Asic
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28 June - 1 July

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28th Conference Asic 2021

BOOK OF ABSTRACTS

Asic · cirad · IRD Institut de Recherche pour le Développement · La Région Occitanie Pyrénées · Méditerranée · l'institut Agro agriculture • alimentation • environnement · SupAgro Montpellier · agropolis fondation · AGROPOLIS INTERNATIONAL · MUSE Montpellier University of Excellence

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Posters

PROGRAM AT A GLANCE

Connecting sustainability and coffee quality

Time CET *	MONDAY 28 JUNE	TUESDAY 29 JUNE
13:00	**OPENING SESSION** Room 1	**POSTER PRESENTATIONS**
	Astrid Nehlig Benoît Bertrand Andrea Illy	SESSION 1 Room 1 SESSION 2 Room 2 SESSION 6 Room 3
14:00	BREAK	BREAK
14:15	**PLENARY SESSION (KN)** Room 1	**PLENARY SESSION (KN)** Room 1
	Hans-Peter Landolt (S9) Karen Garrett & Aaron Plex Sulá (S2)	Robert Henry (S1) Imre Blank (S8)
15:45	BREAK	BREAK
16:00	**ORAL PARALLEL SESSIONS**	**ORAL PARALLEL SESSIONS**
	SESSION 8 Room 1 SESSION 1 SESSION 2 Room 2	SESSION 1 SESSION 2 Room 1 SESSION 7 SESSION 8 Room 2
17:30	BREAK	BREAK
17:45	**POSTER PRESENTATIONS**	**ASIC BOARD MEETING**
	SESSION 7 SESSION 8 Room 1 SESSION 2 Room 2 SESSION 1 Room 3	Astrid Nehlig Benoît Bertrand
18:45		

*Central European Time

Session 1: Plant science
Session 2: Plant pathology & protection
Session 3: Farm management
Session 4: Green coffee processing
Session 5: Sustainability, climate change & labels
Session 6: Biochemistry & biotechnology & composition of green coffee
Session 7: Roasted coffee technology & processing
Session 8: Coffee chemistry & sensory sciences
Session 9: Consumption, Health & Safety

PROGRAM AT A GLANCE

Connecting sustainability and coffee quality

Time CET *	WEDNESDAY 30 JUNE	THURSDAY 1 JULY
13:00		POSTER PRESENTATIONS
		SESSION 1 — Room 1
		SESSION 8 — Room 2
14:00		
		BREAK
14:15	PLENARY SESSION (KN) — Room 1	PLENARY SESSION (KN) — Room 1
	Gerhard Bytof (S4) Piet Van Asten (S5)	Nikolai Kuhnert (S7) Rodrigo Cunha (S9)
15:45	BREAK	BREAK
16:00	ORAL PARALLEL SESSIONS	ORAL PARALLEL SESSIONS
	SESSION 4 SESSION 6 — Room 1	SESSION 3 SESSION 5 — Room 1
	SESSION 5 — Room 2	SESSION 1 — Room 2
17:30	BREAK	BREAK
17:45	POSTER PRESENTATIONS	CLOSING — Room 1
18:00	SESSION 3 — Room 1	General Assembly — Room 1
	SESSION 4 SESSION 6 — Room 2	
	SESSION 5 — Room 3	
18:45		

*Central European Time

Session 1: Plant science
Session 2: Plant pathology & protection
Session 3: Farm management
Session 4: Green coffee processing
Session 5: Sustainability, climate change & labels
Session 6: Biochemistry & biotechnology & composition of green coffee
Session 7: Roasted coffee technology & processing
Session 8: Coffee chemistry & sensory sciences
Session 9: Consumption, Health & Safety

CET (Central European Time)

KN: Keynotes **PO:** Poster presentations
O: Oral presentations **P:** Posters (no oral presentation)

MONDAY 28 JUNE 2021

Opening Session Virtual room 1

13:00-14:00 **Chair: Astrid Nehlig**

13:00-13:30 • **Astrid Nehlig** (ASIC president) and **Benoît Bertrand** (ASIC secretary)

13:30-14:00 • **S0-KN - The future of the coffee industry. A strategy for the Green New Deal**
Andrea Illy (Honorary president)

14:00-14:15 Break

Plenary Session - Keynote lecturers

14:15-15:00 **Session 9: Consumption, Health & Safety** Virtual room 1

Chair: Astrid Nehlig

• **S9-KN - Will it keep me awake? Common coffee / caffeine intake habits and sleep in real life situations**
Dr Hans-Peter Landolt (Institute of Pharmacology and Toxicology - University of Zurich, Zurich, Switzerland)

• **Questions / Answers**

15:00-15:45 **Session 2: Plant pathology & protection**

Chair: Maria Do Céu Silva

• **S2-KN - Global change: Adapting coffee pest and disease management**
Prof. Karen A. Garrett and Aaron I. Plex Sulá (Plant pathology department - University of Florida, Gainesville, USA)

• **Questions / Answers**

15:45-16:00 Break

Oral Parallel Sessions (2 virtual rooms)

16:00-17:30 **Session 8: Coffee chemistry & sensory sciences** Virtual room 1

Chairs: Valérie Leloup & Imre Blank

16:00-16:30 • **S8-O-01 - A New (Sensory and Consumer) Coffee Brewing Control Chart**
Jean-Xavier Guinard (UC Davis Coffee Center, University of California, Davis, California, United States)

• **S8-O-02 - Modelling molecular release in coffee brewing**
John Melrose (Consultant, Church Close Great Bourton, Oxfordshire, United Kingdom)

• **S8-O-03 - The role of ethyl esters in green coffee: flavor or off-flavor precursors?**
Valentina Lonzarich (Aromalab - Ilycaffè spa, Trieste, Italy)

16:30-16:45 • **Questions / Answers**

16:45-17:15 • **S8-O-04 - Furan formation in coffee - Role of precursors and impact of the roast degree on reaction pathways**
Luigi Poisson (Nestlé PTC Beverage Orbe, Société Produits Nestlé SPN, Orbe, Switzerland)

• **S8-O-05 - Identification of coffee compounds that suppress bitterness of the brew**
Chengyu Gao (Department of Food Science and Technology, The Ohio State Univ., Columbus, OH, United States)

• **S8-O-06 - Characterisation of volatile and non-volatile compounds in cascara, a traditional beverage made from dried coffee pulp**
Aileen Pua (Food Science and Technology, National University of Singapore, Singapore, Singapore)

17:15-17:30 • **Questions / Answers**

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16:00-17:30 **Session 1:** Plant science / **Session 2:** Plant pathology & protection — Virtual room 2

Chairs: Alexandre de Kochko & Maria Do Céu Silva

16:00-16:30
- **S1-O-01 - Application of phylogenomics to reconstruct the evolutionary origin of *Coffea arabica***
  Yves Bawin (Plant Sciences Unit, Flanders Research Institute for Agriculture, Fisheries, and Food (ILVO), Melle, Belgium)
- **S1-O-02 - Study of circadian regulation of primary metabolism in coffee plant is key to develop coffee varieties adapted to climate change**
  Jean-Christophe Breitler (CoffeAdapt team, UMR DIADE, CIRAD, Xalapa, Veracruz, Mexico)

16:30-16:45
- **Questions / Answers**

16:45-17:15
- **S2-O-02 - Scaled-up genomic data uncovers a higher level of population genetic structuring in *Hemileia vastatrix***
  Ana Sofia B. Rodrigues (cE3c – Centre for Ecology, Evolution and Environmental Changes, Lisboa, Portugal)
- **S2-O-03 - ExpeRoya, a comprehensive model to forecast the risk of coffee leaf rust on *Coffea arabica* in Central America**
  Natacha Motisi (PRISM/PHIM/BIOS, Univ. Montpellier, CIRAD, Montpellier, France, CIRAD & CATIE, Turrialba, Costa Rica)
- **S2-O-04 - Identification of *Hemileia vastatrix* candidate effectors reveals new ways of promoting pathogen variability through alternative splicing**
  Silvia Tavares (CIFC, LEAF, Instituto Superior de Agronomia, Oeiras, Portugal)

17:15-17:30
- **Questions / Answers**

17:30-17:45 **Break**

Poster presentations (3 virtual rooms)

17:45-18:45 **Session 7:** Roasted coffee technology & processing
Session 8: Coffee chemistry & sensory sciences — Virtual room 1

Chairs: Robert Farr & Chahan Yeretzian

17:45-18:00
- **S8-PO-01 - Critical examination of particle swelling during hydration of ground coffee**
  Sala Kyle (Keurig Dr Pepper, Waterbury Center, Vermont, United States)
- **S8-PO-02 - Spectroscopic methods for discrimination of specialty coffee quality: NIR vs. FTIR**
  Adriana Franca (DEMEC/PPGCA, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil)
- **S8-PO-03 - Predictive Models for Precision Control of Drip Brew Coffee**
  William D. Ristenpart (Coffee Center, University of California Davis, Davis, CA, United States)
- **S8-PO-04 - Effect of storage condition on the shelf life of Liquid Coffee Concentrates and their relation with oxygen contents**
  Mónica Quintero (Research & Development, Colcafé S.A.S., Medellín, Colombia)
- **S8-PO-05 - Brew Temperature Strongly Affects the Color of Brewed Coffee**
  Sara Yeager (UC Davis Coffee Center, Davis, CA, United States)

