically significantly variant in the treatments (p-Value = 0.043). 62% insect damaged scored in naturals compared to 38% and 31% in pulped naturals and fully washed cherries respectively. % moisture was 10.6, 11.3 and 11.5 in naturals, pulped naturals and fully washed respectively.

**CONCLUSIONS & PERSPECTIVES:** 62% insect damage in naturals (unpulped cherries) was noted; implying, 62% loss in US\$ value of green bean resulting from unpulped cherries.

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OIL BODIES AND OLEOSINS IN *Coffea arabica* L. SEEDS:  
EXPERIMENTAL COMPARISON OF TWO METHODS FOR THEIR  
EXTRACTION AND PURIFICATION

MARIN, Veronica<sup>1,2</sup>, DEL TERRA, Lorenzo<sup>1,2</sup>, CRISAFULLI, Paola<sup>1</sup>, NAVARINI, Luciano<sup>1</sup>  
<sup>1</sup>illycaffè S.p.A, via Flavia 110 – 34147 Trieste, Italy; <sup>2</sup> Biolab, illycaffè S.p.A. AREA Science Park,  
Padriciano 99 – 34149 Trieste, Italy.

**RATIONALE:** Oil bodies are spherical organelles involved in seed lipid storage, whose main function is to supply energy to the embryo during germination. They are constituted by lipid droplets surrounded by a phospholipid monolayer and coated with proteins named oleosins. Oleosins have been well-described in oil-rich seeds, but less information is available in *Coffea arabica* seeds, classified as semi-oleic and characterized by a hard endosperm reserve tissue. The lipid fraction in Arabica varies between 12 and 16% w/w depending on botanical variety and geographical origin, and it is well characterized from a chemical point of view, but much less from a plant physiology perspective. This is surprising, given the importance of lipids in contributing to the physical (foam, aroma carrier), chemical (aroma, antioxidant) and physiological (health effects) properties of the coffee beverage. To shed light on this subject, two methods have been adapted to coffee in order to extract and purify oil bodies and oleosins.

**METHODS:** Oil bodies from *C. arabica* seeds of various geographical origins were extracted according to two methods published by Tzen et. al (1990, 1997). Both protocols are based on successive centrifugation steps of the coffee seed homogenate in sucrose density gradients, in order to separate intact oil bodies from the rest of the lipid fraction. Once pure oil bodies are obtained, the embedded oleosins can be isolated by diethyl ether extraction. The second protocol differs from the first in several aspects, supplementing the buffers with 10mM NaHPO<sub>4</sub> (pH 7.5) and employing detergents (0,1% v/v-Triton), denaturing agents (9M urea) and hexane in order to better purify oil bodies from membranes and other lipid/protein debris. For both extractions, oil bodies integrity was observed by an optical microscope, using differential interference-contrast technique. Finally, SDS-PAGE electrophoresis was used to evaluate the purified protein fraction.

**RESULTS:** Better results were obtained using the 1997 protocol in terms of oil body purification and oleosin extraction. Unlike the results of the first method, the alternative protocol substantially removed proteins non-specifically associated with oil bodies, lipid debris and broken oil bodies. After extracting and analyzing multiple samples of Arabica seeds from several origins, major protein bands were displayed between 15 and 20 kDa, matching the predicted oleosin molecular weight.

**CONCLUSIONS & PERSPECTIVES:** Oleosins are the main class of proteins in the oil body membrane and provide a high surface-to-volume ratio that facilitates lipase access during germination. The reported findings confirm that the oleosin protein family is always abundant in coffee seeds. A better understanding of the biology of oil body genesis and degradation in *Coffea arabica* will be of great help in elucidating the role of lipids in green coffee quality.

**REFERENCES** (key words): Tzen *et al.* (1990), Plant Physiol. 94, 1282-1289; Tzen *et al.* (1997), J Biochem.121(4):762-8. (*Coffea arabica*, lipids, oleosins, oil bodies)



STUDY OF STRAINS OF THE GENUS *Aspergillus* SECTION *Nigri*  
PRODUCERS OF OCHRATOXIN A (OTA) ASSOCIATED WITH THE  
COFFEE PRODUCTION (*Coffea arabica*).

NIÑO-RODRIGUEZ, Jorge, SUÁREZ-QUIROZ, Mirna L. and GONZÁLEZ-RIOS Oscar

Tecnológico Nacional de México/Instituto Tecnológico de Veracruz/UNIDA, México

**RATIONALE:** Ochratoxin A (OTA) is the main mycotoxin detected in coffee beans and it is produced by species belonging to the genus *Aspergillus* section *Circumdati*, however, recently in a study it was shown that during storage of green coffee beans, the dominant fungal species belonged to the section *Nigri*. The aim of this work was to isolate and identify species of the section *Nigri* present during the different post-harvest coffee processing and evaluate its diversity by PCR-DGGE.

**METHODS:** Forty two coffee samples (*Coffea arabica*) were obtained from four different treatments: Wet processing (dry and submerged fermentation); mechanical and dry processing. The percentage of fungal infection of moulds belonging to the section *Nigri* was obtained, the strains isolated were molecularly characterized and their ochratoxigenic potential was evaluated. DNA was extracted from samples, purified and PCR-amplified. The PCR products were analysed with PCR-DGGE and statistical analysis of fungal species similarity were obtained for each treatment.

**RESULTS:** Thirty-three strains were isolated and identified (31, *A. niger*; 2, *A. carbonarius* and 1, *A. tubingensis*), 25 strains were OTA producers, of which 4 (2 *A. niger* and 2 *A. carbonarius*) were highly OTA producing (110.8-330.4 ng / g). The process stages with a high incidence of toxigenic strains were the drying and storage from the wet process (dry fermentation). The DGGE profiles showed a low incidence of toxigenic strains of section *Nigri* only in the storage stage. This was possibly due to the limit of detection (Durand et al, 2012) that establishes that the species must be at levels above  $10^4$  spores / mL in order to be detected. The statistical analysis of species similarity showed the influence of the different post-harvest coffee processing on the development of fungal species during each stage.

**CONCLUSIONS & PERSPECTIVES:** The species of the section *Nigri* constitute the main source of OTA production in coffee in Mexico. Therefore, subsequent studies of fungal diversity associated with the production of OTA in coffee should not diminish the importance of the species belonging to the section *Nigri*.

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## Soil carbon dynamics in coffee-avocado intercropping in Coastal California

Fissore, C., Ryals, R., Alcala, S.

The state of California is the top U.S. crop producer (\$30,366,898,000 in crops sold in 2012). The state has long provided a significant proportion of the fruits and nuts consumed in the U.S., and alone accounts for 86% of the national avocado production. Yet, agriculture is extremely competitive in Southern California, and farms are challenged to constantly innovate with alternative crops and cropping systems to remain economically viable. In the last decade, a small number of coastal avocado growers have experimented with coffee-avocado intercropping within their organic production. The intercropping of coffee and avocados can represent a valuable economic solution by securing two high-value crop harvests, without exacerbating the competition for land and resources. However, it remains unknown whether soil health, water use, and plant and root competition for nutrients will be affected by the introduction of coffee in well-established avocado productions. Our work is the first to investigate whether this novel type of intercropping alters functional groups composition in diagnostic soil organic carbon (SOC) fractions and whether there is potential for intercropping to increase SOC sequestration. We collected soil samples from two separate, adjacent management types in Carpinteria, CA: a well-established monocropping avocado and coffee-intercropped avocado trees, in which coffee had been planted four years prior sampling. Both management types were certified organic. We conducted density fractionation to identify diagnostic fractions of SOC, namely a free fraction (fLF), an aggregate-occluded fraction (oLF), and a mineral-associated fraction (HF). We performed total C%, N% and Fourier Infrared Spectroscopy (FTIR) on each fraction to investigate whether intercropping alters SOC functional groups composition and SOC distribution in soil. Our preliminary findings indicate that, four years after the introduction of coffee in avocado production, total SOC content (as  $\text{Mg C ha}^{-1}$ ) does not change significantly and most SOC accumulates in the top 3 cm of soil. Notably, coffee-avocado intercropping leads to greater distribution of more labile forms of C along the soil profile, likely a consequence of greater root presence than in avocado monocropping. Spectroscopy indicates that there is greater accumulation of less decomposed material at depth in intercropping. This may relate to the significantly lower C:N ratio in fLF at 13-25 cm observed for intercropping, which suggests the incorporation of more labile, N-rich material in this fraction. Albeit not significant, intercropping resulted in reduced accumulation of C in the oLF, which may be explained by potential initial soil disturbance at the time of planting and this effect should be further investigated. Our preliminary findings, and those that will follow as our work progresses, are particularly important in the context of the substantial economic and environmental role played by the agricultural sector in California, and the emphasis given by the CA Healthy Soils Initiative to efforts aim at improving soil C sequestration in agricultural soils.



## IMPACTS OF CLIMATE CHANGE IN THE CLIMATE QUALITY INDEX IN ARABIC COFFEE: A CASE STUDY IN THE STATE OF SÃO PAULO, BRAZIL

ALFONSI, Eduardo\*, COLTRI, Priscila\*, PINTO, Hilton\*, MEDEIROS, Yasmin\*, DI BLASIO, Giovanni\*, ALFONSI, Waldenilza\*

\*Center of Meteorological and Climate Researches Applied to Agriculture (CEPAGRI), University of Campinas (UNICAMP), Campinas, São Paulo, Brazil.

### RATIONALE

In the last decades, coffee production has been challenged, which can be intensified in future climate change scenarios. Climatic variables such as temperature and rainfall directly affect the development and productivity, as well as the quality of coffee beverage. Mapping areas with climate potential to produce high quality of coffee beverage is an important requisite to planning coffee cultivation. This is possible by adopting the “Quality Climate Index - QCI” (Camparotto 2012). In this context the aim of this reserach is to analyze and understand the potential impact of future climate change scenario on coffee quality beverage in the state of São Paulo, Brazil.

### METHODS

The study was conducted in São Paulo state, southeast of Brazil, wich is one of the most important coffee region. To analyze the impact of the projections of climate change on coffee arabica, we used the regional climate model Eta-HadGEM2-ES (Chou et al. 2014), with 20km of spatial resolution. We choose the high emission greenhouse gases (GHG) scenario, the Representative Concentration Pathways (RCP) 8.5 W/m<sup>2</sup>, based on the 5th report (AR5) of IPCC, from 2011 to 2040 model data. The study was carried out for the Mundo Novo coffee cultivar with flowering date on September 15th and the flowering-maturity period was estimated using degrees days -GD method (Nunes et al., 2010). To determine the area with potential of coffee quality was used the QCI determined by Camparotto, 2012.

### RESULTS

Analyzing the temperature of the future scenario of Eta-HadGEM2-ES, the annual average temperature, in the study area, could increase in average, 2°C, depending on the region of the state. As a consequence, QCI values might present drastic changes, reducing coffee regions with potential for high quality beverage production in the State of São Paulo. The decrease of the index is related to temperature, and in future climate change scenarios, the temperature increase results in reduction of the flowering-maturation period and consequently the fermentation phases of the pulp occurs faster, being able to reach phases harmful to the quality of coffee.

### CONCLUSIONS & PERSPECTIVES

The increase in temperatures projected by the high emission greenhouse gases (GHG) scenarios of Eta-HadGEM2-ES indicated that areas climatic potential to produce natural coffee beverage qualities in the state of São Paulo will be negatively impacted, resulting in a reduction of high quality production areas.

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## POTENTIAL OF SEVERITY TO COFFEE RUST DISEASE OF COFFEE CROP IN BRAZIL, IN THE SCENARIO OF HIGH CO<sub>2</sub> EMISSIONS

ALFONSI, Waldenilza\*, COLTRI, Priscila\*, ZULLO, Jurandir\*, SHINJI, Kaio\*, PINTO, Hilton\*, MEDEIROS, Yasmin\*, GONÇALVES, Renata\*, KOGA-VICENTE, Andrea\*, ALFONSI, Eduardo\*  
\*Center of Meteorological and Climate Researches Applied to Agriculture (CEPAGRI), University of Campinas (UNICAMP), Campinas, São Paulo, Brazil. walmvital@gmail.com.

### **RATIONALE**

The coffee rust disease, caused by the fungus *Hemileia vastatrix* Berk. & Br, stands out on the world stage because of its devastating power. Losses in production can reach out 50% of total production (Zambolim, 2016). The relationship between the pathogen and the environment is described in the classical phytopathology triangle and, therefore, in future scenarios of climate change, the severity potential and intensity of such disease can be modified, causing new challenges to the coffee sector. In this context, this study has the intent to evaluate the severity potential of coffee rust disease in arabica coffee, in areas climatically suitable for cultivation, in a future scenario.

### **METHODS**

The temperature data of the climatic model Eta-MIROC5 (2011-2040), with 20 km of spatial resolution (Chou et al., 2014), were used in scenarios of high emission of greenhouse gases (GHG), the Representative Concentration Pathways (RCP) 8.5 W/m<sup>2</sup>, based on the 5th report (AR5) of IPCC, to elaborate the distribution and severity of coffee rust disease. The severity potential was estimated using the equation of the incubation period (IP) proposed by (Moraes et al., 1976) in the areas climatically suitable for planting in the states of São Paulo and Minas Gerais (Southeastern of Brazil), bounded by agricultural zoning of climate risks (Alfonsi, 2017). The severity potential was classified according to the method proposed by Alfonsi (2017).

### **RESULTS**

By analyzing the relationship of the pathogen, through the incubation period (IP), with the environment, it was observed that the severity potential of coffee rust disease is increased in a future scenario, since the IP decreased in the states of São Paulo and Minas Gerais. However, when the climatic risk zoning is considered in the analysis, it was observed that coffee rust disease will reduce in the future scenario, since some regions are considered to be of high climatic risk for coffee cultivation. This reduction of the area suitable for coffee planting reduces the area affected by the disease, due to the relation of the disease triangle (pathogen, host and environment).

### **CONCLUSIONS & PERSPECTIVES**

Although coffee rust disease is climatically favorable in future scenarios, it will be limited by the reduction of the area suitable for planting the coffee crop.