18:00-18:15
- **Questions / Answers**

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18:15-18:30
- **S8-PO-07 - Cupping quality evaluation of hybrids of *Coffea arabica***
  Jorge Berny Mier y Teran (World Coffee Research, Portland, United States)
- **S7-PO-01 - Study of the lipid fraction of two hybrid varieties of coffee at different roasting temperature profiles**
  Oscar Gonzalez-Rios (UNIDA/Lab. Tecnología de Café, Tecnológico Nacional de México/Instituto Tecnológico de Veracruz, Veracruz, Veracruz, México)
- **S8-PO-08 - Consumer preferences for black coffee are spread over a wide range of brew strengths and extraction yields**
  Andrew Cotter (Food Science and Technology, University of California, Davis, Davis, CA, United States)
- **S9-PO-01 - α-Glucosidase enzyme inhibition effects, and antioxidant capacity in extracts of *Coffea arabica* leaves**
  Maria Teresa Salles Trevisan (Natural Products and Biotechnology, Univ. Federal do Ceará, Fortaleza, Brazil)

18:30-18:45
- **Questions / Answers**

17:45-18:45 **Session 2:** Plant pathology & protection — Virtual room 2

Chairs: Luc Villain & Jacques Avelino

17:45-18:00
- **S2-PO-01 - Estimating Coffee pest and disease attacks embedded application**
  Fabienne Ribeyre (UPR Bioagresseurs, Cirad/Univ. Montpellier, Montpellier, France)
- **S2-PO-02 - Small-RNA characterization of Coffee Leaf Rust races having different virulence profiles**
  Leonor Guerra-Guimarães (CIFC, LEAF, Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal)
- **S2-PO-03 - New technologies using volatiles for the control of coffee berry borers *(Hypothenemus hampei)***
  Carmenza Góngora (Entomology, Cenicafe. Colombian National Center of Coffee Research, Manizales, Caldas, Colombia)
- **S2-PO-04 - Assessing the involvement of retrotransposons in the genetic and genomic variability of *Hemileia vastatrix***
  Dora Batista (CIFC/LEAF, ISA, Universidade de Lisboa, Oeiras, Portugal)
- **S2-PO-05 - Selection of coffee progenies with multiple resistance to biotic agents**
  Larissa B. Caixeta (Centro de Café Alcides Carvalho, Instituto Agronômico de Campinas, Campinas, São Paulo, Brazil)

18:00-18:15
- **Questions / Answers**

18:15-18:30
- **S2-PO-07 - Crossings compatibility on *Coffea canephora* aiming multiple resistance in clones and full-sib progenies to *Meloidogyne exigua*, *M. incognita* and *M. paranaensis***
  Larissa Caixeta (Coffee Department, IAC - Instituto Agronômico de Campinas, Campinas, SP, Brazil)
- **S2-PO-08 - First steps on the resistance profiling of Kawisari coffee hybrid through cytological and gene expression analyses**
  Inês Diniz (CIFC, LEAF, Instituto Superior de Agronomia, Universidade de Lisboa, Oeiras, Portugal)

18:30-18:45
- **Questions / Answers**

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17:45-18:45 **Session 1:** Plant science — Virtual room 3

Chairs: Thierry Joët & Valérie Poncet

17:45-18:00
- **S1-PO-02 - Microscopy detection of cytoplasmic lipid droplets (LDs) in leaves of different *Coffea* species**
  Paola Crisafulli (illycaffè spa, Trieste, Italy) .../...
- **S1-PO-03 - Development of decaffeinated clonal cultivars of Arabic coffee**
  Julio Cesar Mistro (Instituto Agronômico de Campinas - IAC, Campinas, SP, Brazil)
- **S1-PO-04 - Spatial patterns of apparent fractionation on the subgenome chromosomes in *C. arabica* and gene loss in *C. canephora* and *C. eugenioides***
  David Sankoff (Mathematics and Statistics, University of Ottawa, Ottawa, Ontario, Canada)
- **S1-PO-05 - Warming impact and intraspecific differences in thermoregulation of *Coffea arabica* L. genotypes**
  Antonio Chalfun-Júnior (Plant Physiology Sector/Biology Department, Federal Univ. of Lavras, Lavras, MG, Brazil)

18:00-18:15
- **Questions / Answers**

18:15-18:30
- **S1-PO-06 - Genetic diversity of cultivated and wild *Coffea canephora* trees in Yangambi (DR Congo) and the risk of introgression**
  Lauren Verleysen (Plant, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Melle, Belgium)
- **S1-PO-07 - Genome-wide association study for morphological and yield component traits in *Coffea arabica***
  Eveline Caixeta (Embrapa Café/Bioagro/UFV, Embrapa, Viçosa, Minas Gerais, Brazil)
- **S1-PO-08 - Unravelling the Metabolic and Hormonal Machinery During Key Steps of Somatic Embryogenesis: A Case Study in Coffee**
  Rayan Awada (Nestlé Research, Plant Science Research Unit, Tours, France)
- **S1-PO-09 - Vegetative reproduction of *Coffea canephora* with different cutting standards**
  Fábio Luiz Partelli (Universidade Federal do Espírito Santo, São Mateus, ES, Brazil)
- **S1-PO-10 - Responses of Arabica coffee (*Coffea arabica* L. var. Catuaí) cell suspensions to chemically induced mutagenesis and salinity stress under in vitro culture conditions**
  Andrés Gatica-Arias (Universidad de Costa Rica, San Pedro de Montes de Oca, Costa Rica)

18:30-18:45
- **Questions / Answers**

CET (Central European Time)

KN: Keynotes **PO:** Poster presentations
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TUESDAY 29 JUNE 2021

Poster presentations (3 virtual rooms)

13:00-14:00 **Session 1: Plant science** Virtual room 1

Chairs: Thierry Leroy & Christophe Montagnon

13:00-13:15
- **S1-PO-11 - Coffee Genetic Resources in Yemen, Diversity and Importance for Arabica Coffee Improvement**
  Amin Al-Hakimi (Agronomy, Faculty of Agriculture, Sana'a Universit, Sana'a, Yemen)
- **S1-PO-12 - From the herbarium back to the forest: a successful collection of wild Robusta coffee *(Coffea canephora)* in Guinea**
  Jean-Pierre Labouisse (UMR AGAP, CIRAD, Montpellier, France)
- **S1-PO-13 - A contribution to the future of Robusta coffee by investing in the INERA coffee collection in Yangambi (the Democratic Republic of the Congo)**
  Piet Stoffelen (Meise Botanic Garden, Meise, Belgium)
- **S1-PO-14 - *Coffea* spp. Membrane Responses to Superimposed Elevated [CO_2] and Drought in View of Higher Acclimation Ability**
  Paula Scotti-Campos (UIBRG - Plant Physiology Laboratory, Instituto Nacional de Investigação Agrária e Veterinária, I.P., Oeiras, Portugal)
- **S1-PO-15 - Targeted and untargeted metabolomics for the valorisation of *Coffea anthonyi***
  Andrea Montis (RD3 Unit of Pharmacognosy, Bioanalysis and Drug Discovery, Faculty of Pharmacy, Université libre de Bruxelles, Campus Plaine, Brussels, Belgium)

13:15-13:30
- **Questions / Answers**

13:30-13:45
- **S1-PO-16 - Safeguarding the diversity of species of the genus *Coffea* in Réunion Island**
  Thierry Joët (DIADE, IRD, Université Montpellier, Montpellier, France)
- **S1-PO-17 - Functional characterization of caffeine synthase genes from wild coffee, and discussion of those molecular evolutions**
  Kouichi Mizuno (Akita Prefectural University, Akita, Japan)
- **S1-PO-18 - Studies of the *Baracoffea*: Malagasy coffee trees growing on the West Coast of Madagascar**
  Sylvie Sabatier (UMR AMAP, CIRAD, Montpellier, France)
- **S1-PO-19 - Chromosome-level assembly of allotetraploid Coffea arabica reveals the complex history of a recent allopolyploid**
  Jarkko Salojärvi (School of Biological Sciences, Nanyang Technological University, Singapore, Singapore)
- **S1-PO-20 - The transcriptomic basis for understanding the mitigation of heat impact by elevated [CO_2] in the photosynthetic response of *Coffea arabica* and *C. canephora***
  Isabel Marques (Instituto Superior de Agronomia, Lisbon Portugal)

13:45-14:00
- **Questions / Answers**

.../...

13:00-14:00 **Session 2:** Plant pathology & protection — Virtual room 2

Chairs: Diana Fernandez & Robert Barreto

13:00-13:15
- **S2-PO-10 - Survey of *Hemileia vastatrix* races from Peru to identify potential coffee mutants with disease resistance**
  Maria do Céu Silva (CIFC -Centro de Investigação das Ferrugens do Cafeeiro/LEAF- Linking Landscape, Universidade de Lisboa, Instituto Superior de Agronomia, LISBOA, Portugal)
- **S2-PO-13 - Identification of distinctive transcriptomic profiles among *Hemileia vastatrix* pathotypes throughout key stages of the infection process**
  João Birg (cE3c, FCUL/Universidade de Lisboa, Lisboa, Portugal)

13:15-13:30
- **Questions / Answers**

13:30-13:45
- **S2-PO-14 - Agro-climatic constraints to integrated Coffee Berry Borer Management**
  Bernard Pierre Dufour (UMR PHIM, Plant Health institute/Bios, CIRAD, Montpellier, France)
- **S2-PO-16 - Agrobacterium tumefaciens-mediated transformation revealed an alkaline phytoceramidase that is required in pathogenicity of *Colletotrichum kahawae* to *Coffea arabica***
  Helena Azinheira (DCEB - CIFC, Instituto Superior de Agronomia (ISA), Universidade de Lisboa (ULisboa), Lisboa, Portugal)
- **S2-PO-17 - Characterising tolerance to root knot nematodes in *Coffea canephora* varieties**
  Adam Casey (Centre of plant sciences, University of Leeds, Leeds, United Kingdom)
- **S2-PO-18 - Identification of bacterial endophytes of interest for coffee crop in Vietnam**
  Benoit Duong (UMR LSTM, IRD/AGI, Hanoi, Vietnam)

13:45-14:00
- **Questions / Answers**

13:00-14:00 **Session 6:** Biochemistry & biotechnology & composition of green coffee — Virtual room 3

Chairs: Luciano Navarini & Claudine Campa

13:00-13:15
- **S6-PO-02 - Biochemical characterization of the genetic resources of wild coffee trees collection in Réunion using near infrared spectroscopy**
  Fabrice Davrieux (Qualisud, CIRAD, Saint Pierre, Réunion)
- **S6-PO-03 - Isolation of Mycotoxigenic Fungi and Quantification of Ochratoxin A from Coffee in Ethiopia**
  Legese Hagos (Food Science and Nutrition, Ethiopian Institute of Agricultural Research, Jimma, Oromia, Ethiopia)
- **S6-PO-04 - Production and transfer kinetics of three aroma compounds into the coffee beans during simulated wet processing and their fate after the transfer**
  Fatma Hadj Salem (Umr QualiSud, CIRAD, Montpellier, France)

13:15-13:30
- **Questions / Answers**

13:30-13:45
- **S6-PO-05 - Isolation and characterization of linalool UDP-Glc glycosyltransferases from *Coffea arabica***
  Miho Ida (Faculty of Bioresource Sciences, Akita Prefectural University, Akita, Japan)
- **S6-PO-06 - Substances with physiological effects in several tissues of different coffee species - Part 1 diterpenes**
  Isabelle Kölling-Speer (Prof. für Spezielle Lebensmittelchemie, Technische Univ. Dresden, Dresden, Germany)
- **S6-PO-07 - Application of spent coffee grounds in water treatment for hemodialysis by adsorption of residual chlorine**
  Yoshihiro Tsuji (Department of Medical Engineering, Faculty of Health Sciences, Morinomiya University of Medical Sciences, Osaka-city, Osaka, Japan)
- **S6-PO-08 - Profiling of Robusta coffee *(Coffea canephora)* genotypes in the Democratic Republic of the Congo using untargeted metabolomic analysis on green and roasted coffee**
  Robrecht Bollen (Meise Botanical Garden, Meise, Belgium)

13:45-14:00
- **Questions / Answers**

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14:00-14:15 **Break**

Plenary Session - Keynote lecturers

14:15-15:00 **Session 1:** Plant science — Virtual room 1

Chair: Benoît Bertrand

- **S1-KN - Genomics of Coffee Quality**
  Prof. Robert Henry (Director of the Queensland Alliance for Agriculture and Food Innovation (QAAFI) - University of Queensland, Brisbane, QLD, Australia)

- **Questions / Answers**

15:00-15:45 **Session 8:** Coffee chemistry & sensory sciences

Chair: Valérie Leloup

- **S8-KN - The molecular code of coffee aroma and taste**
  Prof. Imre Blank (Coffee Excellence Center - ZHAW, Zürich, Switzerland)

- **Questions / Answers**

15:45-16:00 **Break**

Oral Parallel Sessions (2 virtual rooms)

16:00-17:30 **Session 1:** Plant science / **Session 2:** Plant pathology & protection — Virtual room 1

Chairs: Thierry Joët & Diana Fernandez

16:00-16:30
- **S1-O-03 - A single polyploidization event at the origin of the tetraploid genome of *Coffea arabica* is responsible for extremely low genetic variation in wild & cultivated germplasm**
  Michele Morgante (Istituto di Genomica Applicata, Udine, Italy)
- **S2-O-05 - The quest for sustainable management of coffee leaf rust with endophyte bodyguards from coffee and mycoparasites of *Hemileia vastatrix***
  Robert Barreto (Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, MG, Brazil)
- **S1-O-04 - Comparative analyses between *Coffea canephora* and *C. humblotiana*, a caffeine-free species, provide insight to determine the origin of the absence of caffeine synthesis**
  Romain Guyot (IRD, Montpellier, France / Universidad autonoma de Manizales, Manizales, Colombia)

16:30-16:45
- **Questions / Answers**

16:45-17:15
- **S2-O-06 - Is the incidence of fungal diseases on Arabica coffee in it's native range related to genetic variation in coffee?**
  Beyene Zewdie Hailu (Department of Ecology, Environment and Plant Sciences, Stockholm Univ., Stockholm, Sweden)
- **S2-O-07 - The mycobiome of wild Rubiaceae to improve the health of coffee plants**
  Priscila Chaverri (CIPRONA, Universidad de Costa Rica, San Pedro, San Jose, Costa Rica)

17:15-17:30
- **Questions / Answers**

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16:00-17:00 **Session 7:** Roasted coffee technology & processing

Session 8: Coffee chemistry & sensory sciences Virtual room 2

Chairs: John Melrose & Nikolai Kuhnert

16:00-16:30
- **S7-O-01 - Coffee bean particle motion: implications for heat transfer during roasting**
  Mark Al-Shemmeri (Jacobs Douwe Egberts, Banbury, United Kingdom)
- **S7-O-02 - Numerical Simulation of Coffee Extraction Based on Mass Transfer**
  Yoshihiko Sano (Department of Mechanical Engineering, Shizuoka University, Hamamatsu, Japan)
- **S7-O-03 - A New Method for Measuring Early Time Scale Coffee Extraction Kinetics in a Well-Stirred Batch Reactor**
  Matthew Maille (Department of Chemical and Biological Engineering, Univ. of Sheffield, Sheffield, United Kingdom)

16:30-16:45
- **Questions / Answers**

16:45-17:15
- **S8-O-07 - Towards smart on-line industrial coffee roasting process control: A new photoionization mass spectrometry (PIMS) for real-time monitoring of roasting process parameters**
  Jan Heide (Analytical Chemistry/CMA, University of Rostock and Helmholtz Zentrum München, Rostock, Germany)
- **S8-O-08 - Sensory Profiles of Cold, Ambient, and Hot Full Immersion Coffee Brews**
  Mackenzie Batali (Food Science and Technology, University of California, Davis, Davis, CA, United States)
- **S8-O-09 - NMR metabolomics as a tool for Arabica green coffee traceability**
  Vincent Portaluri (Eurofins Analytics France, Nantes, France)

17:15-17:30
- **Questions / Answers**

17:30-17:45 **Break**

17:45-18:45 **ASIC Board meeting**
- **Astrid Nehlig**
- **Benoît Bertrand**

CET (Central European Time)

KN: Keynotes **PO:** Poster presentations
O: Oral presentations **P:** Posters (no oral presentation)

WEDNESDAY 30 JUNE 2021

Plenary Session - Keynote lecturers

14:15-15:00 **Session 4:** Green coffee processing — Virtual room 1

Chair: Luciano Navarini

- **S4-KN - Coffee postharvest - a crucial processing step for maintaining green coffee quality - or even more?**
  Dr Gerhard Bytof (Senior Manager Coffee & Science - Tchibo GmbH. Coffee Technology, Science and Research, Hamburg, Germany)
- **Questions / Answers**

15:00-15:45 **Session 5:** Sustainability, climate change & labels

Chair: Philippe Vaast

- **S5-KN - Coffee and Climate Change: the wealthy will win, the poor will lose, and coffee will survive**
  Dr Piet Van Asten (Agronomy Head. Coffee plantations - OLAM International, Kampala, Uganda)
- **Questions / Answers**

15:45-16:00 Break

Oral Parallel Sessions (2 virtual rooms)

16:00-17:30 **Session 4:** Green coffee processing

Session 6: Biochemistry & biotechnology & composition of green coffee — Virtual room 1

Chairs: Gerhard Bytof & Chahan Yeretzian

16:00-16:30
- **S4-O-01 - Untargeted metabolomics of green coffee and correlation to sensory attributes**
  Ric de Vos (Bioscience, Wageningen University & Research, Wageningen, The Netherlands)
- **S4-O-02 - Impact of post-harvest processing on bean composition and in-cup flavor of Arabica and Robusta coffee**
  Johannes Polster (Nestlé Product Technology Centre Beverage, Société des Produits Nestlé SA, Orbe, Switzerland)
- **S6-O-01 - *In vitro* inhibition of *Aspergillus carbonarius* growth and reduction of Ochratoxin A (OTA) contents using *Lactobacillus plantarum* strains isolated from coffee cherries**
  Corinne Beugré (QUALISUD, CIRAD - Université Nangui Abrogoua, Montpellier, France)

16:30-16:45
- **Questions / Answers**

16:45-17:15
- **S6-O-02 - Coffee (*Coffea arabica* L.) bean transcriptome and volatiles under stress**
  Fabián Echeverría-Beirute (CIDASTH, Instituto Tecnologico de Costa Rica, San Carlos, Alajuela, Costa Rica)
- **S6-O-03 - XRF- and ICP-based multi-element and stable isotope profiling can be used to differentiate the geographical origin of Ethiopian coffee**
  Mohammed Worku Adem (Horticulture and Plant Sciences, Jimma University, Jimma, Ethiopia)
- **S6-O-04 - Metabolomic profiling of *Coffea mauritiana* leaf extracts from the Mascarene islands, and comparison with other coffee species for identification of novel biomarkers**
  Laura Lallemand (CYROI, Ste-Clotilde, France ; 2 UMR QualiSud, St-Pierre, France)

17:15-17:30
- **Questions / Answers**

.../...