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## PROFILE OF WOMEN IN THE COFFEE PRODUCTION CHAIN IN THE MUNICIPALITY OF BOM SUCESSO, MINAS GERAIS, BRAZIL

ZENITH, Luiza A.\* , BALIZA, Danielle P.\* , SILVA, Ana P. M.\* , PEREIRA, Sérgio P.\*\*

\*Instituto Federal do Sudeste de Minas Gerais – Campus Avançado Bom Sucesso, Minas Gerais, Brazil, \*\*Instituto Agrônômico de Campinas, São Paulo, Brazil.

### RATIONALE

The agroindustrial system of coffee has shown an expressive growth and with it the participation of women in different segments of the chain. Studies on gender relations within coffee farming are scarce and these imply the work developed by entities involved in the struggle for gender equity in Brazilian coffee. Coffee cultivation is important in the municipality studied, making it necessary to study the profile of the same for an increase in their visibility and appreciation.

### METHODS

A structured questionnaire was applied to 28 women who work in key sectors of the coffee production chain, among them coffee-growing (non cooperative and cooperative members to the Joint Cooperative of Rural Bom Sucesso Producers (COOPERBOM)), rural workers and employees of COOPERBOM then being selected seven women in each sector. The questionnaire was composed of questions common to all interviewees, including personal data, work in the coffee production chain, work / family relationship, women in coffee (professional achievement, visibility, challenges, difficulties, among other issues). After the questionnaires were applied, the results were tabulated and analyzed statistically.

### RESULTS

Among the results obtained, the racial question was startled, with most of the interviewees self-declared as brown, with the exception of coffee growers associated with the cooperative, in which the majority declared themselves as white (71%). Another relevant finding is related to the age at which the younger women are employees of the cooperative, while the coffee growers were those with the highest percentage of older women (26 - 35 and 36-59, respectively). It was reported that among the segments of the coffee production chain, rural women workers have the lowest level of education and the lowest monthly income, which is the most vulnerable among the respondents.

### CONCLUSIONS & PERSPECTIVES

It is important to increase the visibility and awareness of the importance of the work carried out by these women for the success and sustainable development of coffee production in the municipality of Bom Sucesso, Minas Gerais, Brazil.

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## SUSTAINABILITY LABELS: A COMPETITIVE STRATEGY IN BRAZILIAN COFFEE PRODUCTION

BLISKA, Adriano A.\*; AGGIO, Gustavo O.\*; SILVEIRA, Jose Maria F. J.\*; BLISKA JUNIOR, Antonio\*\*, BLISKA, Flavia M. M.\*\*\*

\* Institute of Economics/University of Campinas, \*\*Faculty of Agricultural Engineering/University of Campinas, \*\*\* Agronomic Institute, Campinas, Brazil.

### RATIONALE

The labels used in the coffee segment convey to consumers an image of reliability and assurance of product attributes. They meet the growing consumption of "sustainable products" and add value to coffee. "Sustainability labels" attest that the production process uses technologies that result in positive and significant impacts on the health of workers, entrepreneurs and consumers, and which minimize waste, losses and unnecessary costs, contributing to sustainable sectoral development. Its benefits include greater market access for companies, rational use of production factors, and increased environmental protection. These benefits are related to the management of coffee companies and contribute to local and regional sustainability. The objective of this study is to verify if the adoption of labels is associated with companies with more efficient management systems and/or is associated with coffee regions where specific competitive strategies predominate in order to characterize clusters.

### METHODS

The Method Identification of Management Degree - MIGG Coffee (BLISKA JÚNIOR et al, 2015) was used to evaluate the levels of management of 1122 coffee companies. The method consists of a questionnaire that evaluates 64 management indicators, grouped in eight criteria, which classifies the management in levels from "one" (lowest) to "nine" (highest). The technique of Multiple Correspondence Analysis - MCA (DI FRANCO, 2016) was used in cluster analysis.

### RESULTS

The levels of management in Brazilian rural coffee firms are significantly higher in those that adopt some kind of label or certification. Multiple labels result in even better management levels. In general, the entrepreneurs consider only the premiums per bag produced, they do not take into account the rationalization of the production and processing, the reduction of costs, the increase of productivity and in the quality of the grains, which are not perceived as advantages of certification. As the goals and targets of the labels are incorporated, they come to recognize their contribution to the competitiveness of their companies. In some regions there is a predominance of homogeneous competitive strategies, which characterize clusters, such as the West of Bahia and Alta Mogiana Paulista, which have already obtained or are searching for the origin indication label. Those regions are characterized mainly by the specialization of the productive process, intensive in technology, resulting in high productivity and quality.

### CONCLUSIONS & PERSPECTIVES

In the Brazilian coffee production, the sustainability labels express clusters with effective competitive strategies, compatible with the concept of strategic management.

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## METHODS

MEASUREMENT OF NO<sub>x</sub>, CO<sub>2</sub> AND N<sub>2</sub>O EMISSIONS IN DIFFERENT COFFEE PULP TREATMENT SYSTEMS, BY COMPOSTING AND GASIFICATION PROCESS.

## RESULTS

Composting movement process is a practice with a positive effect on the generation of GHG, since the aeration of the system favors anaerobic decomposition, which encourages the generation of carbon dioxide. Not so in systems where movement is not applied, where an anaerobic environment generates more methane production.

- The comparison in terms of GHG generated by both technologies (composting vrs gasification) showed decreases in generation for the gasification system, in the Methane and Nitrous Oxide pollutants, not so in the case of Carbon Dioxide.
- With respect to the emission factor, generated for the composting system in the selected Mills, nitrous oxide is in a range of 0.0-0.2 g N<sub>2</sub>O / kg of pulp. While the methane is between 0.0 and 6 g CH<sub>4</sub> / kg of pulp.

## CONCLUSIONS & PERSPECTIVES

When you do movement composting process not only helps to improve the mixing process of the compost and favors the decomposition, it also generates greater emissions product of the fragmentation that occurs in the material, allowing a greater diffusion of the gases from inside the mound of compost towards the atmosphere. According to the data obtained, the gasification systems show a reduction in the emissions of greenhouse gases if you compare composting process with and without movement and is a alternative to the coffee pulp treatment and low gas emissions in the sector.

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COLTRI, Priscila P. \*, GONÇALVES, Renata R. do Valle\*, PINTO, Hilton, S. \*, SHINJI, Kaio\*  
\*University of Campinas, BRA, Center of Meteorological and Climatic Research Applied to Agriculture.

## RATIONALE

Quantifying the dynamics of land cover change in coffee growing areas is necessary to understanding the Brazilian coffee sector, one of the most important in the world. Investigating how coffee plantations are distributed and how they are changing in the last decades is essential to decision making, policy interventions, coffee economy, ecological systems, climate change and integrative approaches. In this context, this research aimed to quantify and analyse the dynamic of changes in land cover coffee growing areas in the South-central of Brazil.

## METHODS

We used 25 year of Brazilian official data of coffee planted area (Brazilian Institute of Geography and Statistics or IBGE), from 1990 to 2015. The study was carried out in the South-central of the country, including the main producers states -Paraná, São Paulo and Minas Gerais- and the expansion area: Mato Grosso, Goiás, Bahia, Espírito Santo and Rio de Janeiro. We divided the official data into four analyses group: zero hectares (abandoned area), 1 to 49 ha (smallholders farmers), 50 to 499 ha (medium producers) and 500 to 120000 ha (large producers). We calculated the percentage of the total growth rate and the annual growth rate. This analyses was realized individually (to each state) and to the total area. Coffee land cover maps were created indicating the different behaviours of the studied region.

## RESULTS

The results demonstrated that, in these 25 years studied, 32% of the coffee area in the study region was abandoned, with a annual growth rate reduction of 2%. The main producer region, that includes Minas Gerais, São Paulo and Paraná state, presented positive growth rate to abandoned area, while the other states, presented decreases in this class, being considered expansion area. However, the abandonment of the coffee areas were greater than the expansion. Additionally, we also verified that medium and large producers, decreased, with a rate of 1.6% and 3.4% per year, respectively. In the total period, medium producers decreased 20.22%, and large producers, decreased 39% of the total area. On the other hand, smallholders increased 3.7% in the period, with a annual growth rate of 0.25%. The only exception was Minas Gerais state that decreased smallholders farmers by 7.11% per year. The overall results suggest that medium and large coffee properties are decreasing or disintegrating in smallholders.

## CONCLUSIONS & PERSPECTIVES

The coffee area in the South-central region of Brazil was significantly reduced in the period from 1990 to 2015. Medium and large properties reduce significantly as the abandoned coffee areas increased in this period. At the same time, smallholders farms increased. These analyses suggests that the national coffee cultivation has experienced substantial changes in the last two decades and the principal perspectives are to understand the main drivers responsible for these changes.



## COFFEE TREES INTERCROPPING WITH TREES SPECIES OF ECONOMIC INTEREST IN BRAZIL: REPRODUCTIVE DEVELOPMENT AND DRINK QUALITY.

CUNHA, Rodrigo L.\*, VENTURIN, Regis P\*, CARVALHO, Vicente L.\*, FREITAS, Ana F.\*\*,  
ALACÂNTARA, Elifas N.\*

\*Minas Gerais Agricultural Research Institution, Lavras, Minas Gerais, Brazil, FAFEMIG's scholarship holders, \*\*Federal University of Lavras, Lavras, Minas Gerais, Brazil, Capes scholarship holders.

### RATIONALE

The arborization with species for multiple uses in the coffee farm becomes an interesting option as it can minimize climate changes, being able to influence the ripening of the fruits and still represent an option of profit to the producer. The objective of the work was to evaluate the stages of the reproductive development of coffee under the influence of different tree species compared to full sunshine coffees.

### METHODS

The trial was installed in the region of Santo Antonio do Amparo. The plots consisted of shaded coffee trees on every three rows of coffee trees by tree species such as cedar (*Acrocarpus fraxinifolium* ARN.), mahogany (*Khaya ivorensis* A. CHEV.), acacia (*Acacia mangium* WILLD.), avocado (*Persea americana* MILL.), teak (*Tectona grandis* LF) and macadamia (*Macadamia integrifolia* M. and B.). The coffee tree was planted in the 3.4m by 0.65m spacing and the tree species in 9m by 13m spacing. Reproductive development stage coffee evaluations were carried out, according to Pezzopane et al., (2003) proposals: development, expansion and maturation of the fruits from July 2017 to May 2018 were made and also the drinking quality and physical test.

### RESULTS

In 2017, there were prolonged drought (from July to September), in this situation coffee trees in full sun shine and shaded environment, remained in with dormant buds, and after the first rains were they developed the blossoming stages, small green, fruit expansion, green fruit, green-yellow, cherry, dried cherry and dried fruits. In May 2018, near the coffee harvesting, a greater cherry fruit percentages were observed in consortiums with Cedar, Acacia, Avocado and Macadamia compared to Teak and Mahogany and the check treatment, due to the greater height and tree canopy.

### CONCLUSIONS & PERSPECTIVES

Coffee trees in shaded environment by Cedar, Acacia, Avocado and Macadamia presented greater cherry fruits percentage. Shading, provided by tree species did not affect the coffee drinking quality and beans size.

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## MITIGATING CLIMATE CHANGE EFFECTS IN AN AGROFORESTRY SYSTEM OF COFFEE AND JANGADA TREE (*Heliocarpus popayensis*) IN SOUTHERN BRAZIL

MORAIS, Heverly\*, SOUZA, Marcos A.\*, SANTORO, Patricia H.\*

\*Agronomic Institute of Parana, Londrina, BR

### RATIONALE

Global climate changes could affect coffee production in Brazil (Assad et al., 2004). Coffee cultivated in agroforestry system is a promising alternative to reduce the impacts of climate changes, especially high temperatures (Moreira et al., 2018). The potential for an agroforestry system with a native species, jangada tree (*Heliocarpus popayensis*) was examined in order to mitigate impacts on coffee by reducing maximal air temperature and global radiation. The goal of this work was to compare radiation and temperatures in coffee agroforestry system with jangada tree and in unshaded coffee, during the Summer in Southern Brazil.

### METHODS

The treatments were coffee agroforestry with jangada tree and unshaded coffee. Coffee was planted in April 2012 using a 2.5 x 0.6 m spacing and the jangada trees spacing were 2.5 x 6.0 m between plants at the coffee row. One weather station was installed in each treatment to collect solar radiation and air temperature. The data were collected during a hot Summer, from December 21st 2017 to March 19th 2018 in Londrina, Parana State, Southern Brazil.

### RESULTS

The measurements of solar radiation indicated that the mean shading in coffee plants under jangada trees, during the Summer, was 56%, which may be considered as a moderate shadowing. In terms of temperature, the unshaded system of coffee showed higher daily maximum, medium and minimum air temperature compared to the shaded system. The mean of maximum air temperature (32.0 °C) was recorded in the unshaded coffee plants, surpassing the mean maximum temperature observed in the shaded coffee (28.8 °C). On March 12, when the highest air temperature was recorded, the unshaded coffee had air temperature maximum of 37.3 °C, while in the shaded treatment the maximum reached 34.5 °C. The highest difference in maximum air temperature between treatments was 4.9 °C on February 24, with values of 35.5 °C and 30.6 °C in shaded and non-shaded coffee trees, respectively. Variation among treatments in minimum air temperature was minor. The shaded area minimum temperatures were 0.9 to 1.1 °C lower than the unshaded coffee plants. Mean air temperature during the studied period was 23.9 °C in the unshaded treatment and 23.0 °C in the shaded one.

### CONCLUSIONS & PERSPECTIVES

Jangada trees modified the microclimate of the coffee crop in the agroforestry system, providing reduction of the maximum, mean and minimum air temperatures and the intensity of radiation. The agroforestry system of coffee intercropped with jangada trees may be a mitigation strategy against future climatic variability and impacts related to high temperatures.

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## The Benefits of Public Private Partnership financing model to coffee cooperative societies in Kenya

MUGO, Harrison\*, LUBABALI, Hudson\*, SALASYA, Beatrice\*\*

\*Kenya Agricultural and Livestock Research Organization – Coffee Research Institute (KALRO-CRI), Ruiru, KENYA, \*\* Kenya Agricultural and Livestock Research Organization , Nairobi, KENYA.