16:00-17:30 **Session 5:** Sustainability, climate change & labels — Virtual room 2

Chairs: Piet van Asten & Bruno Rapidel

16:00-16:30
- **S5-O-01 - Sustainability projects - sustaining old governance structures?**
  Aske Skovmand Bosselmann (Department of Food and Resource Economics, University of Copenhagen, Frederiksberg C, Denmark)
- **S1-O-05 - Enhancing the adoption worldwide of Arabica hybrids through implementation of on-farm trials, transfer of propagation techniques and stakeholder dialog platforms**
  Hervé Etienne (CoffeeAdapt team, UMR DIADE, BIOS department, CIRAD, Montpellier, France)

16:30-16:45
- **Questions / Answers**

16:45-17:15
- **S5-O-02 - Learnings of 15 years of coffee Cup of Excellence competition in 15 countries: Specific insight on the importance of varieties**
  Christophe Montagnon (RD2 Vision, Valflaunes, France)
- **S5-O-03 - Gorongosa Coffee: Sustainable coffee production in the Gorongosa National Park in the context of deforestation, climate change and food security**
  Ana I. Ribeiro-Barros (Forest Research Center, University of Lisbon, School of Agriculture, Lisbon, Portugal)
- **S5-O-04 - The Tanzanian smallholder coffee growers investment decisions in coffee production**
  Leonard Kiwelu (Special Project Unit, Tanzania Coffee Research Institution (TaCRI), Moshi, Kilimanjaro, Moshi, Tanzania)

17:15-17:30
- **Questions / Answers**

17:30-17:45 **Break**

Poster presentations (3 virtual rooms)

17:45-18:45 **Session 3:** Farm management — Virtual room 1

Chairs: Christophe Montagnon & Anders Raebild

17:45-18:00
- **S3-PO-01 - k-SCAS: Framework for a Knowledge-Driven Specialty Coffee Agribusiness System**
  Eduardo Trauer (ESAG / Business Administration / P.G.P. Engineering and Knowledge Management, State University of Santa Catarina / Federal University of Santa Catarina, Florianópolis, SC, Brazil)
- **S3-PO-02 - From Simulation Game to Early Warning System: an interactive Agent-Based Model to fight coffee rust in Central America**
  Pierre Bommel (Green RU 47, Univ Montpellier, CIRAD, Montpellier, France / CIRAD & CATIE, Turrialba, Costa Rica)
- **S3-PO-03 - Identification of constraints affecting the coffee productivity and quality in Burundi**
  Alain Kagisye (UCLouvain, Ottignies-Louvain-La-Neuve, Louvain-La-Neuve, Belgium)
- **S3-PO-04 - Inclusion of farmers in *Coffea canephora* selection process through surveys**
  Claire Ged (Coffee and Cocoa, Nestlé Research, Tours, France)
- **S3-PO-05 - Influence of Balanced Nutrition on quality of *Coffee Arabica* L.**
  Victor Hugo Ramirez-B. (Yara-International, Research Center for Crop Nutrition and Environment, Dülmen, North Rine Westfalen, Germany)

18:00-18:15
- **Questions / Answers**

.../...

18:15-18:30
- **S3-PO-08 - Grafting as a way to modulate expression of physiological and biochemical parameters linked to drought tolerance in *Coffea canephora***
  Jerome Spiral (Plan Science Research Unit, Nestlé Research Tours, Notre Dame d'Oé, France)
- **S3-PO-09 - First results on growth and yields of *Coffea arabica* L. var. Laurina plants grafted on 5 different rootstocks**
  Gian Luca Malvicini (Coffee Procurement Dept., illycaffè S.p.A., Trieste, Italy)
- **S3-PO-10 - Using local knowledge to identify shade tree species that best suit farmer's needs in coffee farms in Western highlands of Cameroon**
  Baptiste Camus (ISTOM, Angers, France / UMR AGAP Institut, Univ. Montpellier, CIRAD, INRAE, Institut Agro, Montpellier, France)

18:30-18:45
- **Questions / Answers**

17:45-18:15 **Session 4:** Green coffee processing

Session 6: Biochemistry & biotechnology & composition of green coffee — Virtual room 2

Chairs: Marino Petracco & Noël Durand

17:45-18:00
- **S4-PO-01 - The influence of microorganism succession at different coffee drying stages in beverage quality**
  Aldir Teixeira (Experimental Agricola do Brasil, São Paulo, SP, Brazil)
- **S4-PO-03 - La Cumplida Refinada: sustainable coffee fermentation**
  Frederic Mestdagh (Green Coffee Quality and Development, Nestlé Nespresso, Romont, Switzerland)
- **S6-PO-09 - Deep-dive into the role of coffee microorganisms on flavor generation during post-harvest processing**
  Cyril Moccand (Department of Biology, Nestlé Research, Lausanne, Switzerland)

18:00-18:15
- **Questions / Answers**

18:15-18:30
- **S6-PO-10 - Transcriptome and biochemical analyses of diterpene synthases from *Coffea arabica* L.**
  Suzana T. Ivamoto-Suzuki (Laboratório de Ecofisiologia e Biotecnologia Agrícola, Universidade Estadual de Londrina, Londrina, Paraná, Brazil)
- **S6-PO-11 - Changes in the sensory and volatile characteristics of coffee quality during storage in modified atmospheres**
  Oscar González-Ríos (UNIDA/Tecnología del Café, Tecnológico Nacional de México/Instituto Tecnológico de Veracruz, Veracruz, Veracruz, México)
- **S6-PO-12 - Quality comparison of Arabusta and Robusta grown in French Guiana**
  Cécile Abdallah (UMR DIADE, Univ. Montpellier, IRD, CIRAD, Montpellier, France)
- **S6-PO-13 - Genomics, lipidomics and metabolomics profiling of *Coffea canephora* L. cultivated and conserved in South-western Nigeria**
  Chinyere Anagbogu (Plant Breeding, Cocoa Research Institute of Nigeria, Ibadan, Oyo, Nigeria)

18:30-18:45
- **Questions / Answers**

.../...

17:45-18:15 **Session 5:** Sustainability, climate change & labels — Virtual room 3

Chairs: Aske Bosselmann & Bruno Rapidel

17:45-18:00
- **S5-PO-01 - Local agroforestry knowledge and development of an online decision-support tool (shadetreeadvice.org) for selection of trees to be associated to coffee in North Vietnam**
  Philippe Vaast (UMR Eco&Sols, CIRAD, Hanoi, Vietnam)
- **S5-PO-02 - Shaping climate-smart coffee landscapes to unite farm-based climate-smart practices with landscape scale benefits**
  Paul Günter Schmidt (Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid (UPM), Madrid, España)
- **S5-PO-03 - A new approach to detecting deforestation in coffee growing regions**
  David Browning (Enveritas, Old Greenwich, CT, United States)
- **S5-PO-04 - Statistical analysis of the weather impact on Robusta coffee yield in Vietnam**
  Thi Lan Anh Dinh (LERMA, Observatoire de Paris, Paris, France)
- **S5-PO-05 - Coffee cultivation at Mt. Elgon: perspectives and challenges in a changing climate**
  Alejandra Sarmiento Soler (Tropical Plant Production and Agricultural Systems Modelling, University of Göttingen, Göttingen, Germany)

18:00-18:15
- **Questions / Answers**

18:15-18:30
- **S5-PO-07 - Cost Structure of Specialty Coffee Production in Honduras and El Salvador**
  Carlos Carpio (Texas Tech University, Lubbock, Texas, United States)
- **S5-PO-08 - Impact of high atmospheric CO_2 concentration on the seasonality of gas exchange and carbohydrate metabolism in coffee trees under field conditions**
  Emerson Alves da Silva (Fisiologia e Bioquímica de Plantas, Instituto de Botânica, São Paulo, SP, Brazil)
- **S5-PO-10 - Ecophysiological performance of *Coffea arabica* L. under agroforestry system on Gorongosa mountain, Mozambique**
  Crimildo Cassamo (PlantStress & Biodiversity Lab, LEAF or CEF, Instituto Superior de Agronomia, Universidade de Lisboa, Oeiras and Lisboa, Portugal)

18:30-18:45
- **Questions / Answers**

CET (Central European Time)

KN: Keynotes **PO:** Poster presentations
O: Oral presentations **P:** Posters (no oral presentation)

THURSDAY 1 JULY 2021

Poster presentations (2 virtual rooms)

13:00-14:00 **Session 1: Plant science** Virtual room 1

Chairs: Jose Rafael Chan & Alexandre de Kochko

13:00-13:15
- **S1-PO-22 - Root Microbiome of Arabica Coffee Plant Grown in Different Geographical Location**
  Luciano Navarini **(illycaffè spa, Trieste, Italy)**
- **S1-PO-23 - Seed purity of the first two commercial coffee hybrid varieties *(C. arabica)* Star2 and Star3 in Ecuador**
  Victoria Berry (Plant Science Research Unit, Nestlé R&D Tours, Notre Dame d'Oé, France)
- **S1-PO-25 - Exploring drought tolerance variation in Ugandan *Coffea canephora***
  Catherine Kiwuka (Centre for crop systems analysis, Wageningen University and Research, Wageningen, Netherlands)

13:15-13:30
- **Questions / Answers**

13:30-13:45
- **S1-PO-26 - Water-use efficiency of new *Coffea arabica* F1 hybrids undergoing different water availability in an agroforestry system**
  Thuan Sarzynski (DIADE, University Montpellier, CIRAD, IRD, Montpellier, France / NOMAFSI, Son La, Vietnam)
- **S1-PO-27 - Genomic characterization of 10 Vietnamese elite clones of Robusta *(Coffea canephora)***
  Bao Tram Vi (UMR DIADE, Univ. Montpellier, CIRAD, IRD, Montpellier, France / AGI, Hanoi, Vietnam)
- **S1-PO-28 - WCSdb: A database of Wild Coffea Species**
  Romain Guyot (UMR DIADE, IRD, Montpellier, France)
- **S1-PO-29 - Genetic Variability and Genetic Structure of Thai Arabica Coffee hybrids (*Coffea arabica* L.) Based on SSR markers and A Model-based Genetic Clustering Method**
  Chatnapa Khomarwut (Ministry of Agricultural and Cooperative, Department of Agriculture (DOA), Bangkok, Thailand)
- **S1-PO-30 - Tailoring the creation of next generation of coffee varieties in Rwanda**
  Simon Martin Mvuyekure (Rwanda Agriculture Board, Kigali, Rwanda)

13:45-14:00
- **Questions / Answers**

13:00-14:00 **Session 8: Coffee chemistry & sensory sciences + others** Virtual room 2

Chairs: Imre Blank & Robert Farr

13:00-13:15
- **S8-PO-09 - Optimization of espresso coffee extraction to lower the amount of coffee**
  Simone Angeloni (School of Pharmacy, University of Camerino, Camerino, Italy / RICH - Research and Innovation Coffee Hub, Belforte del Chienti, Italy)
- **S8-PO-10 - A metabolomics approach to discriminate which compounds contribute to the sensory characters of coffee brew**
  Taku Hanzawa (Research & Development Department, UCC Ueshima Coffee Co., Ltd, kobe, Japan)
- **S8-PO-11 - A simple predictive model for the espresso coffee Extraction Yield**
  Alessia Perticarini (School of Sciences and Technology - Mathematics Division, University of Camerino, Camerino, Italy / RICH - Research and Innovation Coffee Hub, Belforte del Chienti, Italy)

13:15-13:30
- **Questions / Answers**

.../...

13:30-13:45
- **S8-PO-13 - The role of chemometrics in the characterization of coffee quality**
  Erica Liberto (Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Torino, Italy)
- **S8-PO-14 - Developing a milk coffee flavor wheel for Japanese consumers**
  Shinichiro Hatakeyama (Food Research & Development Institute, Morinaga Milk Industry Co., Ltd, Zama, Kanagawa, Japan)

13:45-14:00
- **Questions / Answers**

14:00-14:15 **Break**

Plenary Session - Keynote lecturers

14:15-15:00 **Session 7:** Roasted coffee technology & processing — Virtual room 1

Chair: Chahan Yeretzian

- **S7-KN - Mass spectrometry based profiling of coffee chlorogenic acids**
  Prof. Nikolai Kuhnert (Department of Life Science and Chemistry, Jacobs University Bremen, Bremen, Germany)
- **Questions / Answers**

15:00-15:45 **Session 9:** Consumption, Health & Safety

Chair: Astrid Nehlig

- **S9-KN - A cup of coffee for a healthier aging**
  Prof. Rodrigo Cunha (Center for Neuroscience and Cell Biology - Multidisciplinary Institute of Ageing MIA - Faculty of Medicine, University of Coimbra, Coimbra, Portugal)
- **Questions / Answers**

15:45-16:00 **Break**

Oral Parallel Sessions (2 virtual rooms)

16:00-17:30 **Session 3:** Farm management / **Session 5:** Sustainability, climate change & labels — Virtual room 1

Chairs: Anders Raebild & Philippe Vaast

16:00-16:30
- **S5-O-05 - Patterns of Global Collaboration in Research and Innovation in Coffee Genetic Diversity and Breeding for Enhanced Sustainability**
  Selim Louafi (BIOS, CIRAD, Montpellier, France)
- **S5-O-07 - Elevated [CO_2] buffers the effects of severe soil drought cycles on water status, photosynthesis and growth of *Coffea arabica***
  Ana Karla Lobo (Plant Genomics and Transcriptomes Group/Department of Botany, Sao Paulo State University, Rio Claro, Sao Paulo, Brazil)

16:30-16:45
- **Questions / Answers**

16:45-17:15
- **S3-O-02 - Effects of water stress on Arabica coffee production**
  Emily Pappo (School of Natural Resources and the Environment, University of Florida, Gainesville, FL, USA)
- **S5-O-08 - Young shade trees buffer extreme climatic events and maintain high coffee yield and quality under their canopies in Yunnan, China**
  Clément Rigal (CIRAD, UMR-Absys, Montpellier, France)
- **S3-O-03 - Application times and glyphosate residue on coffee beans**
  Cesar Augusto Candiano (Technical Group, Experimental Agricola do Brasil Ltda., Sao Sebastiao do Paraiso, Minas Gerais, Brazil)

17:15-17:30
- **Questions / Answers**

.../...

16:00-17:30 **Session 1: Plant science** Virtual room 2

Chairs: Valérie Poncet & Jose Rafael Chan

16:00-16:30
- **S1-O-06 - Stenophylla coffee *(Coffea stenophylla)*: the forgotten coffee crop species of West Africa**
  Aaron Davis (Natural Capital & Plant Health, Royal Botanic Gardens, Kew, Richmond, Surrey, United Kingdom)
- **S1-O-07 - Evolution of *Coffea* leaf functional traits in relation to climate**
  Filip Vandelook (Living Collections, Meise Botanic Garden, Meise, Belgium)
- **S1-O-08 - Elevated air [CO_2] partly mitigates drought impact in *Coffea* spp. cultivars**
  José N. Semedo (UIBRG - Plant Ecophysiology Laboratory, INIAV IP (Ministry of Agriculture), Oeiras, Portugal)

16:30-16:45
- **Questions / Answers**

16:45-17:15
- **S1-O-09 - Genome Sequence Assembly of Coffea arabica variety Geisha (UCDv1.0)**
  Juan F. Medrano (Dept of Animal Science, University of California, Davis, Davis, CA, United States)
- **S1-O-10 - Evaluation of F1 hybrid (*C. arabica* L.) performances and farmer acceptance on-farm conditions in three producing regions in Ecuador**
  Juan Carlos Herrera (Plant Science Research Unit, Nestlé Research, Tours, France)

17:15-17:30
- **Questions / Answers**

17:30-17:45 Break

Closing Virtual room 1
- **Astrid Nehlig**
- **Benoît Bertrand**

18:00-18:45 **General Assembly** Virtual room 1

ABSTRACTS MONDAY 28 JUNE

PLENARY SESSION - KEYNOTE LECTURERS

Session 9: Health & safety, consumption, quality & trends

Session 2: Plant pathology & protection

S9-KN

Will it keep me awake? Common coffee / caffeine intake habits and sleep in real life situations

Landolt Hans-Peter (landolt@pharma.uzh.ch)

Institute of Pharmacology and Toxicology, University of Zürich, Zürich, Switzerland

With coffee being the most common dietary source, many people consume caffeine daily, aimed to reduce sleepiness and promote vigilance when sleep deprived or upon waking. Acute caffeine enhances vigilance and promotes wakefulness yet can delay sleep initiation and reduce electroencephalographic (EEG) markers of sleep intensity. It is unclear whether these effects of coffee/caffeine are retained or disappear during chronic consumption, which is typical in the general population.
We recently conducted several studies to address these questions. We examined whether repeated coffee intake in a dose and timing mimicking 'real world' habits maintains simple and complex attentional processes during chronic sleep restriction, such as during a busy work week. We found in genetically caffeine-sensitive individuals that regular coffee (300 mg caffeine/day) benefits most attentional tasks for 3-4 days when compared to decaffeinated coffee. Genetic variants were also used in the population-based HypnoLaus cohort, to investigate whether habitual caffeine consumption causally affects time to fall asleep, number of awakenings during sleep, and EEG-derived sleep intensity. Multi-level statistical analyses consistently showed that sleep quality is virtually unaffected when > 3 caffeine-containing beverages/ day are compared to 0-3 beverages/day. This conclusion was further corroborated by an experiment, in which sleep was quantified in the laboratory in habitual caffeine consumers. Indeed, when compared to placebo, daily intake of 3 x 150 mg caffeine over 10 days did not strongly impair nocturnal sleep nor subjective sleep quality in good sleepers. Finally, we tested whether a delayed, time-controlled, pulsatile caffeine-release formula can improve the quality of awakening in sleep-restricted volunteers. We found that 160 mg caffeine taken at bedtime ameliorated the quality of awakening, increased positive and reduced negative affect scores, and promoted sustained attention immediately upon scheduled wake-up. The data indicate that time-controlled caffeine release towards the end of a sleep episode could prevent over-night caffeine withdrawal and provide a proactive strategy to attenuate disabling sleep inertia.
Although coffee/caffeine does not compensate for chronic sleep loss, the data suggest that coffee/caffeine intake can transiently attenuate detrimental neurobehavioral consequences of reduced sleep and, when administered in time-controlled fashion, improve the quality of awakening upon sleep restriction without disturbing sleep in conditions that are common in society.

References:

- Weibel et al. 2020 Progress in Neuropsychopharmacology and Biological Psychiatry https://doi.org/10.1016/j.pnpbp.2019.109851
- Baur et al. 2021 Progress in Neuropsychopharmacology and Biological Psychiatry https://doi.org/10.1016/j.pnpbp.2020.110232
- Weibel et al., 2021 Scientific Reports https://doi.org/10.1038/s41598-021-84088-x