### *Abstract*

Coffee production in Kenya plays a key role as source of foreign exchange earnings to the Country. It's produced by both small and large - scale farmers in 32 coffee growing Counties. In Kenya, the production of coffee is constrained by a number of factors. Among these, shortage of coffee planting materials of improved coffee varieties (Ruiru 11 and Batian) released to farmers by Kenya Agricultural and Livestock Research Organization - Coffee Research Institute (KALRO-CRI) is a major challenge. Both the Ruiru 11 and Batian varieties are resistant to Coffee berry disease (CBD) and Coffee leaf rust (CLR) that are major diseases of coffee in Kenya with an estimated crop loss at 80% if not managed. The shortage of planting materials was addressed by developing Coffee Productivity Project (CPP) that aimed to expand coffee nursery capacities of Cooperative Societies to produce seedlings of Ruiru 11 and Batian for the farmers through Public Private Partnership (PPP). The study was conducted in eight (8) coffee Farmers Cooperative Societies (FCS) under the CPP from eight (8) coffee growing Counties. The data was collected using questionnaires that were uploaded in Open Data Collection Kit (ODK). Of the societies surveyed, 100% indicated that their staff were capacity build with 100% of them stating that the trainings were useful in their respective Cooperatives. The coffee nurseries in the Cooperatives were expanded. The annual mean coffee seedlings produced per society increased by 366% from 15,000 to 70,000 seedlings. The sales of seedlings created sources of revenue generation to Societies. Annually, there was an increase in revenue generation by 400% from an average of Kshs 200,000 to 1,000,000 per society. The average membership per society increased by 15% from 2444 to 2807 members due to availability and accessibility of seedlings. The mean acreage under coffee increased by 18.9% from 435.2 to 517.3 acres per society. On average, the coffee cherry delivered per society increased by 25% from 228 to 286 metric tons with average cherry payment per kg increasing by 100% from Kshs 36.74 to 73.64. The CPP created employment of temporary workers in the Cooperatives. On average, nine (9) employees per society were engaged to provide labour to the coffee nurseries constructed for seedlings production. Evidently, this Public Private Partnership model under coffee enterprise, positively impacted on coffee business in the Cooperatives. A model that can be applied on other enterprises to improve the livelihoods of the society.





## PROFILE AND AGRICULTURAL PRACTICES OF COFFEE GROWERS A FAIRTRADE POOL IN BRAZIL

PEREIRA, S. P \* , OLIVEIRA, E \*\* , AGUIAR, CM G \*\* \* , BALIZA, DP \*\*\* , OLIVEIRA, EF \* \* , AOUN, LA \* \*

\* Agronomic Institute (IAC) \*\* Coffee Research Consortium, \*\* \* Federal University of Lavras (UFLA) \*\*\* \* Federal Institute of Southeast Minas Gerais (Southeast IF MG). [Brazil].

### RATIONALE

The consumers concern with the environment and the food safety It has created market segments which differentiate the quality of agricultural products considering environmental aspects in their production. In this context observing "Good Agricultural Practices" in food production has become indispensable to meet the market demand for more sustainable products. Good Agricultural Practices ( GAPS ) are based on the principles of food safety and environmental preservation and social conditions that respect those involved in the production process, integrating agronomic and market requirements under a single concept. The objective of the research was to describe the profile of the producers of the city of Divinolândia, State of São Paulo – Brazil, linked to the APROD ( Divinolândia Mountain Coffee Growers Association) and separating them into groups of according with the similarity and relation with the Good Agricultural Practices in the coffee production.

### METHODS

The research was conducted in coffee properties associates of the Aprod based in the city of Divinolândia-SP in 2018 with 53 farmers, where was used a questionnaire type Survey containing 182 questions. The questionnaire was based on the main standards and codes of conduct of certification programs and current laws in the country about agricultural issue in particular coffee cultivation. After to apply the questionnaire with the producers the data were tabulated and analyzed by SPSS software (Statistical Package for the Social Science). Besides descriptive statistics with frequency and percentage of answers It was did the statistical analysis multivariate Cluster that objective to group the individuals (cases) with characteristics similar in function of a set of selected variables.

### RESULTS

The first part of the questionnaire (31 questions) that speak about the characterization of the coffee grower and your property, It was used to do an overview of the group of coffee growers connected to APROD and your properties. In the second part of the questionnaire (151 questions) that speak about the survey of GAPS proceeded a descriptive presentation of the results and cluster analysis was carried out. The purpose of this analysis was to organize the data within a certain structure to allow to do groupings and whose elements had certain similarities. Subsequently was realized a crosstabs between variables of the first part of the questionnaire with the cluster generated. This procedure was adopted with the intention to characterize through the socioeconomic variables the two groups generated with performance distinct in relation to Good Agricultural Practices.

### CONCLUSIONS & PERSPECTIVES

The rural properties of APROD linked coffee farmers show heterogeneous performance in relation to the different dimensions analyzed in this research. It was observed that there were significant differences between the groups of properties formed from the cluster analysis and it was possible to separate the set of properties in groups different. The methodology proposed was able to categorize groups of coffee farms according to their performance in relation to Good Agricultural Practices.



## WHATSAPP USERS PROFILE IN THE AGRO-INDUSTRIAL COFFEE SYSTEM OF SÃO PAULO - BRAZIL

PEREIRA, S.P \*, AGUIAR, C.M.G \*\*, BALIZA, D.P \*\*\*, OLIVEIRA, E.F \*\*\*\*, OLIVEIRA, E \*\*\*\*, AOUN, L.A \*\*\*\*

\* Agronomic Institute (IAC), \*\* Federal University of Lavras (UFLA), \*\*\* Federal Institute of Southeast Minas Gerais (IF Southeast MG), \*\*\*\* Coffee Research Consortium. [Brazil]

### RATIONALE

The State of São Paulo is the third largest coffee producer in Brazil and has the largest industrial roasting plant in the country, as well as the largest export port in Santos city. The changes in the telecommunication benefited the coffee industry, facilitating the access popular platforms like WhatsApp, Facebook and YouTube. The user can to create groups with specific topics and share files or information to help in the diffusion of technologies and good practices. The objective of the present study is to analyze the profile of the users of WhatsApp groups that speak about coffee, of the seed to cup.

### METHODS

It was created a structured questionnaire (survey) and It was available in Google Forms Platform. The link was shared in about twenty WhatsApp groups connected coffee industry. Between June 25 and July 3, 2017, It were interview one hundred forty-seven coffee growers than work in State of São Paulo. The data were transcribed for SPSS software used in social sciences statistics, where the frequency and percentage of responses were analyzed.

### RESULTS

The results obtained in the present research show profile of WhatsApp groups inserted in the agro-industrial system coffee of the State of São Paulo in relation to questions of gender, age group, schooling level, higher performance segment, search frequency for technical information and news. The main sources of information, credibility, dissemination, sharing and if this knowledge has added positive points in your area of operation.

### CONCLUSIONS & PERSPECTIVES

The internet and the WhatsApp are part of the daily routine of the agents that compose the Agro-industrial System Coffee in the State of São Paulo. Many WhatsApp groups with the coffee theme are created daily and increase the communication and exchange information across sectors. The majority of users are male and have a high academic background and they are in the economically active age range. They use the internet and the WhatsApp how first option to get news and technical information. Most people consider themselves well informed and believe in information depending on the source. The technical information and news brings benefits to your coffee activity. The present research give indications of the communication models that have been used within the agro-industrial system coffee of Brazil. The largest producer and exporter of coffee and second largest consumer in the world.



## CARBON ASSESSMENT FOR ROBUSTA COFFEE (*COFFEA CANEPHORA*) PRODUCTION SYSTEMS IN THE CENTRAL HIGHLANDS OF VIETNAM

NGUYEN, Nhiem<sup>1</sup>, TALSMA Tiffany<sup>1</sup>, NGUYEN, Kien<sup>1</sup>, DO, Chung<sup>2</sup>, D'HAENZE, Dave<sup>2</sup>, LÄDERACH Peter<sup>1</sup>

<sup>1</sup>International Center for Tropical Agriculture (CIAT) Asia, Hanoi, Vietnam. <sup>2</sup>Hanns R. Neumann Stiftung Asia Pacific, Dak Lak, Vietnam

### RATIONALE

The high yield and production of Robusta coffee in Vietnam has been attributed to intensive fertilizer use and the expansion of monoculture coffee growing area with many cases linked to deforestation in recent decades, which consequently contribute significant greenhouse gas (GHG) emission to the atmosphere, adversely affecting the environment. In this study, we evaluated the climate impact of Robusta production via quantification of carbon stock and GHG emissions in the intensive shaded and unshaded coffee farms of the world's largest Robusta producing region, Vietnam's Central Highlands.

### METHODS

Data on (a) tree diameter and height via tree inventory, and (b) on-farm practices for the calendar year 2015 including fertilizer use, crop residue management, energy use (for irrigation, weeding, spraying, and coffee processing), and transportation of inputs and outputs were collected from 46 (23 shaded and 23 unshaded) coffee farms in November of 2016. Carbon (C) stocks in biomass (trees) were estimated using species-specific and generic allometric equations based on (a) and wood density while GHG emissions from farm management practices were estimated based on (b) using CoolFarmTool, an online GHG calculator.

### RESULTS

There was no significant difference in green coffee bean yield and total GHG emissions between shaded and unshaded systems due to similar input use intensity and management practices. In both systems inorganic fertilizer production and application contributed the largest part of about 54% to the total GHG emissions. Shaded systems had significantly higher annual C stock (with a C stock share of 64% by shade trees at average age of 9.6 years and a density of  $85 \pm 35$  trees ha<sup>-1</sup>) than that of unshaded system. Accounting for sequestered carbon, shaded system had negative net GHG emissions ( $-2.4 \pm 6.1$  kg CO<sub>2e</sub> kg<sup>-1</sup> coffee) while unshaded system had a positive value ( $1.0 \pm 1.4$  kg CO<sub>2e</sub> kg<sup>-1</sup> coffee). Approximately 20% of our sample farmers reported having cleared secondary forest to plant coffee within 20 years of 2015. Consequently, an estimated carbon loss of 30.3 Mg CO<sub>2e</sub> ha<sup>-1</sup> year<sup>-1</sup> has been incurred since deforestation occurred, which is about 5 times higher than that of carbon sequestered in shaded systems. We found that farmers prefer high value fruit trees (especially avocado, durian, mango, and cashew), in addition to *Cassia siamea* as shade trees for intercropping with coffee.

### CONCLUSIONS & PERSPECTIVES

Growing shade trees can help off-set GHG emissions produced by coffee management practices via their provision of carbon sequestration services while not significantly reducing coffee yield. For each and every hectare coffee converted from forest, a minimum of 5 hectares of bare land has to be converted into shaded coffee systems with similar annual carbon stock accumulation rates as the shaded plots in our sample to compensate for carbon loss. Fruit trees and *Cassia siamea* are recommended shade trees for intercropping project in Vietnam's Central Highlands as they are preferred by farmers.

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## *Coffea Arabica* water use in open versus shaded systems under smallholder's farm conditions in Eastern Uganda

Alejandra Sarmiento-Soler<sup>1,5</sup>, Philippe Vaast<sup>2,3</sup>, Munir P. Hoffmann<sup>1</sup>, Reimund P. Rötter<sup>1,4</sup>, Laurence Jassogne<sup>5</sup>, Piet J.A. van Asten<sup>5,6</sup> and Sophie Graefe<sup>7,8</sup>

<sup>1</sup>Tropical Plant Production and Agricultural Systems Modelling (TROPAGS), University of Goettingen, Grisebachstraße 6, 37077 Göttingen, Germany

<sup>2</sup>UMR Eco&Sols, Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Université Montpellier, Montpellier, France

<sup>3</sup>World Agroforestry Centre (ICRAF), Hanoi, Vietnam

<sup>4</sup>Center for Biodiversity and Land Use (CBL); University of Goettingen, Buesgenweg 1, 37077 Göttingen, Germany

<sup>5</sup>International Institute of Tropical Agriculture (IITA), Kampala, Uganda

<sup>6</sup>Olam International Ltd, Kampala, Uganda

<sup>7</sup>Tropical Silviculture and Forest Ecology, University of Goettingen

<sup>8</sup>Organic Plant Production and Agroecosystems Research in the Tropics and Subtropics (OPATS), University of Kassel, Kassel, Germany

### RATIONALE

**Increasing temperatures and water scarcity related to climate change bring new challenges to coffee production. Therefore, sound scientific understanding of the functioning of current coffee cultivation systems is crucial to guide and implement adaptation strategies to climate change.**

### METHODS

We monitored coffee water use (l day<sup>-1</sup>) and certain microclimate parameters (rainfall, temperature, relative humidity, radiation and soil moisture) during one year (March 2015 – April 2016) in coffee-open systems and coffee-shade systems at Mt. Elgon, Uganda.

### RESULTS

Coffee daily water use per plant ( $1.2 \pm 0.64$  l d<sup>-1</sup>) and coffee transpiration per leaf area ( $0.30 \pm 0.15$  mm day<sup>-1</sup>) did not differ significantly between cultivation systems. Nevertheless, system transpiration was on average higher in shaded systems ( $0.63 \pm 0.25$  mm d<sup>-1</sup>) than in open systems ( $0.27 \pm 0.1$  mm d<sup>-1</sup>). Consequently soil water content was reduced in shaded systems up to 50 %.

### CONCLUSIONS & PERSPECTIVES

Coffee water use was not affected by the presence of shade trees. Hence, we could not prove water competition or complementarity between coffee and shade trees. Further research is needed to quantify water limitations under more extreme environmental conditions and the verstatility of several management practices (mulching, pruning, thinning) to reduce water limitations.

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## **Hyperspectral imaging for compositional and functional analysis of green coffee beans**

Ian D. Fisk, Nicola Caporaso, Martin B. Whitworth

*Abstract for the ASIC conference, October 2018, USA*

Coffee quality is strongly dependent upon the composition of the green coffee beans. The study and analysis of this composition is therefore of critical importance in defining the quality of the final coffee brew. The lipid fraction of green coffee beans is of importance especially for espresso-like preparations, where it gives additional volatile compounds arising from fatty acid degradation, as well as the typical crema and round mouthfeel.