S2-KN

Global change: Adapting coffee pest and disease management

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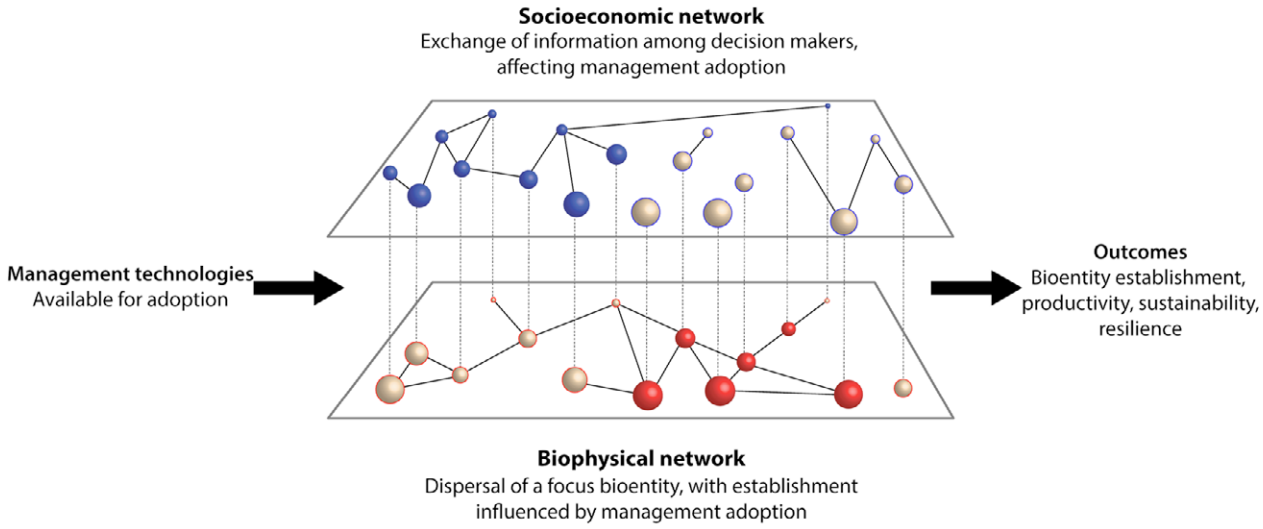
Global change presents challenges for coffee pest and disease management, in terms of changing climate and the threat of pest movement through trade (Ziska et al. 2018). Where pest and disease risk increase through these global processes, adaptation can proceed through use of resistant varieties, improved on-farm management strategies, and financial buffering through, for example, insurance programs. For regional management, targeting of efforts for surveillance and mitigation can draw on models of climate-based risk combined with models of cropland connectivity (Xing et al. 2020) for coffee, to identify key locations. Coordinated efforts can be important for regional management to ensure that management is sufficient to counterbalance increasing pest and disease risk. One approach to studying regional systems is impact network analysis (INA; Garrett 2021), a method for evaluating likely system outcomes in terms of the interactions between the quality of available management techniques, the socioeconomic network of managers making decisions about whether to use these techniques, and their links to the epidemic network through which pathogens spread.



The framework for impact network analysis (INA) to evaluate how a system, such as regional coffee pest and disease management, can adapt to climate change (from Garrett 2021).

References:

- Garrett, K. A., Methods in Ecology and Evolution, 2021, doi:10.1111/2041-210X.13655
- Xing, Y., et al., BioScience, 2020, 70:744-758.
- Ziska, L.H., et al., Agronomy, 2018, 8:152.

ORAL PARALLEL SESSIONS

Session 8: Coffee chemistry & sensory sciences

S8-O-01

A New (Sensory and Consumer) Coffee Brewing Control Chart

Guinard Jean-Xavier (jxguinard@ucdavis.edu), Frost Scott, Batali Mackenzie, Cotter Andrew, Ristenpart William

UC Davis Coffee Center, University of California, Davis, Davis, California, United States

RATIONALE

We propose a new and improved Coffee Brewing Control Chart to update Lockhart's 1959 iconic chart. The new chart is based on our research on the effects of beverage strength - total dissolved solids or TDS (%) and brewing yield or Percent Extraction PE (%), as effected by roast (light, medium, dark), grind size (fine, coarse), basket geometry (flat, cone), water pulsing cycle, and temperature (87, 90, 93oC), on the sensory profile and consumer acceptance of drip brewed coffee (Frost et al., 2019, 2020; Batali et al., 2020; Cotter et al., 2020).

METHODS

Coffees were brewed across the 1.00 to 1.50% TDS and 16 to 24% PE ranges according to factorial designs of the brewing variables listed above. The intensities of the sensory attributes of the coffees in the experimental designs were measured using descriptive analysis with trained panels. Their consumer acceptance was then measured on the 9-point hedonic scale from 1='dislike extremely' to 9='like extremely', with 5='neither like nor dislike', with 118 Northern California black coffee drinkers.

RESULTS

Across the 1.00 to 1.50% TDS and 16 to 24% PE ranges, the new chart shows (1) the dominant and most variable sensory attributes across the chart – sour, astringent and citrus on the upper left side; bitter, roasted, ashy and body on the upper right side; sweet and fruity on the lower left side, and tea-like and floral on the lower right side; and (2) the response surface areas of highest liking for the two preference segments we uncovered among black coffee drinkers – one (n=67) that preferred low TDS and low-medium PE coffee, and another (n=51) that liked most either medium-high TDS and low PE coffee or medium TDS and high PE coffee. Whereas a response surface of the mean hedonic ratings vs. TDS and PE would have had an optimum relatively close to the 'Ideal' label in Lockhart's Brewing Control Chart, plotting it would have meant to ignore preference segmentation and would therefore have been both misleading and incorrect.

CONCLUSIONS & PERSPECTIVES

The new chart will improve the ability of coffee roasters and baristas to deliver specific sensory profiles to match the different likes and dislikes of consumers through the proper navigation of TDS and PE ranges as effected by the manipulation of key brewing variables.

References:

- Frost et al. 2019 Journal of Food Science DOI:10.1111/1750-3841.14696.
- Frost et al. 2020 Journal of Food Science DOI:10.1111/1750-3841.15326.
- Batali et al. 2020 Scientific Reports DOI:10.1038/s41598-020-73341-4.
- Cotter et al. 2020 Journal of Food Science DOI:10.1111/1750-3841.15561.

S8-O-02

Modelling molecular release in coffee brewing

Melrose John (jrmelrose@gmail.com)

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RATIONALE

In recent years, there has been significant progress in the measurement of brew concentrations over time during coffee brewing from packed beds of coffee grinds. To understand this data, it is crucial to quantify those physical chemistry effects that occur internal to coffee grounds but which modulate the release out of the grounds.

METHODS

A multi-scale modelling tool is used to give insights into the underlying physical chemistry inside coffee grounds by fitting measured concentrations kinetics during both brewing and gas stripping (used in instant coffee production). The model includes source and sink terms internal to grounds to model adsorption and partitioning to phases within in grounds and models for hindered diffusion due to co-solute and grounds structure. Melrose (2020)

RESULTS

Good fits to literature data will be reported for total yield, Caffeine, Acetaldehyde, Acetic acid and a range of molecules with varying polarity (data from Khun et al 2017, Mestdagh et al 2014 and others). From the total yield data, a bi-modal distribution of release rates was found. For the individual molecules, the modelling quantifies: the relative roles of hindered diffusion within coffee particles due to structure and co-solutes; the neutralisation effect of coffee bases on organic acids; and, for non-polar molecules, the effect on brew extraction rates of partitioning between water and oil phases internal to coffee grounds. Co-solutes have a strong effect on hindrance in the case of gas stripping from wet grounds.

CONCLUSIONS AND PERSPECTIVES

Most parameters in the model are taken from physical chemistry data sets (e.g. partition coefficients) for the individual molecules and coffee brews. The main fit parameters are structural hinderance and choice of representative grain size from the measured PSD, however a good degree of accuracy can be achieved using a combination of independently measured parameters and some fit – the latter however have expected values. This opens the route for combining modelling and experiment to systematically explore and manipulate effects within grounds which modulate brew composition.

References:

- Melrose J. R 2020 Eds Megan Povey In IOP E-Book PiFM Conf. 2020.
- Kuhn et al. J. Food Eng., 2017 206, 37-47.
- Mestdagh et al. Food Res. Int. 2014 63, 271–274.

S8-O-03

The role of ethyl esters in green coffee: flavor or off-flavor precursors?

Lonzarich Valentina[1] (valentina.lonzarich@illy.com), Crisafulli Paola[2], Del Terra Lorenzo[2], Teixeira Aldir Alves[3], Teixeira Regina[3], Teixeira Monteiro Allan[3], Navarini Luciano[2]

[1] *Aromalab - illycaffè spa, Trieste, Italy* ; [2] *illycaffè spa, Trieste, Italy* ; [3] *Experimental Agricola do Brasil Ltda, Sao Paulo, Brazil*

RATIONALE

One of the most feared defect in coffee is represented by the so called fermented/over-fermented off-flavor or "stinker" (Bade-Wegner et al.,1997). The occurrence of "stinker" beans is associated with an intolerable fruity, overripe fruit, silage-like and even rotten flavor. In wet-processing, in addition to an immediate pulping, the fermentation process must be well controlled to ensure a high-quality beverage. "Over-fermentation" (beyond that necessary to loosen the mucilage) is generally considered detrimental to coffee quality. The stinker off-flavor is inevitably produced in case of uncontrolled fermentation or prolonged fermentation of beans trapped in the interstices of pulping machines, fermentation tanks and other sites. Specific ethyl esters have been reported as key aroma compounds responsible for the over-fermented flavor defect of coffee. Ethyl-2-methylbutanoate, ethyl-3-methylbutanoate (ethyl isovalerate) in addition to other compounds representing typical end-products of the alcoholic fermentation, have been recognized as key volatiles at the origin of the perceived "stinker" off-flavor. Recently it has been reported that longer-fermented coffee offers more sensory notes that are preferred by consumers and that green coffee beans from extended fermentation processing are characterized by positive fruity flavor thanks to the presence of esters (mainly ethyl 3-methylbutanoate) in higher concentrations than in standard processed green coffee beans (Zhang et al.,2019). Thus, the same precursors are related to both bad processing/unintentional fermentation and intentional extended fermentation. This crucial point will be discussed resorting to some selected examples.

METHODS

Several coffee green bean samples, both properly chosen and *ad hoc* prepared, have been chemically characterized by SPME headspace-GC-MS/MS focusing the attention on ethyl esters.

RESULTS

The presence of ethyl esters related to "stinker" defect has been systematically detected in samples obtained under simulated unproper storage conditions or in samples sensorially characterized by off-flavors.

CONCLUSIONS & PERSPECTIVES

To define the difference between an over-fermented off-flavor and a deep fruity flavor, it could be proposed an objective criterium and in particular a threshold of the content of some ethyl esters in green coffee beans. In the lack of any regulation, it is not clear how to discriminate fermented flavor deriving from an intentional practice from that deriving from incorrect processing. It could be a practical option then, to set to zero the target content of ethyl esters like ethyl-2-methylbutanoate and ethyl isovalerate in premium quality green beans.

References:

- Bade-Wegner et al., 1997. In proceedings of the 17th ASIC Colloquium: Nairobi (Kenya). Paris: ASIC. p. 176.
- Zhang et al., 2019., Applied and Environmental Microbiology, 85, Issue 6 e02635-18.

S8-O-04

Furan formation in coffee – Role of precursors and impact of the roast degree on reaction pathways

Poisson Luigi (Luigi.Poisson@rdor.nestle.com), Auzanneau Noémie, Schaerer Ania, Davidek Tomas

Nestlé PTC Beverage Orbe, Société Produits Nestlé SPN, Orbe, Switzerland

RATIONALE

In the last two decades, furan received considerable attention due to its classification in group 2B as 'possibly carcinogenic to humans' by the International Agency for Research on Cancer. Similar physiological effects are also debated for its derivatives like 2-methylfuran (2-MF). Previous studies with model and real food systems showed that furans can be formed from multiple precursors such as saccharides, amino acids, polyunsaturated fatty acids, and ascorbic acid.[1] The role of furfuryl alcohol and 2-furoic acid as potential precursors of these furans through oxidation/decarboxylation during coffee roasting was also discussed.[2] In addition, the transfer of a methyl group from trigonelline through 1-methylpyridinium to furan was considered in the formation of 2-MF.[3]

METHODS

The role of sucrose and specific amino acids in the formation of furan and 2-methylfuran upon coffee roasting was investigated in a kinetic study applying ^{13}C- and ^{15}N-labeled precursors in biomimetic in-bean experiments. In addition, the formation mechanisms of potential intermediate compounds like furfural and furfuryl alcohol were evaluated, and their degradation mechanisms studied in roasting experiments performed under inert gas atmosphere. Additional omission experiments were performed to elucidate the role of trigonelline in the formation of 2-MF.

RESULTS

The results highlighted similar formation kinetic for furan and 2-methylfuran. Their generation requests quite high energy, consequently the formation kinetics shows exponential behavior with a strong increase at the later stage of roasting. Sucrose contribution to both furans was important mainly in the middle roasting stages, while main amounts of furan and 2-MF in the final roasted product predominantly stem from other precursors (polysaccharides, lipids, etc.). In contrast, the formation kinetics and pathways of furfural and furfuryl alcohol were found very different, indicating no direct link to the formation of the studied furans. A methylation of furan from trigonelline to form 2-methylfuran could not be corroborated.

CONCLUSIONS & PERSPECTIVES

The combination of kinetic studies with labeling experiments in the coffee bean system was evidenced as a very powerful tool to elucidate the origin and possible formation pathways of furans upon coffee roasting. Understanding of the reaction pathways upon coffee roasting is crucial to enhance generation of beneficial compounds (aroma, taste, bioactives) while mitigating the undesirable ones.

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S8-O-05

Identification of coffee compounds that suppress bitterness of the brew

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RATIONALE

Sensory-guided fractionation methods have been traditionally used to identify taste compounds, such as bitterness in coffee, however this analytical approach can overlook contextual interactions such as compounds with modifying properties. To overcome this analytical limitation, the current study utilized an untargeted LC/MS chemical profiling flavoromics approach to identify compounds that contribute to coffee brew bitterness.

METHODS

The untargeted chemical profiles of fourteen coffee brew samples were collected by UPLC/MS-QToF. The bitter intensity of same coffee brews was determined by a descriptive sensory panel consisting of 8 trained panelists. Then, the correlation between chemical profiles and the bitter intensity of coffee brews were modeled by orthogonal partial least squares (OPLS). The chemical markers that were highly predictive of bitter intensity were subsequently purified by multi-dimensional preparative LC/MS for quantification, sensory recombination testing, and compound identification.

RESULTS

Significant differences in the perceived bitterness intensity ($p < 0.05$) were noted among the fourteen coffee brew samples, ranging from 5.0 to 9.4, with no significant correlation observed to the quantified caffeine content ($R^2 = 0.07$). The untargeted UPLC/MS-QToF chemical profiling obtained 1680 chemical features after data processing. The OPLS model for bitterness intensity was established with good fit ($R^2Y > 0.9$) and predictive ability ($Q^2 > 0.9$). Ten highly predictive compounds negatively correlated to coffee bitterness were selected for sensory recombination testing and when added to a control brew significantly reduced bitter intensity by 1.5 points ($p < 0.05$). Three compounds, namely 4-caffeoylquinic acid, 5-caffeoylquinic acid, and 2-*O*-β-D-glucopyranosyl-atractyligenin were identified as main bitter modulators in coffee, and individually significantly decreased the perceived bitterness intensity of the brew between 0.6-0.8 points.

CONCLUSIONS & PERSPECTIVES

The application of untargeted flavoromics methods enabled the discovery of flavor modulators, 4-CQA, 5-CQA, and 2-*O*-β-D-glucopyranosyl-atractyligenin, that suppressed the bitter taste of coffee brew. These results support the importance of taste modulators to the flavor perception of coffee brew.

S8-O-06

Characterisation of volatile and non-volatile compounds in cascara, a traditional beverage made from dried coffee pulp

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RATIONALE

Cascara, historically consumed in several Middle Eastern cultures, has risen in popularity in recent years as an emerging hot infusion beverage. This upsurge is of interest to the coffee industry due to its potential in reducing some of its main agricultural by-products (husk, pulp). While many look to novel techniques for waste upcycling, cascara is an excellent traditional biovalorised product. Apart from its pleasant organoleptic qualities, cascara possess a high antioxidant capacity which may impart desirable nutritional value. Under the current market pressure, it would be of great interest to develop cascara products to enhance the market value of wasted pulp to supplement the income of farmers.

METHODS

In this study, we analysed five cascaras from *Coffea arabica*. Using solvent assisted flavour evaporation (SAFE) and headspace-solid phase microextraction (HS-SPME), we profiled their volatiles composition via a gas chromatography-mass spectrometry/flame ionisation detector (GC-MS/FID). Key odourants were elucidated via GC-olfactometry (GC-O) and aroma extract dilution analysis (AEDA). The non-volatiles composition of these samples were also profiled via liquid chromatography-quadrupole time-of-flight (LC-QTOF) and high performance LC (HPLC). Three colorimetric antioxidant assays were conducted.

RESULTS

189 volatile compounds were identified across the five cascaras and confirmed with standards, highlighting the complexity of cascara aroma. In terms of volatiles extraction, SAFE proved to be the most efficient in features extraction, and thus the most suitable for studying cascara brews. 18 odourants were elucidated from the GC-O, and sweet-like odours were suggested by AEDA to be the most potent and therefore central to the characteristic smell of cascara. Non-volatile compounds were identified of various classes (sugars, acids, flavonoids, and other phenolic compounds), and some were correlated to the natural variation in antioxidant capacity of cascara.

CONCLUSIONS & PERSPECTIVES

These analyses presented the range of compounds present in cascara and observed large natural variations in the concentrations of some of these compounds. Apart from providing insight into cascara composition and its relation to some physical and organoleptic properties, this study also pinpoints several compounds of interest that may be studied in greater detail to improve the processing and flavour of cascara.

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ORAL PARALLEL SESSIONS

Session 1: Plant science

Session 2: Plant pathology & protection

S1-O-01

Application of phylogenomics to reconstruct the evolutionary origin of *Coffea arabica*

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RATIONALE

Coffea arabica is an allotetraploid species that emerged after a natural hybridization event between two *Coffea* species (Lashermes *et al.,*1999). The identity of these progenitor species, the age of the *C. arabica* hybridization event, and the evolutionary trajectory towards self-compatibility in *C. arabica* remained a subject of debate (*e.g.* Tesfaye *et al.,* 2007). Here, we applied a phylogenomic approach to assess the evolutionary origin of *C. arabica*.

METHODS

Leaf samples were collected from 23 *Coffea* species, including all known close relatives of *C. arabica.* Genotyping-by-sequencing (GBS) was applied to obtain genome-wide polymorphic molecular markers. The GBS alleles from the two *C. arabica* subgenomes were separated from each other using a two-step approach. First, the close relatives of *C. arabica* were partitioned into two groups according to their phylogenetic relatedness. Second, the *C. arabica* alleles were assigned to one of these two groups based on genetic similarity. Phylogenetic relationships were modelled with a time-calibrated multi-labelled tree with the subgenomes of *C. arabica* separately positioned as if they were two independent species. In addition, the ancestral states of self-compatibility in *Coffea* were reconstructed using the multi-labelled tree and a maximum likelihood method.

RESULTS

Coffea canephora and *C. eugenioides* were confirmed as the closest extant relatives of *C. arabica,* whereas the age of the *C. arabica* hybridization event was estimated between 1.08 and 0.54 million years ago. Self-compatibility presumably evolved independently in *C. arabica*, although the 'Eugenioides' subgenome was found to be closely related to other *Coffea* species that are assumed to be self-compatible (*i.e. C. anthonyi* and *C. heterocalyx*).

CONCLUSIONS & PERSPECTIVES

The hybridization event at the origin of *C. arabica* presumably took place in a time of environmental upheaval, in which the creation of genomic novelty may have been beneficial for survival. The close relatedness between the 'Eugenioides' subgenome and the self-compatible species suggests that *C. arabica* may have been evolutionary predisposed to develop self-compatibility. Our study provides new insights in the origin of *C. arabica*, strongly enhancing the knowledge on the evolution of this species (Bawin *et al.,* 2020).

References:

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- Tesfaye et al. 2007 Genome 1112-1129.

S1-O-02

Study of circadian regulation of primary metabolism in coffee plant is key to develop coffee varieties adapted to climate change

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RATIONALE

The circadian clock (CC) is a critical regulator to optimize the plant physiology and metabolism to the correct time of the day, providing plants with the ability to anticipate daily and seasonal environmental changes. Plant transcriptome is massively modulated by the CC which is an integrator of many environmental cues. Through several experiments we show here how the CC of Arabica is modulated by the environment and how the genetic background can also modulate the CC with huge impact on primary metabolism.