Food constituents have been shown to be analysable in granular food commodities without the need of grinding using Near-Infrared Spectroscopy (NIRS)-based techniques. An advance of conventional NIRS is based on adding the spatial domain to the spectral data, named hyperspectral imaging (HSI), in order to analyse and understand the spatial variation of a target food constituent.

In this research, we have shown the feasibility of using HSI for the non-destructive rapid prediction of green coffee constituents, analysed on a single bean basis. The application of HSI allowed analysing multiple individual beans at a time, thus allowing high efficiency and the possibility of screening larger amount of product.

A wide range of green coffee batches were sampled from 17 producing countries, by obtaining both Arabica and Robusta varieties and samples treated using the wet- and dry-processing, in order to obtain the largest natural variation expected in the commercial product. Samples were not submitted to any treatment and ten single beans were selected from each batch. Beans were scanned on both sides using an HSI system able to acquire spectra in the range ~900-2500 nm, in the push-broom acquisition mode. The pixels belonging to each bean were selected in order to export the average spectrum for each bean. The samples were then individually analysed by wet chemistry to obtain reference measurement of the total lipid content in each coffee bean, using an NMR-based technique. Multivariate statistical analysis was used (mostly PLS regression) in order to build quantitative prediction models on single seeds. The results were expressed either on wet-basis or dry basis, using the reference moisture content measurement.

The results have shown that lipid content greatly varies between samples, not only the expected difference between Arabica and Robusta coffees was verified, but it was noticed that large variation is obtained also within batches, probably due to the natural variation arising from the soil, location and even variation within plants.

The prediction models had excellent performance and were comparable to NIRS models in which the ground samples were used. On the contrary, these models were built on a moving stage in a non-contact manner on the whole coffee beans. Despite these conditions, HSI was still able to provide a calibration performance with  $R^2$  of 0.89 and 0.90, for the wet- and dry-basis models, respectively. The cross-validation performance had  $R^2=0.88$  and 0.89, respectively. The prediction error was 0.90 and 0.98% (wet- and dry-basis, respectively), which is completely acceptable for screening purposes, considering the range of natural variation being from 8.1 to 22.7 % (dry-basis). The second derivative spectral pre-treatment gave better results compared to other pre-treatments (first derivative, standard normal variate, etc.).

As HSI uses a large number of spectral bands, new models were generated using a lower number of variables (from 6 to 22), selected on the basis of their importance. These models still showed promising results for screening purposes, allowing using less expensive cameras and lower computational time.

In conclusion, HSI has been shown to have potential for rapid, non-destructive analysis of green coffee, utilisable for research laboratories, plant physiologists and geneticists in plant breeding programmes, and the food industry.

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Modelling moisture loss in roasting beans.

FADAI, Nabil T.\* , AKRAM, Zahra\*\* , GUILMINEAU, Fabien\*\* , MELROSE, John\*\* , PLEASE, Colin P.\* , VAN GORDER, Robert A.\* .

\*Mathematical Institute, University of Oxford, Andrew Wiles Building, Radcliffe Observatory Quarter, Woodstock Road, Oxford OX2 6GG United Kingdom

\*\*Jacobs Douwe Egberts R&D UK Ltd, Ruscote Avenue, Banbury OX16 2QU United Kingdom  
Koninklijke Douwe Egberts B.V., Oosterdoksstraat 80, 1011 DK Amsterdam Netherlands

A model (1) is developed to give insight into the evolution of the profiles of temperature and moisture (liquid and vapour phases and transition between) internal to a roasting bean. Modified evaporation rates and chemical reaction groups improve existing mathematical models (2). We model the phase change from liquid to vapour water within the bean during roasting using first-order Arrhenius-like global reactions. For other components of the bean, we consider a three-component solid phase model for organic compounds which allows for porosity of the solid matrix to vary during the roasting process. Simulations are compared against data for the overall moisture loss data of roasting of whole beans and chopped beans. It is found that the multiphase model with global water reactions and three-component solid phase reactions agree with experimental data for the average moisture content in whole beans and chopped bean, but that the data allows for a range of possible parameter values. It is discussed what experimental data might be collected to more firmly determine the parameters.

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Poster presented by John Melrose.



## EFFECT OF PROBIOTIC CULTURES ON CHLOROGENIC ACIDS IN A SOY-COFFEE FERMENTED PRODUCT DURING COLD STORAGE

DUARTE, Giselle;<sup>a,b</sup> LIMA, Juliana;<sup>a</sup> FELBERG, Ilana;<sup>c</sup> MIGUEL, Marco Antônio;<sup>d</sup> FARAH, Adriana.<sup>a</sup>

<sup>a</sup> Núcleo de Pesquisa em Café (NUPECAFÉ), Instituto de Nutrição, Universidade Federal do Rio de Janeiro (UFRJ), Brazil, <sup>b</sup>Instituto Federal do Rio de Janeiro, Brazil, <sup>c</sup>Embrapa Agroindústria de Alimentos, RJ, Brazil; <sup>d</sup>Laboratório de Microbiologia de Alimentos, Instituto de Microbiologia, UFRJ, Brazil.

### RATIONALE

Chlorogenic acids are among the main bioactive compounds in coffee. The use of coffee chlorogenic acids (CGA) and polysaccharides by colonic bacteria with bifidogenic and immunostimulatory effect has been reported in the literature. In the present work, we aimed to evaluate the effect of probiotic cultures on coffee CGA in a soy-coffee fermented product during cold storage.

### METHODS

An aqueous soybean extract at 10% was heated at 80°C in water bath with 15% sucrose, guar and xantham gums. Following, the mixture was inoculated at 42°C with the probiotic starter culture (10<sup>6</sup> CFU) (*Lactobacillus acidophilus* (LA) and *Bifidobacterium animalis subsp. Lactis* (BA) from Christian Hansen, SP, Brazil), and incubated for 6h at (at 40°C). Following, light-medium roasted instant arabica coffee (1%) was added and the fermented product (pH4.67) and stored at 7-10 °C. The same product was reproduced with addition of 1% cooked soy hull. Samples were collected prior and after 7, 14 and 21 days storage. Chromatographic analyses of CGA and metabolites were performed in duplicate according to Duarte and Farah (2011).<sup>1</sup> ANOVA and unpaired T-test were used to treat results with differences considered when  $p \leq 0.05$ .

### RESULTS

After 21 days storage (pH 4.60), the original content of CGA prior to storage (24.2 mg/100g) was reduced to 18.2 mg/100g, a reduction of 19.5%, with no difference between the products with and without soy hull. Average dicaffeoylquinic acids (diCQA) content was reduced by 36%, while caffeoylquinic acids (CQA) were reduced only by 6.9% probably due to an increase derived from hydrolysis of diCQA, confirmed by release of caffeic acid. Feruloylquinic acids (FQA) content were also decreased by 20%. The following phenolic metabolites were identified after 21 days storage in order of abundance: caffeic acid; dihydrocaffeic acid; *p*-coumaric acid; 4-hydroxyacetic acid; 3,4-dihydroxyacetic acid; ferulic acid, syringic acid, and vanilic acid. Viability results and evaluation of yeast, mold and other bacteria counts indicate that BA was the main strain responsible for those changes.

### CONCLUSIONS & PERSPECTIVES

Even during cold storage probiotic bacteria changed CGA content and profile. The fact that no change was observed in metabolites production when soy hull was added to the mixture indicates that phenolic compounds were preferred as source of energy, with a reduction in the total content of these compounds and production of metabolites. Additional unshown results indicate soy isoflavones were also used as source of energy. The effect of phenolic profile changes on the product bioactivity should be evaluated and the use of coffee polysaccharides by the bacteria should be also monitored.

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## USING BIOINFORMATICS ASSOCIATED WITH REAL TIME PCR AS A SIMPLE STRATEGY TO DISCRIMINATE COFFEA ARABICA AND COFFEA CANEPHORA SPECIES IN GROUND ROASTED COFFEES.

FERREIRA, Thiago<sup>\*</sup>,<sup>\*\*</sup> FARAH, Adriana<sup>\*</sup>, OLIVEIRA, Tatiane C.<sup>\*\*</sup> OLIVEIRA, Edna Maria M.<sup>\*\*</sup>  
<sup>\*\*</sup> Food Chemistry and Bioactivity Lab & Coffee Research Core (NUPECAFÉ), Nutrition Institute, UFRJ, Brazil. <sup>\*\*</sup>Molecular Diagnostic Laboratory, Brazilian Agricultural Research Corporation, Brazil

### RATIONALE

Coffee beverage is widely appreciated worldwide. The main traded species are *Coffea arabica* and *Coffea canephora*. The first has richer aroma and pleasant taste, and consequently higher acceptability and market value. In most countries, blends using exclusively *C. arabica* beans are labelled “100% Arabica coffee” in order to attract consumer’s attention for their high quality. Nevertheless, *C. canephora* beans can be used to dilute such brands under the same label to increase profit. Discriminating both species after coffee is roasted and ground is a hard task because they share most chemical components and physical and genetic characteristics. This study presents a simple and useful approach based on bioinformatics tools and real time PCR for this purpose.

### METHODS

*In silico analyses*- In order to discriminate *C. arabica* and *C. canephora* species, the similarity between *C. arabica* sequence (GW441275.1) and *C. canephora* genome was assessed in Coffee Genome Hub database (CGH), using an internal similarity search tool (Blast). By using CGH, Basic Local Alignment Search Tool and *in silico* PCR, the primers ARA (for *C. arabica*) and EST (for *C. canephora*) were drawn.

*In vitro analyses* -*C. arabica*, cv. Catuaí and *C. canephora* cv. conilon seeds originated from the states of Minas Gerais and Espírito Santo, Brazil, respectively, were roasted to reach medium roast degree (# 55 Agtrom, SCAA). DNA extraction was adapted from Ferreira *et al.* (2016). DNA concentrations of the extracts were assessed by UV absorbance at 260 nm. Real-time PCR runs were carried out by using the 7500 Real-Time PCR System (Applied Biosystems, USA). 7500 SDS v1.4.1 software (Applied Biosystems, USA) was used to read the amplification signals, and to analyse the endpoint data. In order to estimate the amount of *C. canephora* added to *C. arabica*, serial dilution of DNAs isolated from green coffee samples and from ground and roasted blends of *C. canephora* and *C. arabica* were made and compared to commercial samples.

### RESULTS

*In silico* primers showed high specificity. ARA primers reached a specific melting temperature at  $75.96 \pm 0.26$  °C. In Real time PCR runs, performed in both raw and roasted samples, the number of cycles decreased as the amount of *C. canephora* increased. The method was able to detect the presence of *Coffea canephora* in proportions down to 25%. The robustness of the method is being tested for detection of concentrations down to 5%.

### CONCLUSIONS

The association of bioinformatics tools with real time PCR was shown to be a simple, appropriate and useful approach for detection of *C. canephora* species in ground and roasted coffee blends.

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## Impact of post-harvest processes on green coffee bean composition and in-cup flavor

POLSTER, Johannes\*, MESTDAGH, Frédéric\*\*, DAVIDEK, Tomas\*

\* Nestec Ltd. Nestlé Product Technology Centre Beverage, 1350 Orbe, Switzerland

\*\* Nestlé Nespresso S.A., 1007 Lausanne, Switzerland

### RATIONALE

Post-harvest treatment of coffee cherries significantly impact the aroma and taste of the final beverage<sup>1</sup>. However, in contrast to the extensive knowledge on coffee roasting, only few systematic studies were performed monitoring green coffee bean changes along post-harvest treatments and linking these physical and chemical changes with an altered in-cup flavor.

Thus, the aim of the present study was i) to systematically analyze how various post-harvest processes can modulate the chemical composition of green coffee beans, ii) to reveal how these changes are influencing in-cup flavor and iii) to identify suitable markers to monitor and optimize post-harvest processing.

### METHODS

Different post-harvest treatments, namely wet and dry processing, were applied to freshly harvested Nicaraguan Arabica coffee cherries. To follow the physical and chemical changes, samples were taken during the post-harvest process as well as before and after roasting. A large number of metabolites, e.g., sugars, amino acids, organic acids as well as aroma and taste compounds, were analyzed by using state-of-the-art analytics and the data obtained were correlated with the sensorial changes.

### RESULTS

The results obtained give a clear insight how different post-harvest treatments were influencing the chemical composition of green coffee beans. Suitable marker compounds were identified and the enhanced formation of specific aroma compounds, such as various esters during dry coffee processing, could be clearly correlated with the respective sensory profiles.

### CONCLUSIONS & PERSPECTIVES

In summary, opportunities of post-harvest processing for modification of the green bean composition in connection with in-cup flavor modulation were shown. Analytical tools applied in this study can be used to monitor and to optimize effectiveness of post-harvest treatments.

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LIU Chujiao, FISK Ian, LINFORTH Robert, YANG Ni  
Division of Food Sciences, University of Nottingham, Sutton Bonington Campus, Loughborough,  
UK

Coffee is one of the most important international trade products. The species of coffee dominating the world market are Arabica and Robusta, which account for 61% and 38% respectively of the coffee production worldwide. Arabica, perceived with a smooth and rich flavor, is usually more desirable than Robusta that is described as having a strongly muddy odour. Many studies have focused on roasting as the key process step to optimise the coffee aroma profile [1], and some researchers have explored the possibility of pre-treating green Robusta coffee beans to reduce bitterness [2].

#### RATIONALE

Cold plasma is an emerging technology that has been recently applied in the food industry and processing. Currently, cold plasma has been widely used mainly for toxin degradation, food decontamination and product shelf life enhancement. Due to the interactions of energetically highly reactive plasma with phytochemical compounds, the cold plasma treatment could also affect on the food product quality. No work has been previously carried out to understand the effect of cold plasma pre-treatment on aroma generation in Robusta coffee beans. Therefore, cold atmospheric plasma technology was applied in this study, with the aim to change the chemical reaction pathways during thermal treatment (roasting) and ultimately regulate aroma formation during the coffee roasting.

#### METHODS

Green coffee beans with the same origin were used either as a control sample or subject to pre-treatment. Cold atmospheric plasma was used for pre-treat samples with different periods of time. After treatment, samples were subjected to equal thermal treatment (200 °C static oven roasting for 20 min) and ground to comparable particle size distributions. Aroma compounds generated during roasting (n=4) were evaluated by headspace analysis using SPME-GC-MS, and colour was analysed by Hunter Lab.

#### RESULTS

Fifteen volatile compounds were identified and selected as the key compounds, including aldehyde, ether, furans, ketones, acid, phenolic compounds and sulfur-containing compounds. All the key compounds significantly increased in the cold atmospheric plasma pre-treated Robusta coffee compare with the non-treated Robusta coffee ( $p < 0.05$ ). These volatile compounds are associated with sensory odour description such as sweet, malty, grassy, sour, burnt, and smoky.

#### CONCLUSIONS & PERSPECTIVES

The analytical results indicated that several key compounds could be regulated in the Robusta coffee beans by cold atmospheric plasma pre-treatment. Therefore, this novel technology could be used to modify the quality of Robusta coffee.

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## DEVELOPMENT OF NEW CHOCOLATE-LIKE PRODUCT USING COFFEE MATERIALS

KOBAYASHI, Tsukasa\*, IWAI, Kazuya\*, NARITA, Yusaku\*, HANZAWA, Taku\*,  
FUJIMOTO, Hirofumi\*, KAKIUCHI, Misako\*, FUKUNAGA, Taiji\*  
\*Innovation Center, UCC Ueshima Coffee Co., Ltd., Hyogo, Japan.

### **RATIONALE**

Coffee beans are one of the most important agricultural crops in the world. After roasting and grinding, most coffee beans are treated with hot or cold water to produce beverages, but in some cases they are taken directly as sweets. To increase the variety of ways to enjoy coffee beans, we investigated the use of coffee beans instead of cocoa beans, which have many similarities with coffee, such as growing areas and processing processes. In particular, we focused on chocolate, which is a typical product of cacao beans.

In this research, we developed a new chocolate-like product using coffee materials and named it “solid coffee”. Specifically, we replaced all of the cacao-derived ingredients in chocolate with materials from coffee beans and then evaluated the physicochemical properties of the product.

### **METHODS**

We made chocolate using coffee beans (*Coffea arabica*, Brazil) and coffee oil instead of cacao mass and cacao butter, respectively. Initially, sugar, vegetable oil, finely ground coffee beans, and powdered milk were mixed and kneaded for 2 h. Subsequently, vegetable oil, emulsifier, and coffee oil were added, and the mixtures were kneaded again. Finally, kneaded samples were molded and cooled. To evaluate the resulting physicochemical properties, we prepared a control sample using finely grounded cacao mass and cacao butter. Physical properties and major components were analyzed, and sensory evaluations were performed.

### **RESULTS**

Solid coffee samples were easily melted compared to the cacao-based control samples. In analyses of main components, solid coffee was rich in dietary fiber and did not contain the heavy metal cadmium. High levels of caffeine and diterpenes were detected in solid coffee, although caffeine contents could be reduced by using decaffeinated coffee beans.

### **CONCLUSIONS & PERSPECTIVES**

We succeeded to develop a new chocolate-like product using coffee materials instead of cacao, and did not use any other flavoring materials. This solid coffee form provides a strong coffee feeling in one bite, and expands the opportunities to enjoy coffee beans.



IKEDA, Michio\*, AKIYAMA, Masayuki\*, HIRANO, Yuta\*, MIYAJI, Kazuhiro\*,  
SUGAWARA, Yasunori\*\*, TAKAHASHI, Kana\*, KOIZUMI, Reiko\*, IMAYOSHI, Yuriko\*\*,  
IWABUCHI, Hisakatsu\*\*, ONODERA, Takeshi\*\*\*, TOKO, Kiyoshi\*\*\*

\*Morinaga Milk Industry Co., Ltd., Zama, Kanagawa, Japan, \*\*San-Ei Gen F.F.I., Inc.,  
Toyonaka, Osaka, Japan, \*\*\*Kyushu University, Nishi Ward, Fukuoka, Japan

#### RATIONALE

With the aim of developing a ready-to-drink (RTD) milk-coffee beverage that preserves the original coffee flavor, we investigated the effects of the manufacturing process on retronasalaroma (RA) odorants and sensory characteristics based on our previous study<sup>1</sup>.

#### METHODS

Samples: Sodium carbonate was used to adjust the pH of coffee samples to 6.8. Reconstituted (r-) milk samples were prepared by blending concentrated skim milk, cream, and water. The conventional blending-before-sterilization (BBS) process was used to prepare milk-coffee samples by blending r-milk with sugar-added coffee extract with pH adjustment, and then sterilizing the blend (pH 6.8) using an indirect plate (PLT-) sterilizer. The newly developed blending-after-sterilization (BAS) process was used to prepare milk-coffee samples (pH 6.4) by blending r-milk, sterilized using a PLT- or direct infusion (INF-) sterilizer, with sugar-added coffee (pH 5.1) without pH adjustment, sterilized using a PLT-sterilizer at  $\leq 10^{\circ}\text{C}$ . Homemade milk-coffee samples were prepared by blending PLT- or INF-sterilized milk and unsterilized sugar-added coffee without pH adjustment at  $\leq 10^{\circ}\text{C}$ . Analyses: RA odorants were analyzed by gas chromatography-olfactometry (CharmAnalysis<sup>TM</sup>) using an RA simulator (RAS)<sup>2</sup>. Sensory characteristics were evaluated by quantitative descriptive analysis (QDA)<sup>®</sup>.

#### RESULTS

A total of 33 RAS odorants were detected in the RAS effluent and classified into 19 odor-description groups. Principal component analysis (PCA) of odor-description intensities indicated that the effect of coffee pH adjustment on odor characteristics was greater than that of sterilization. Furthermore, BAS and BBS samples differed in intensity, and BAS samples prepared using INF-sterilized r-milk was more similar to homemade samples than BAS samples prepared using PLT-sterilized r-milk. PCA of sensory scores showed that BAS and homemade samples prepared using a PLT sterilizer were stronger in coffee flavor than BBS samples, and that the sensory characteristics of a BAS sample were similar to those of a homemade sample.

#### CONCLUSIONS & PERSPECTIVES

Adjusting the pH of the coffee extract when the RTD milk-coffee beverage is being processed significantly affects RAS odorants and should be avoided. RTD milk-coffee beverages produced by conventional BBS processes do not preserve the original coffee flavor following adjustment of the pH of the coffee during manufacturing. Use of new BAS processes without pH adjustment of coffee produces RTD milk-coffee beverages with a flavor more similar to that of homemade milk-coffee. In conclusion, INF sterilization of r-milk is preferable to PLT sterilization in the BAS process. The BAS process is being used in Japan to produce RTD milk-coffee beverages.

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BENSON, Courtney A.\*, EHLERT, Sven\*\*, CZECH, Hendryk\*\*\*, ZIMMERMANN, Ralf\*\*\*, HOWELL, Jessalin\*

\*The J.M. Smucker Company, Orrville, OH; \*\*Photonion GmbH, Schwerin, Germany; \*\*\*Joint Mass Spectrometry Centre (University of Rostock), Rostock, Germany

## RATIONALE

Coffee roasting is an important process transforming green coffee, which is weak and hay-like, into the rich and flavorful beverage that many people enjoy each day. During the roasting process, chemical changes occur in the coffee beans beginning with the loss of moisture. In the final stages of roasting, a robust chemical and flavor profile is developed. PIMS is a method for the investigation of complex gas mixtures such as coffee roast gases.

## METHODS

A Single Photon Ionization – Time of Flight Mass Spectrometer (SPI-TOFMS) was coupled to a lab scale roaster. Two different bean types: Arabica and Robusta, at three different roast profiles: slow, medium and fast, were monitored. The final time and temperature for each of the specific roast programs was extended beyond a typical endpoint, essentially over-roasting the coffee to obtain the full chemical off gas profile of the roast. Once cooled, the coffee samples were ground and the color of the final samples was measured on a colorimeter.

## RESULTS

Using principle components analysis (PCA) to analyze the SPI mass spectra collected, bean color and type are noted as factors that influence the variation in the chemical profile of the roast gases. In general, the mass spectra from the coffee roast gases can be used to indicate the reproducibility among the roasts. Non-Negative Matrix Factorization (NNMF) is the multivariate technique applied to characterize the compounds being released at different phases in the roast process. Using NNMF, the chemical profile of the coffee roast gases for each program is divided into four “phases” of the roasting process. The overall concentration of the chemical profile is increasing and highest in the final phase of the roast.

## CONCLUSIONS & PERSPECTIVES

The formation and degradation of specific compounds during roasting was observed using SPI. Fatty acids are released from the bean during the early evaporation phase, followed by carbohydrate degradation in the later phases. In general, this method is useful to gain insight into the formation of flavor during coffee roasting.

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The Modelling of Coffee Brew Yield.

MELROSE, John\*, ROMAN- CORROCHANO, Borja\*, BAKALIS, Serafim\*\*.

\*Jacobs Douwe Egberts R&D UK Ltd, Ruscote Avenue, Banbury OX16 2QU United Kingdom  
Koninklijke Douwe Egberts B.V., Oosterdoksstraat 80, 1011 DK Amsterdam Netherlands

\*\*Faculty of Engineering, Nottingham University, Nottingham NG7 2RD, United Kingdom.

Models of coffee brewing have been developed recently (1,2). A key question is how to parameterise such models against controlled experiments allowing predictions to be made on a wide set of brew conditions. Brew experiments were performed on a range of grind sizes from 200 to 1000  $\mu\text{m}$ ) in both dilute batch conditions and flow through a packed bed for a range of flow rates in conditions similar to that of espresso brewing (3). Using a model based on diffusion out of particles (1), the yield vs time data can be fit using a single particle length scale and two diffusion constants. In reality, however, coffee grinds are bi-modal in particle size with fine and coarse distributions. Furthermore, diffusive release from fines and the outer rim of coarse particles can be expected to be with bulk solution diffusion constants whilst that from the core of coarse particles to with be hindered (lower) values. A bi-modal model with fine and coarse particles was also used to fit the data set, with hindered diffusion constants in the range expected, however, the distribution of hindered diffusion constants was itself bi-modal.

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Poster presented by John Melrose.





KHAMITOVA, Gulzhan<sup>\*,\*\*</sup>, ANGELONI, Simone<sup>\*,\*\*</sup>, CAPRIOLI, Giovanni<sup>\*</sup>, SAGRATINI, Gianni<sup>\*</sup>, VITTORI, Sauro. <sup>\*,\*\*</sup>

<sup>\*</sup>University of Camerino, Camerino, MC, Italy,

<sup>\*\*</sup>International HUB for Coffee Research and Innovation.

#### RATIONALE

Espresso coffee (EC) extraction is a complex process where physical and chemical variables clearly impact on the final cup. Besides these parameters, the size of grinded coffee plays a crucial role to extract good espresso with tantalizing aroma, dense cream and mouthfeel flavor [1]. Coffee particles are made by a large number of compounds. An understanding of these compounds can be helpful for process optimization and quality control. This research aims to analyze the levels of compounds' variation at specific particle size distribution (200-400 and 400-1000 microns).

#### METHODS

Quantitative and qualitative analyses are carried out through HPLC-VWD and GC-MS [2]. The espresso machine settings are maintained at 9 bar and  $92 \pm 2^\circ\text{C}$ . Particle sizes between 200-400 and 400-1000 microns are used to prepare EC.

#### RESULTS

EC extraction time, kinetics of extraction and its dependence on water pressure and temperature were controlled during the analyses. Analytes as caffeine, trigonelline, nicotinic acid, chlorogenic acids (CQAs) and volatiles were identified. EC extraction were performed in duplicate for each particle size. Results confirmed a good extraction efficiency of caffeine, which accounted for 170 mg at 200-400 microns and 90 mg per cup at 400-1000 microns. Likewise, the amount of all other bioactive compounds increased when extracting coffee with smaller particle sizes.

#### CONCLUSIONS & PERSPECTIVES

EC extraction at different particle sizes was evaluated by analyzing the content in bioactive components and volatiles that were found in EC. Extraction optimization will be developed by modifying extraction variables.

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Mizue\_Kishigami<sup>1,2</sup>, Marco Wellinger<sup>1</sup>, Chahan Yeretizian<sup>1</sup>

<sup>1</sup> Zurich University of Applied Science (ZHAW), Institute of Chemistry and Biotechnology, <sup>2</sup> Suntory Beverage & Food Limited, Japan

## RATIONALE

The extraction process of coffee is dependent on a series of factors, such as water temperature, contact time and particle size distribution. These interact with each other and make up the flavor of the final beverage. Currently, there is lack of understanding about the influence and interactions of the various control parameters. Extraction is mostly discussed using the Coffee Brewing Control Chart, which plots the strength of a coffee beverage against its extraction yield. The aim of this study was to work towards a more refined insight on the extraction process.

## METHODS

The study was conducted on a MoccaMaster machine to produce filter coffee. Fractions were taken at fixed time intervals. For each fraction, we measured % TDS by refractometry and acidity by titration. Caffeine, chlorogenic acids and melanoidines were measured by HPLC. Additionally, the influence of carbon dioxide on the extraction rate was also evaluated by changing the CO<sub>2</sub> concentration in ground beans and the alkalinity of water.

## RESULTS

Results are plotted on a modified Coffee Control Brewing Chart, to represent the time-evolution of extraction for the various chemical compounds.

The coarser the grind was, the faster the extraction progressed to a certain Extraction yield. In particular, %TDS of the first few fractions was very high for the coarsely ground coffee compared to the finer ground coffee. Furthermore, for ground coffee where part of the CO<sub>2</sub> has been removed, the extraction was progressing more rapidly.

## CONCLUSIONS & PERSPECTIVES

It was found that grind size significantly impacts the initial speed of extraction. One of the reasons is believed to be the difference of degassing speed. As the previous study showed, if the grind is fine, degassing progresses more quickly, which in turn decreases wettability of the ground coffee during extraction.

The main goal of this project is to characterize the dynamics of the coffee extraction process more closely, while still using the well-known Coffee Control Brewing Chart. Further parameters to be included are aroma composition and sensory evaluations, completing the view on the influence of different extraction parameters.



## QUANTIFICATION OF CAFFEINE AND CHLOROGENIC ACID (5-CQA) IN ARABICA COFFEE BEAN SAMPLES AT THREE DIFFERENT ROASTING LEVELS USING HPLC

LEBLANC, Corrine\*, MORENO, Carlos\*, WEIKART, Daphne\*, WATKINS, Ruth\*, HARRIS, Gabriel\*

\*North Carolina State University, Raleigh, North Carolina, USA

### RATIONALE

Coffee is a part of many Americans' morning routine, with over 150 million consumers drinking an average of 3.1 cups daily. While coffee consumption is driven by consumer liking, the potential health promoting properties of the compounds caffeine and chlorogenic acid are also of interest. Roasting coffee beans can destroy these compounds and produce other chemical and physical changes that have yet to be fully described. This research was conducted to characterize the effect of roast level on various physical and chemical factors in coffee.

### METHODS

Arabica beans from Colombia, New Guinea, and Ethiopia were analyzed for caffeine and chlorogenic acid content at different roast levels using green beans as a control. Roast levels were quantified using Agron values. Bean densities were obtained for each roast level for more in depth analysis of compositional changes in coffee beans throughout the roasting process. Beans were ground using a hand mill followed by a spice grinder and brewed as per (Fujioka and Shibamoto, 2008). Brewed coffee was collected from each batch and analyzed via HPLC using a C-18 column.

### RESULTS

Agron values for light roasts were 100, or underdeveloped. Medium roasted Colombia, New Guinea, and Ethiopia beans had Agron values of 90.33, 82.33, and 79.33, respectively. Dark roasted Agron values were 32.67, 32.33, and 36.00, respectively. Bulk bean density decreased as roast level increased. The bulk density of green Colombia, New Guinea, and Ethiopia beans was 0.68 g/mL, 0.67 g/mL, and 1.00 g/mL, respectively. The bulk density of the light roast beans was 0.64 g/mL, 0.61 g/mL, and 0.66 g/mL. The medium roast bulk densities were 1.41 g/mL, 0.37 g/mL, and 0.45 g/mL. The dark roast bulk densities were 0.24 g/mL, 0.25 g/mL, and 0.24 g/mL. On a g per 100 g "wet weight" basis, caffeine content increased from 1.41 in green beans to 1.90 in the medium roast and decreased to 1.39 in the dark roast. Chlorogenic acid decreased from 5.13 g per 100 g in green beans to 3.80 in the medium roast to 0.03 in the dark roast.

### CONCLUSIONS & PERSPECTIVES

While caffeine, chlorogenic acid content, and density are all affected by roasting conditions, chlorogenic acid is most strongly affected. Future studies will examine coffee roasting effects on acrylamide formation and green coffee bean moisture content effects on chemical changes during roasting.

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Towards a comprehensive definition of “Sensory Literacies”: Meaning and implications of the concept when thinking about sensory communication.

MORALES VARGAS, Javier\*

\*M.Sc in Anthropology student; University of Costa Rica, San José, Costa Rica.

*“Literacy is one of those mischievous concepts, like virtuousness and craftsmanship, that appear to denote capacities but that actually convey value judgments” (C. H. Knoublauch, p. 74).*

While designing an anthropological research project about the daily use of sensory language among specialty coffee cupers in Costa Rica, “sensory literacy” was chosen as a concept for analysis. It was found in some sensory science’s papers and even in one of the talks during Re:Co Symposium, Budapest 2017. After a deep search for any discussion around the term, no satisfactory result was found. The usual attempts were only vague notions about “knowing with the senses”, “reading the sensory world” or “learning how to perceive”, not a definition. The most developed notion was found on Brian Kennedy’s talk (2013), but it is far from complete.

Considering how much the concept of literacy has been used in common language —e.g. political literacy, health literacy, digital literacy, scientific literacy, etc.— one possible hypothesis would be that the definition of sensory literacy is somehow intuitive, hence the lack of a more strict definition. However, that “intuitiveness” must be placed under debate. Using a bibliographical approach to different disciplines that have studied writing literacy (education, anthropology, sociology and linguistics) and then triangulating with preliminary data from ethnographic observations during coffee cupings and conversations with coffee professionals; a first attempt to build a comprehensive definition is made.

Sensory literacy is proposed as being a complex body of ideas, theories, beliefs and categorizations of the senses held by a community (social focus); a teaching process guiding to certain expected degree of communication competence (individual focus) and also a daily practice influenced by social factors. Since each community thinks different about the senses, the plural “sensory literacies” is suggested. Further implications of using this concept in terms of communication among a transnational community like the specialty coffee industry, both positive and negative, are discussed.

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## DIGESTION OF BODY-COMPLEX INNER COFFEE BEANS-CELLS USING SOME SELECTED PROTEASES

NAKAJIMA Yuri\*, MINOSAWA Mai\*, YAMAMOTO Ryohei\*\*, and KASAI Naoya\*

\*Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka JAPAN.

\*\*College of Bioscience and Biotechnology, Chubu University, Aichi JAPAN.

### RATIONALE

Coffee beans cell-wall have the body-complex as a preservation structure of oil and protein bodies in the cells. The body have been never isolated from the cells, the characteristics also are not investigated. We have been studying on the isolation and the high digestion of soybeans seeds, and found the body of the cells are similar structure in the microscopic investigation. From considering these results, the body could be isolated and digested, and then give the rich peptides in the digestion. This treatment could be useful for more rich roasting or new high treatment. Therefore, we tried the isolation of the body using cellulase collapsing the cell walls, and the body digestion test were done using various proteases for food treatment use and our selected effective proteases produced by *Aspergillus niger*.

### METHODS

Coffee beans were gifted from UCC corporation of Japan. Food treatment proteases were gifted from enzyme makers of Japan. The beans was cracked and soaked with 5-fold deionized water, and heated to inactivate the coffee enzymes such as polyphenol oxidase at 100 or 121°C, 20min. Ten or 20% Cellulase (*Trichoderma reesei*, GODO-TCF, Japan, 0.1M HAc buffer pH 5.0) was mixed the treated beans to digest at 48°C for 3-7days. The body suspended soup of the reaction mixture was used for the proteases selection. The test was done using the suspension of the soup and the various 1% proteases for over night at 48°C in a 96 well plate or PP micro tubes. The reaction mixtures were estimated the digestion as the turbidity using plate reader at 630 nm. The digestive structures were investigated in the phase-constant microscopy.

### RESULTS

The body digestion was found and selected in the 3 kinds of protease: 2 acidic proteases and 1 neutral protease. Additionally, the soybean body high digesting enzyme, it would be a kind of acidic peptidases, produced by *Aspergillus niger* also was effective. The body was digested, and the turbidity was decreased.

The structure was solubilized and decreased off, and the residual oil drops were found. The effective proteases are not all the same proteases of soybean body-complex digestable.

### CONCLUSIONS & PERSPECTIVES

Coffee cells-body was isolated using the selected collapsing cellulase digestion. The body shape and the structures or the characteristics are similar that of soybeans. The body was digested and high solubilized by some proteases. The effective proteases are not all the same ones. The high digestion and solubilization of the coffee beans cell-walls give the rich oligo saccharides, and these proteases or peptidases digesting the coffee beans give the rich peptides. These treatment could be a useful treatment for the rich and high use roasting and a new treatment.

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## IMPACT OF BEVERAGE TEMPERATURE ON INTERFACIAL PROPERTIES & FOAMING OF COFFEE BEVERAGES

Deepak Sahai\*, Xiaoping Fu\*, and Alexander Sher\*;  
Nestlé Development Center, Marysville, Ohio 43016, USA

Foaming characteristics of coffee and the quality of the foam like the stiffness is highly dependent on the beverage temperature. Surface tension of coffee decreases linearly with temperature and can be correlated with coffee foamability. Lower beverage temperature results in a stiffer and a more stable foam.

### RATIONALE

Understanding the impact of physicochemical parameters on foaming properties is important to control the creation of crema on top of coffee. Crema is an important preference driver for consumers in coffee.

### METHODS

The foaming properties of a black coffee beverage were evaluated using a “Foam Tube” device<sup>(1)</sup>. Foamability and foam stability was expressed as foam to liquid ratio (F/L) after 10 seconds and 5 minutes respectively. The dynamic interfacial tension was measured using a bubble pressure tensiometer (SITA, Germany).

### RESULTS

Surface tension was reduced from 51 mN/m to about 42 mN/m as temperature increased from 20° to 75°C. A lower interfacial tension implies a higher propensity to create foam at higher coffee temperatures (better foamability).

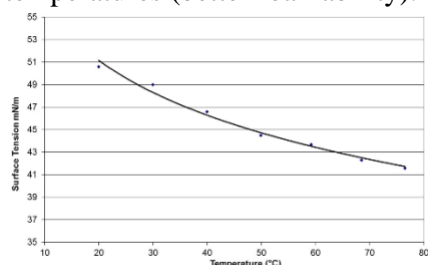


Fig. 1. Change in Interfacial Tension of a coffee solution with increasing temperatures.

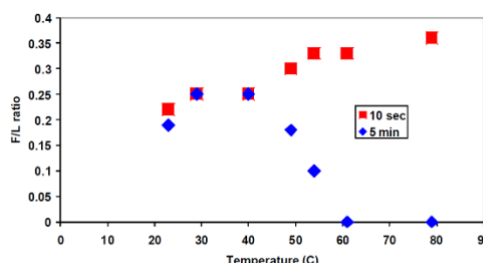


Fig. 2. Foamability (FLR-10 sec) and Foam stability (FLR-5 min) of instant coffee at different temperatures.

It was observed that the coffee exhibited a tendency to generate more foam with increase in temperature. However, at temperatures above 30°C, the foam became increasingly unstable. The coffee foam generated at temperatures below 40°C had much smaller bubble size and significantly higher stiffness.

### CONCLUSIONS & PERSPECTIVES

Dynamic interfacial tension of a coffee solution decreases with increase in temperature. Improvement in foamability was observed when foaming coffee at increasing temperatures. But the stability of the coffee foam followed an opposite trend. Coffee foam became significantly less stable at temperatures above 40°C. Factors such as disproportionation, larger bubbles and lowering of viscosity at the air liquid interface have a negative impact on coffee foam stability.

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## IMPACT OF COFFEE TYPE (ROBUSTA VS ARABICA) AND DEGREE OF ROASTING ON FOAMING PROPERTIES OF COFFEE BREW.

Deepak Sahai\*, Xiaoping Fu\*, and Alexander Sher\*  
Nestlé Development Center, Marysville, Ohio 43016, US

Robusta coffee showed better foaming properties, both in terms of foamability and foam stability than Arabica. Darker roast coffees showed better overall foaming performance than medium or lighter roast coffees. It was concluded that presence of high molecular weight surface active melanoidine material in coffee was conducive to coffee foaming and foam stability.

### RATIONALE

Understanding the impact of type of coffee (Robusta Vs Arabica) and the degree of roasting of the coffee beans is critical to understand the foaming properties of the brew.

### METHODS

The foaming property were evaluated using a whipping “Test Rig” device. Foamability and foam stability was expressed as foam to liquid ratio at 1 minute (FLR-1) and after 5 minutes (FLR-5) of foaming. Melanoidines the browning polymers in the coffee samples were profiled using size exclusion chromatography and detection at 280 nm.

### RESULTS

Test Rig” results showed that Robusta coffee showed a higher propensity to form foams as compared to Arabica coffee. Robusta foams were more stable as compared to Arabica coffee foams at all roast levels. Coffee foamability and foam stability also increased with darker roasting.

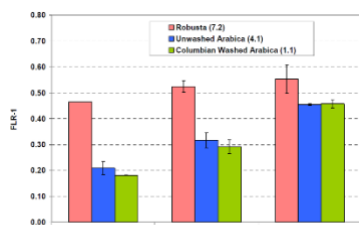


Fig. 1. Foam stability (FLR-5) of different coffee brews as impacted by degree of roasting

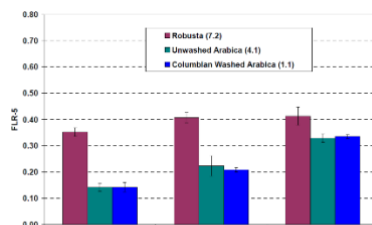


Fig. 2. Foam stability (FLR-1) of different coffee brews as impacted by degree of roasting

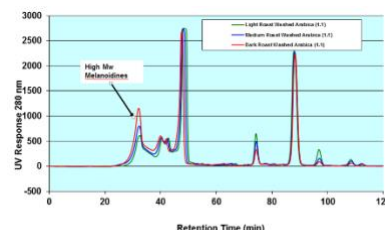


Fig. 3. Melanoidine profile of a brew with degree of roasting

The foaming and foam stability could be correlated to the relative concentration of melanoidine present in the coffee brew.

### CONCLUSIONS & PERSPECTIVES

- Robusta coffee brew showed higher foam volumes and better stability than Arabica coffee.
- Darker roasting for all coffee types improved propensity to form foams and foam stability.
- High molecular weight surface active browning polymers formed during roasting appear to play a role in foam stability. These browning polymers migrate to the air liquid interface and prevent drainage of liquid thus stabilizing the foam.

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KITZBERGER, CINTIA S.G.\*, GOMES-SANTIS, VALÉRIA B.\*, SERA, GUSTAVO H.\*, SCHOLZ, MARIA BRÍGIDA S\*.

\* Instituto Agronômico do Paraná, Londrina, IAPAR, BR.

## RATIONALE

The quality of the coffee beverage has been determined by various methods and a description of the sensory attributes of these brews is a way to attract attention to coffees with potential to specialty coffees. Coffees composition in relation to the chlorogenic isomers acids (CGA isomers) has attracted attention and can provide additional information on these coffees besides of the main aroma precursor generally studied. The objective of this work was associated the sensory description of the coffee beverages with the perfil of the main CGA isomers.

## METHODS

Cherry fruits from the following twenty arabica coffee genotypes were harvested from May to July 2015 in three locals denominated C (Congoinhas), A (Assaí) and L (Londrina) in Paraná-Brazil, washed and sun-dried on a patio: Catuaí, Iapar 59, IPR 97, IPR 98, IPR 99, IPR 100, IPR 101, IPR 102, IPR 103, IPR 104, IPR 105, IPR 106, IPR 107 and IPR108. Samples were standardized in a sieve (6.5 mm) and defective beans were removed. Fruit ripening, post-harvesting and drying processes were standardized to avoid interference in the descriptions of the sensory attributes of coffee beverages. Free choice profiling (FCP) a descriptive sensory analysis was applied and is based on the principle that people perceive the same characteristics in foods but express themselves differently to describe them [1]. The following classes of CGA isomers were detected, caffeoylquinic (3-CQA, 5-CQA, and 4-CQA), feruloylquinic (5-FQA), and dicaffeoylquinic (3,5; 3,4-diCQA and 4,5-diCQA) acids by HPLC-RP [2]. Principal component analysis and Procrustes generalizated analysis was used to analyze the data by XLSTAT statistical software program.

## RESULTS

The coffees Iapar 59 C, Catuaí C, IPR 97 L, IPR 98 L, IPR 100 L, IPR 101 L, IPR 102 L, IPR 103 L and IPR 104 L were characterized by attributes of coffee color appearance, turbidity, coffee, chocolate, sweet, leaf, burnt, off-flavor and acid, astringent, bitter, caramel and sweet taste and textured body. This coffees presented sensory description with positive connotations attributes and with potential to be explored as special. Catuaí A, IPR 98 A, IPR 99 A and IPR 107 A and Iapar 59 L, Catuaí L, IPR 99 L, IPR 105 L, IPR 106 L, IPR 107 L and IPR 108 L were characterized by coffee color and transparency appearance, and coffee, sweet, off-flavor, green and caramel aroma and bitter, sweet taste, and body texture. Although they present some attributes of positive connotation but in general presented a sensory description with few attributes and some of negative character. We can verify that the coffees with a greater number of positive attributes presented the lowest contents of all the CGA isomers, except for the IPR coffees 100, 101, 102, 103 and 104 that presented the highest values of 3-ACQ and 4-ACQ. This fact may be due to a more complete beans maturation, which can be evidenced by the higher ACQ/DCQA ratios presented by these coffees (values 3.72 to 5.33), which shows a relationship directly proportional to coffee beans maturation.

## CONCLUSIONS & PERSPECTIVES

Perfil of CGA isomers was associated to sensory attributes and positive connotations were correlating to lower values of main CGA isomers.

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## HIGH DIGESTION OF COFFEE BEANS USING COMBINED COFFEE AGP DEGRADING ENZYME AND VARIOUS CELLULASES, AND ITS INVESTIGATION FOR USE IN CAFFEIC ACID AND CAFFEINE ANALYSIS

SHIBATA Akira\*, MATSUNO Yoshiharu\*, YAMASHITA Ayu\*, NISHIKAWA Chiharu\*, YAMAMOTO Ryohei\*\*, YAMAMOTO Atsushi\*\*, and KASAI Naoya\*

\*Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka JAPAN ,

\*\*College of Bioscience and Biotechnology, Chubu University, Aichi JAPAN.

### RATIONALE

Coffee beans cell-wall is mainly consisted of arabinogalactanprotein (AGP), galactomannan (GM), and cellulose (C), and these are coagurated deeply and each other. We have found the selected effective cellulase, the one has the enzyme activity fractions of arabinofuranosidase, mannanase, and glucanase were concereted effective to cause the collapse, and then postulated that the AGP inhibits the cellulase digestion. Recently we have found a AGP degrading enzyme from microorganisms. In this time, the combination of various cellulases and the AGP degrading enzyme effect was investigated, and also done the test of the high-digestion is effective or not to improve extraction of the coffee acid and caffeine from coffee beans.

### METHODS

Coffee beans were gifted from UCC corporation of Japan. Various food use-cellulases were gifted from some enzyme makers in Japan. The beans was cracked and deffated with hexane soaking, the inner proteins was solubilized by 1N NaOH, 120°C, 20min. 1% Cellulase (*Trichoderma reesei*, GODO-TCF, Japan, 0.1M HAc buffer pH 5.0) was mixed the treated beans to digest at 40°C for over night. The cellulase was precipitated by heating at 121°C, 20min and removed. The supernatant was condensed using 100kDa membrane filter, the AGP was precipitated in 50% ethanol. The AGP degrading enzyme was produced by cultivation of AGP degrading bacterium of *Chitinophaga* sp. KSM45 using medium of the AGP and polypeptone. The section of coffee beans were soaked with pH 5.8 HAc buffer systems, at 30°C for 1week in a 96-well plate or PP tubes. The effects were investigated using phase-constant microscope. The analysis of coffee acid and caffeine of the digested coffee beans were also evaluated on HPLC system.

### RESULTS

The AGP degrading enzyme was effective to degrade the coffee cell walls, and the cell walls of the sectioned coffee beans were not stained by the beta Yariv reagent. The combined the AGP degrading enzyme of the *Chitinophaga* sp. KSM45 and some cellulases could digest the coffee sections effectively. The cellulose of the cell wall was digested to give glucose in the digestion of the combination of the enzymes. AGP is barrier for the cellulase digestion, and it was clearly shown that the AGP inhibit the digestion of cellulases. Extraction improvement of the coffee acid or caffeine from the beans using these enzymes was expected, but rather these compounds were decreased in the sample of the enzymes.

### CONCLUSIONS & PERSPECTIVES

AGP degrading enzyme was effective to digest AGP in the cell walls, and some cellulases combined with the AGP degrading enzymes were effective to digest, but were not in the all ones. The high digestion using the combined these enzymes could give a new or high treatment of a rich coffee roasting. The high digestion was not effective to improve for the extraction of caffeic acid or caffeine. These decreasing of the compounds were also found in the only addition of the enzymes.

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Modelling molecular release during brewing.

MELROSE, John\*, SCHLAURI, Paul\*\*, WELLINGER, Marco\*\*, YERETZIAN, Chahan\*\*.

\*Church Close House, Church Close, Great Bourton, Banbury OX17 1RW.

\*\*Institute of Chemistry and Biotechnology, Coffee Excellence Center, Zurich University of Applied Sciences, Einsiedlerstrasse 31, CH-8820 Wädenswil, Switzerland.

The measurement of releasing species over time can give new insight into the brewing of coffee (1-4). A model for coffee brewing (5) is used to fit data for caffeine, chlorogenic acids and melanoidins brewed from a packed bed in a Semi-Automatic brewing machine. For caffeine, where diffusion constants are known, good fits are found using diffusion constants with expected values. Fits for the other species will be presented. Using a model for the total yield the release of individual species against totally yield can be modelled. Composition (relative proportions of individual species) and yield are not one to one, coffee prepared to the same yield can have different compositions and the degree of variation possible will be estimated by modelling. Release of species may not always be pure diffusion, modelling of release under various interaction scenarios will be presented.

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Poster presented by John Melrose.



SMRKE, Samo\* ,LIU, Chujiao\*\*, STURROCK, Craig J.\*\*\*, FISK, Ian\*\*, YERETZIAN, Chahan.\*

\*Zurich University of Applied Sciences, Institute of Chemistry and Biotechnology, Coffee Excellence Center, Einsiedlerstrasse 31, 8820 Wädenswil, Switzerland.

\*\*Division of Food Sciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, United Kingdom

\*\*\*Agricultural and Environmental Sciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, United Kingdom

During coffee roasting a complex array of chemical reactions occur, the products of which are responsible for the generation of the much appreciated coffee aroma. The roasting process not only generates aroma, but also large amounts of gas is formed and released from the coffee beans such as CO<sub>2</sub> generated by Maillard reactions. Furthermore water is generated from chemical reaction, adding to the water from the initial moisture of the green beans. At the early stages of roasting the coffee beans undergoes a transition to a rubbery state [1] where its structure becomes soft and the escaping gases (mainly steam) cause the beans' volume to increase by 50% or more [2]. The consequence of this phenomenon is that the structure of the bean changes from a dense solid structure of the state of green beans to a highly porous structure of roasted beans. After the initial moisture level is driven down sufficiently, the beans transition from a rubbery state back to a glassy one, become brittle and due to the force of continuing release of gasses the beans' structure cracks, which is heard as audible pops, also called as the "first crack". At the late stages of roasting the rate of gas release increases to a level where the brittle structure of the beans starts to crack again causing the so called "second crack".

To explore the changes in the structure of the coffee bean and the impact of different roast profiles on the structural porosity, the roasted coffee beans were imaged using 3D X-ray computer tomography (CT) at 5 μm resolution. The coffee beans were roasted to three different roast degrees (light – just after first crack, medium, dark – at second crack). The X-ray CT data was reconstructed into images that sliced the bean along the long axis of the bean. Each of the slices was analysed using ImageJ software particle analysis to assess the size distribution of cross section pore area for each slice. To avoid analysing cracks in the bean's structure pore cross sections larger than 10 000 μm<sup>2</sup> were excluded from the dataset. The pore volume was modelled assuming a spherical shape. Volume weighted mean and mode of the pore size distribution was calculated from the dataset.

The pores size distribution of roasted coffee beans did not change drastically from light to dark roast. The maximum of the pore size distribution (volume weighted mode of diameter distribution) was calculated at 44.1 ± 0.2 μm (n=3), 47.1 ± 1.1 μm (n=5) and 49.1 ± 1.8 μm (n=3) for light, medium and dark roast, respectively. The volume weighted mean for light, medium and dark roasts was larger at 56.0 ± 1.7 μm, 62.1 ± 4.1 μm and 63.3 ± 4.8 μm. These results indicate a relatively large increase of pore volume from light to dark roast degree (about 40%). A typical increase in bulk volume of the beans from light to dark roast for the roasts conducted for these experiments was about 10%. The increase of measured mean pore size is potentially caused by shrinkage in the interstitial material though loss of organic material or by formation of cracks when roasting from light to dark roast, since both would also cause two adjacent pores being more often identified as a single pore.

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## A SIMPLE AND RAPID PRETREATMENT OF COFFEE BEANS WITH SERUM ALBUMIN FOR OCHRATOXIN A HPLC ANALYSIS

YAMATO Naoki\*, NAGAYA Atsumi\*, YAMAMOTO Ryohei\*, YAMAMOTO Atsushi\*, and KASAI Naoya\*\*

\* College of Bioscience and Biotechnology, Chubu University, Aichi JAPAN. \*\* Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka JAPAN.

### RATIONALE

We developed a rapid analytical method for ochratoxine A (OTA) in coffee beans. OTA is a mycotoxin that is produced by several *Aspergillus* and *Penicillium* species. Food commodities, including coffee beans, can be easily contaminated by OTA prior to harvest or, more commonly, during storage. In mammals, OTA is nephrotoxic and causes chronic tubulointerstitial nephropathy, which is known as a Balkan endemic nephropathy. Neurotoxicity, teratogenicity, and immunotoxicity were also observed by animal and cell experiments. The International Agency for Research on Cancer (IARC) has classified OTA into Group 2B (possibly carcinogenic to humans). The analytical methodology for OTA usually includes extraction, clean-up and separation/detection. Recently, an immunoaffinity column came to be frequently used in the sample clean-up of the OTA, and it represents the state of the art. However, this methodology is high-cost and tedious. We developed alternative pretreatment method using serum albumin instead of antibody. Serum albumin is known to capture OTA selectively.

### METHODS

Coffee beans were digested with cellulase to extract OTA and liquid-liquid partition of the extract with ethyl acetate was done as partial clean-up step. Then, enough amount of bovine serum albumin (BSA) was added into the reconstituted aqueous solution from organic layer. OTA was bound to BSA and the complex was separated by the ultrafiltration to remove low molecular impurities. OTA was eluted with methanol from the complex retained on the ultrafiltration membrane. Pyrene-1-carboxylic acid was used as an internal standard to compensate the volume of methanol eluent. The eluant was directly injected on the HPLC with fluorometric detection.

### RESULTS

We got clear HPLC chromatograms of OTA by above clean-up/pretreatment method. The sensitivity of the method was below 1 ng OTA/g coffee bean. The proposed clean-up method with BSA and ultrafiltration was useful tool for the practical monitoring of OTA in various coffee samples, such as raw coffee beans, roasted coffee beans and coffee beverages.

### CONCLUSIONS & PERSPECTIVES

We developed a simple and rapid clean-up method with BSA, which is a versatile reagent, for OTA analysis. In addition, the enzyme digestion enabled effective extraction of OTA from coffee beans. Consequently, the proposal method is an exceptionally useful for the practical monitoring of OTA, especially for solid sample like coffee beans, for example, wheat and soybeans.

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YOSHII Takaaki, IWASA Keiko, YAMADA Daisuke, NAKAHARA Koichi  
Research Institute, Suntory Global Innovation Center Limited, Kyoto, Japan

### RATIONALE

A bean is a bio-reactor, in which diverse flavor and aroma compounds are generated during roasting. A bean pops towards the end of roasting, because the bean can't withstand the rise in internal pressure due to the steam and organic gasses generated. The popping induces the volatilization of steam and organic gasses. So we hypothesize that this steam participate in the hydrolysis of chemical compounds in the bean.

On the other hand, we previously reported that the hydrolysis of glicoside and polyphenols in barely malts are a key reaction for beer quality in the subcritical water conditions [1, 2]. In this report, we study the water (steam) state in a bean and the hydrolysis, which impact on coffee quality.

### METHODS

We measured the moisture content in one coffee green bean. 10 g of green beans were set in the sealed vessel (32.2 mL) and heated in the oven. The pressure in the vessel was monitored at temperature from room temperature up to 220 °C. The compounds were also analysed during roasting.

### RESULTS

Moisture content in a green bean was 11.2 %. The water density in a bean was culculated based on the moisture content. It was under the saturated steam density curve in roasting condition. This result showed that water-containing state in a bean was saturated during roasting. To understand the physical property of water in a bean, we calculated the differential pressure between the pressure in the vessel during roasting and saturated steam pressure. The differential pressure had the inflect point approximately 200°C. So, it suggested that the popping occured at that temperature and that subcritical water presented in a bean . The information of analyses data validated that the hydrolysis by subcritical water generated the compounds, contributing to the flavor and aroma quality.

### CONCLUSIONS & PERSPECTIVES

We revealed the state of water inside bean in the sealed vessel during roasting. As a result of compounds analysis, it became clear that hydrolysis in beans proceeds by subcritical water. The new finding may be used to control hydrolysis and make flavor and aroma more diverse.

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## VACUUM PHOTOIONIZATION TOF-MS: A POWERFUL TOOL FOR THE INVESTIGATION OF COFFEE ROAST GAS COMPOSITION AND ON-LINE PROCESS CONTROL IN REAL-TIME

ZIMMERMANN, Ralf\*, HEIDE, Jan\*, EHLERT, Sven\*\*, CZECH, Hendryk\*, WALTE, Andreas\*\*, KOZIOROWSKI, Thomas\*\*\*.

\*University of Rostock, Joint Mass Spectrometry Centre, Rostock, GERMANY, \*\*Photonion GmbH, Schwerin, GERMANY, \*\*\*PROBAT GmbH, Emmerich am Rhein, GERMANY.

### RATIONALE

The steps required to turn green coffee into a popular beverage are highly complex. Especially the roast process decides upon the quality and flavor of the coffee in the final cup. Photoionization MS (PIMS) allows to monitor and to control this roasting process on-line and in real time, whereby time resolutions in the range of seconds enable the investigation of fast and dynamic processes.

### METHODS

Single-photon ionization (SPI) can be performed either with lamps or lasers as VUV-light source. The emitted wavelength limits the range of analytes to ionization energies from approximately 7 to 11 eV (equal 177 nm to 112 nm). Because most matrix gases such as oxygen, nitrogen, carbon dioxide, and especially water vapor have higher ionization energies than 11 eV, they do not appear in the mass spectrum. In resonance-enhanced multi-photon ionization (REMPI) at least two UV-photons are used for ionization. This ionization mechanism requires a stable excitable intermediate state, which determines the ionization selectivity. Accordingly, REMPI is generally selective for aromatic chemical structures and additionally for aliphatic amines in the lower UV range while it still suppresses the ionization of other roast gas constituents and especially background gases. In particular, the complementary use of SPI and REMPI can access new information.

### RESULTS

PIMS was used to investigate the coffee roasting process in from a single bean up to batch sizes in plant scale. By thermogravimetry (TG), the roasting process of single coffee beans was simulated and the evolved gases were monitored [1]. It is also possible to have a “chemical look” to the inside of a single coffee bean while it was roasted [2]. On a larger scale, statistical methods help to better understand the processes that occur during coffee roasting. [3] SPI and REMPI at multiple wavelengths were discussed regarding their benefits to detect roasting phases under different roasting conditions.

### CONCLUSIONS & PERSPECTIVES

PIMS is not only well-suited for the roast gas analysis, but also for other industrial roasting processes, such as of cocoa or nuts. It can also be applied in other research and industrial application focusing on a fast, time resolved analysis of complex gas mixtures.

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## EFFECTS OF COFFEE CONSUMPTION ON BRAIN POTENTIAL RELATED TO MOTOR PREPARATION AND ANTICIPATORY ATTENTION ASSOCIATED WITH POSTURAL CONTROL IN STANDING

MAEDA, Kaoru\*, KUDO, Shintaro\*, KANAO, Kenro\*, HORI, Ruji\*, KAKUDA, Akihiro\*, IWAI, Kazuya\*\*, FUKUNAGA, Taiji\*\*

\*Morinomiya University of Medical Science, Osaka, Japan, \*\*UCC Ueshima Coffee Co., Ltd., Osaka, Japan.

### RATIONALE

Postural control in standing human is reportedly affected by anticipatory and attentional process [1]. This process changes according to arousal level [2] which is reportedly increased by muscle contraction, somatosensory and auditory stimuli. However, few research has been investigated how the coffee consumption affects the brain activity during the preparatory period for postural control. In addition, whether decaffe affects postural control or not is a matter of significance in terms of prevention of excessive drug ingestion.

### METHODS

Thirteen subjects (mean age: 23.2 (SD = 4.3) years) were classified as either regular-coffee (N=6) or decaffe (N=7) group in a double-blinded fashion. Standing subjects rapidly flex their shoulder bilaterally from 0 to 90 degrees with sub-maximal speed in response to a second auditory signal (S2) presented 2 sec after the first signal (S1). This trial was repeated 15-20 times. Next, the subjects sat on a chair and drank a cup of coffee (the regular or decaffe). Ten minutes after the coffee consumption another 15-20 trials of shoulder flexion were performed. Electroencephalography (EEG) recorded between S1 and S2 was averaged (baseline was a mean amplitude of 500 ms-period before S1) and a negative shift of EEG (contingent negative variation; CNV) was obtained for the pre- and post-consumption trials, and a peak of CNV was detected. The peak latency of CNV was indicated by the time difference from S2 onset and the peak amplitude of CNV was measured from the baseline. Third, difference in the latency (Ld) and the amplitude (Ad) between pre- and post-consumption was calculated for each group (i.e. regular-coffee and decaffe group). Positive value of Ld corresponds to a delay of the peak emergence. Two sample *t*-test (alpha level = 0.05) was used to assess the significance of difference.

### RESULTS

The number of subject who exhibited CNV both pre- and post-consumption of coffee was 3 and 5 in regular-coffee and decaffe group, respectively. Ld in decaffe group was significantly larger than regular-coffee group ( $p < 0.05$ ). CNV peak amplitude showed no significant difference between pre- and post-consumption in both group.

### CONCLUSIONS & PERSPECTIVES

While coffee consumption has no effect on brain activity related to preparation for postural control and anticipatory attention removal of caffeine presumably leads to an extension of time required to reach a peak of the brain activity.

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## Effect of coffee aqueous extracts AND BIOACTIVE COMPOUNDS on probiotic bacteria GROWTH

SALES, Amanda\*, MIGUEL, Marco Antonio L.\*\*\*, FARAH, Adriana\*

\*Food Chemistry and Bioactivity Lab&Coffee Research Core (NUPECAFÉ), Nutrition Institute, Federal University of Rio de Janeiro (UFRJ), Brazil; \*\*\* Food Microbiology Lab, Microbiology Institute, UFRJ, Brazil. [afarah@nutricao.ufrj.br](mailto:afarah@nutricao.ufrj.br), [marco.miguel@micro.ufrj.br](mailto:marco.miguel@micro.ufrj.br)

**RATIONALE:** Non-digestible or partially digestible compounds in coffee such as polysaccharides (galactomannan-GM and type 2 arabionogalactan-AG) and chlorogenic acid (5-CQA) have been studied as enhancing agents for beneficial microbiota growth, especially bifidobacteria, but results involving lactobacilli strains and roasting degree are unclear. Among bioactive compounds in coffee are also caffeine-CAF and trigonelline-TG, which have showed selective antimicrobial effect *in vitro* for pathogenic bacteria. The present study aimed to investigate the effect of regular and decaffeinated (decaf) medium and dark roasted *Coffea arabica* and *C. canephora* aqueous extracts as well as coffee bioactive compounds on probiotic strains of *Lactobacillus* sp. and *Bifidobacterium* sp.

**METHODS:** The growth (24h and 48h) of *Lactobacillus rhamnosus* GG ATCC 53103 (GG), *L. acidophilus* LA-5 (LA), *Bifidobacterium animalis* DN-173010 (BA), and *B. animalis* subsp. *lactis* BB12 (BB12) was evaluated, separately, in a low-carbon sources culture medium enriched with 0.5, 1 and 1.5 g/100mL of soluble coffees from regular and decaf medium and dark roasted *C. arabica* and *C. canephora* seeds or with 50, 100, 200, 400 and 800mg/100mL of 5-CQA, TG, CAF, GM, and AG. The growth of non-probiotic *Escherichia coli* ATCC 25922 was also evaluated after incubation with the same extracts and compounds. Plain low-carbon sources culture medium was used as negative control (NC) and plain conventional MRS medium as positive control (PC) for probiotic bacteria. PC for *E.coli* was plain conventional Luria Bertani medium. Experiments were performed three times in duplicate. ANOVA was used to compare results followed by Tukey test ( $p \leq 0.05$ ).

**RESULTS:** After 48h incubation, NC and PC increased the count of probiotic tested strains (mean = 7.32 and 8.50 log CFU/mL, respectively). Compared to NC, regular *C. arabica* extract (regardless of roasting degree) increased the count of GG and LA in all concentrations (mean  $\Delta = 1.08 \log$  CFU/mL), and their growth was similar to PC. *C. arabica* extract also increased BA and BB12 growth (mean  $\Delta = 0.46$  and  $0.86 \log$  CFU/mL, respectively). Regular *C. canephora* and both decaf extracts increased the growth of probiotic bacteria similar to NC. Regarding bioactive compounds, the best *Lactobacillus* strain growth was observed for GG in 50mg of caffeine ( $\Delta = 1.64 \log$  CFU/mL), followed by all 5-CQA and TG concentrations ( $\Delta = 1.13$  and  $0.93 \log$  CFU/mL, respectively). No expressive growth was observed for LA when incubated with all bioactive compounds individually. For the *Bifidobacterium* strains, the greatest growth was observed for BA in all 5-CQA concentrations ( $\Delta = 0.17 \log$  CFU/mL) and in 400 and 800mg of GM ( $\Delta = 0.55 \log$  CFU/mL). For BB12, the greatest growth occurred in 200, 400 and 800mg of GM ( $\Delta = 0.69 \log$  CFU/mL) and in all concentrations of AG ( $\Delta = 0.76 \log$  CFU/mL). No growth was observed when *E. coli* was incubated with coffee extracts and bioactive compounds.

**CONCLUSIONS:** In this study, regular Arabica coffee improved the growth of probiotic strains of *Bifidobacterium* and *Lactobacillus* *in vitro* regardless of roasting degree. Different bioactive compounds (especially chlorogenic acids and polysaccharides) contributed to this potential prebiotic effect, which seems to be strain-dependent. Although caffeine and trigonelline can be well absorbed, they can also potentially improve the growth of *Lactobacillus rhamnosus* GG throughout the digestive tract, when ingested in physiological concentrations.



Bioactive AND POTENTIALLY DETRIMENTAL compounds in  
PERFORMANCE AND TRADITIONAL coffees from THE USA retail  
market

dePAULA, Juliana\*, REVI, Ildi\*\*, BATISTA, Alessandro Maia\*, FARAH, Adriana\*  
\*NutritionInstitute, Federal University of Rio de Janeiro, Brazil\* \*\* PurityCoffee, USA

**RATIONALE:** The USA stands out as the second largest coffee importer and the main consumer. Particularly the consumption of specialty coffee has been growing considerably in the last years. Coffee is considered by many as a functional food due to the high levels of bioactive compounds, especially chlorogenic acids (CGA) and lactones (CGL), caffeine and trigonelline, which are known to exert positive impacts on health. However, incidental carcinogenic contaminants such as mycotoxins produced by molds should be avoided and, potentially carcinogenic compounds like acrylamide should be minimized. The objective of this study was to investigate the levels of bioactive and detrimental compounds in a sample of coffees commercially available in the USA market.

**METHODS:** Sixteen ground roasted coffees were purchased in Greenville, South Carolina and via internet. They were divided into traditional (n=6) and performance (health claims stated on the labels, n=10). CGA&CGL<sup>1</sup>, caffeine<sup>1</sup>, trigonelline<sup>1</sup> and Ochratoxin A (AOAC method 2000.03) were analyzed by HPLC. Total aflatoxins was determined by immunoaffinity column (AOAC method 991.31) and acrylamide, by GC-MS<sup>2</sup>. Yeasts and molds counts were performed by optical microscopy. Cupping was performed by one cupper using Specialty Coffee Association (SCA) method. Unpaired *t*-test was used to compare group results, at 95% significance (GPad Prism v.7.0)

**RESULTS:** Nine isomers of CGA and two CGL were identified and quantified in all samples. The mean total CGA&CGL content in performance coffee samples was not different ( $1.3 \pm 0.4$  g/100 g, ranging from 0.5 to 2.1 g/100g) when compared to traditional ones ( $1.2 \pm 0.4$  g/100g, ranging from 0.8 to 1.7 g/100g). Regarding caffeine, the average contents in performance coffees ( $1.2 \pm 0.2$ g/100g, ranging from 0.9 to 1.5g/100g) were similar to traditional ones ( $1.3 \pm 0.2$ g/100g, ranging from 1.1 to 1.7g/100g). Like caffeine, trigonelline mean contents were similar in performance ( $0.9 \pm 0.2$ g/100g, ranging from 0.5 to 1.2g/100g) and traditional ( $1.0 \pm 0.1$ g/100g, ranging from 0.8 to 1.1g/100g) coffees. Performance coffees presented mean acrylamide contents of  $152.2 \pm 202.5$   $\mu$ g/kg, similar to traditional coffees ( $173.9 \pm 116.0$   $\mu$ g/kg). There is no regulation on the maximum limit of acrylamide allowed for coffees, however, in California (USA) it was suggested by Proposition 65 list, that if a food contains more than 275  $\mu$ g/kg of the contaminant, an alert must be placed on the label that the product contains acrylamide. Regarding mycotoxins, FDA has not established maximum limits in foods but the levels were in conformity with regulatory agencies from other countries, for example 10  $\mu$ g/kg in Brazil<sup>3</sup>. Additionally, regarding molds and yeasts, all traditional coffee samples were in conformity with current good manufacturing practices as required by the Food Safety Modernization Act (FSMA), while 4 samples (40%) among performance coffees presented levels that indicate non-conformity with good manufacturing practices. The cupping scores varied between 53 and 83 for performance coffees and from <40 to 73 for traditional coffees, suggesting that traditional coffees have inferior sensory quality and a higher number of defects when compared to performance ones which nevertheless did not present the expected high quality.

**CONCLUSIONS:** Although the number of samples was not representative of what is available in the market and more samples should be evaluated, there are not many brands in the performance category. The low levels of bioactive compounds associated with the molds and yeast counts in some of the samples did not reflect the health promotion claims stated in their labels.

**REFERENCES:** <sup>1</sup>Farah *et al.* *Food Chemistry*, 2006, 98(2), 373. <sup>2</sup>Soares *et al.* *Food Additives and Contaminants*, 2006, 23(12), 1276. <sup>3</sup>ANVISA, RDC07/2011