METHODS

RNA sequencing was systematically used to study gene expression over 24h in combination with metabolomical and physiological analysis. Then we have integrated information of multiple circadian RNA-seq diurnal time-course in order to estimate the fraction of the Arabica transcriptome that is circadian regulated. Comparison of all the rhythmic genes identified in coffee genome with those of *Arabidopsis thaliana* revealed they are identical at almost 90%.

RESULTS

We present how photoperiod, full-moon light, and thermos-cycles, led to changes in transcript abundance of thousand of genes during the diurnal cycle altered the CC amplitude which in cascade modify the expression of thousand of rythmic genes. Moreover, we will explain how the genetic background can change the CC and the implication in terms of primary metabolism, physiological behaviour and disease resistance.

In this presentation we pay special attention to three major genes of the core clock for which we recall how important they are for the regulation of the transcriptome and *in fine* on the development of the plant and the major agronomic traits.

CONCLUSIONS & PERSPECTIVES

Variations in the environment and/or genetic background have a major impact on the rhythmicity and daily amplitude of expression of a large number of Arabica coffee genes. The same trends as in model plants (notably Arabidopsis) are found here.

The study of variations in the expression of the main core clock genes is fundamental and needs to be refined. A major question that needs to be addressed is: can we find allelic variation in circadian genes in the Arabica species and can we use it to breed news varieties with higher performances.

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S2-O-02

Scaled-up genomic data uncovers a higher level of population genetic structuring in *Hemileia vastatrix*

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RATIONALE

Coffee Leaf Rust, caused by *Hemileia vastatrix* (*Hv*), has been the major constraint to global coffee production. Only recently the evolutionary history of this pathogen began to be dissected. Silva et al. (2018) found for the first time the species to be structured into three divergent genetic lineages with marked host tropism (C1 and C2 infecting diploid coffee species; and C3 infecting tetraploid coffee species). Nevertheless, no significant structuring was found within the C3 lineage, which represent the most widespread and epidemiological relevant *Hv* group. Here, we extended the investigation to a worldwide scale sampling for obtaining a deeper insight on the dynamics and adaptive patterns of *Hv* populations.

METHODS

RADseq and analysis of 99 *Hv* isolates from 23 geographical regions, different pathotypes and coffee hosts were performed. Phylogenetic reconstructions, PCA, assessment of population structure and outlier loci detection analyses were carried out.

RESULTS

Phylogenetic analyses corroborated the existence of the previous *Hv* groups, but furthermore showed a well-supported structuring within C3, with three main lineages (I,II,III). This pattern seems to reflect *Hv* geographical origin associated to the historical distribution and exchange of coffee materials. Lineage I comprises the higher number of isolates, mainly of Asian origin, and exhibits a ladder-like diversification pattern, suggesting rapid evolution and population expansion. On the contrary, Lineage II (comprising mainly *Hv* isolates of African origin) and Lineage III (including only Timor *Hv* isolates) appear to be more restricted, revealing a higher degree of differentiation. Signals of selection were detected in 75 loci while a total of 84 loci were found to be associated with C3 lineages by single and multi-association analyses. From the total of 112 putatively adaptive loci found, 29 loci were significant in all the analyses, within which exclusive SNP alleles were detected in Timor and African lineages. The results show that these loci seem to contribute strongly for shaping *Hv* genetic structure.

CONCLUSIONS & PERSPECTIVES

Our study provides a higher-resolution perspective on the evolutionary history of *Hv*, revealing for the first time a clear structuring within rusts infecting tetraploid coffee hosts, which seems to follow an adaptive pattern, with coffee host as a major selective pressure. In addition, lineage diagnostic SNPs were found that could assist in future discrimination of *Hv* isolates.

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References:

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S2-O-03

ExpeRoya, a comprehensive model to forecast the risk of coffee leaf rust on *Coffea arabica* in Central America

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RATIONALE
To anticipate socio-economic crises linked to epidemics of coffee leaf rust (CLR), we developed a bio-physical model to forecast the risk of increase of CLR at the territorial and plot scales.
METHODS
This model was built using the IPSIM (Injury Profile SIMulator) methodology, which has the advantage of representing in a simplified way the complex relationships that exist within agro-ecosystems and allows easy appropriation of the tool by the actors of the sector. ExpeRoya combines ordinal qualitative variables that describe the interactions between weather, pathosystem and crop management that affect CLR risk of increase each month.
RESULTS
Firstly, this model was socially evaluated in national workshops in Central America and Dominican Republic and through a survey with international rust experts. Secondly, it was numerically evaluated on CLR monitoring data. As the model fitted well the data it is currently used to produce monthly alerts in Honduras and Dominican Republic through “Pergamino”, an online platform.
CONCLUSIONS & PERSPECTIVES
ExpeRoya is a new promising tool to be used in association with a socio-economic model to form a risk management system of coffee crop.

ExpeRoya accessible on the Pergamino platform (http://www.redpergamino.net/). After uploading the epidemiological data and the weather data, the system variables are filled in the plateform, e.g. the fruit load, the variety, the calendar for pesticide applications, the level of nutrition of coffee trees, the density of shade, the date of flowering, the date of beginning and end of harvest. The graph shows an example of forecast of the risk of growth of CLR for the 2017 monitoring campaign in Honduras. The boxplots represent the monthly monitored CLR incidences in the country and the red arrows represent the risk of change in the incidence forecasted by ExpeRoya for the next month. The horizontal blue, green, yellow, orange and red zones represent, in growing order, the alert levels, which may change according to (i) the periods considered (flowering and harvest) and (ii) the level of susceptibility of the varieties. The rules for changing the alert levels are specified in the box “Colores de alerta”.

S2-O-04

Identification of *Hemileia vastatrix* candidate effectors reveals new ways of promoting pathogen variability through alternative splicing

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RATIONALE

Coffee leaf rust (CLR), caused by the biotrophic fungus *Hemileia vastatrix* (Hv), is the most important disease of *Coffea arabica*. The recent outbreak of CLR in central America highlighted the importance of the disease, and reinforced the need for better and faster breeding techniques in order to obtain new coffee resistant varieties. The identification of rust effectors has been single out as a shortcut to the identification of plant proteins that can guide the selection of new resistant genotypes.

METHODS

To identify candidate effectors we used a bioinformatic pipeline aimed at selecting putatively secreted protein sequences, followed by the application of the MEME algorithm to identify new motifs between the sequences (Talhinhas et al. 2014). Protein localization in coffee infected tissue was achieved by immunolocalization using specific antibodies raised against the candidate effectors (Kemen et al. 2005). Agroinfiltration of *Nicotiana benthamiana* leaves with different cDNAs sequences was used to identified the subcellular compartment target by the candidate effector proteins.

RESULTS

We choose four small proteins (<200 amino acids) predicted to be secreted, rich in cysteines and that share three amino acid motifs. The amplified genomic sequences also showed a similar structure. The transcript level of the four sequences was upregulated in infected coffee leaves, peaking seven days after inoculation, when haustoria were the predominant fungal structure observed. The candidate effectors were localized inside of intercellular hyphae and haustoria from *H. vastatrix* in infected leaves. Surprisingly, different transcripts were obtained for single candidate effectors when PCR amplified from infected coffee leaves, therefore revealing splicing variants. These sequences had a distinct transcription profile in leaves of *Coffea* spp. inoculated with different *H. vastatrix* isolates, and a different subcellular localization in agroinfiltrated *N. benthamiana* leaves.

CONCLUSIONS & PERSPECTIVES

The selected sequences showed the hallmark of true effectors, which needs to be supported by the ongoing effort of identifying the plant proteins interactors. The splicing variants identified can contribute to increase the 'arsenal' diversity that *H. vastatrix* employs in the 'arms race' against *Coffea* spp. immunity, and may contribute to the diversification of *H. vastatrix* virulence potential.

Acknowledgments: This work was funded with national funds through Foundation for Science and Technology (FCT) UNIT LEAF (UID/AGR/04129/2020).

References:

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POSTER PRESENTATIONS

Session 7: Roasted coffee technology & processing

Session 8: Coffee chemistry & sensory sciences

S8-PO-01

Critical examination of particle swelling during hydration of ground coffee

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RATIONALE

Water imbibition into coffee particles is of interest for understanding extraction, particularly as it relates to diffusion, which is the limiting factor for the rate of solids extraction. Coffee swelling during extraction has been widely reported, and it has been hypothesized that swelling could restrict water flow through the bed, limiting diffusion and extraction of solubles (Corrochano et al. 2015). Therefore we characterized the amount of particle swelling of ground coffee during hydration on the timescale of typical brew methods (0.5 - 5 minutes).

METHODS

Previous studies of swelling have generally looked at the coffee bed in aggregate, or the distribution of particle sizes, rather than individual particles. In this study we used laser diffraction and optical microscopy to look for particle swelling of samples and of individual particles after exposure to water. Most of the experiments used room temperature water due to equipment constraints, but several microscopy measurements on particles immersed in 90 ºC water for over 5 minutes yielded the same results.

RESULTS

Laser diffraction measurement of particle size requires the sample to be suspended in liquid, and larger particles of ground coffee tend to float due to air trapped in the pores. This effect initially skews the results towards smaller size, but the median size appears to increase over time as the liquid wets the particles, allowing the larger particles to be included in the measurement. Pre-wetting sample with isopropyl alcohol prior to addition to water greatly reduces this artefact, and measurements of swelling appear to be zero (no statistically significant change in size versus time). Likewise, optical microscopy of individual particles shows no clear evidence of swelling after immersion, even when the particles are placed in 90 ºC water for 5-10 minutes. Seven types of ground coffee were measured for various water chemistries, and none exhibited swelling when placed in water.

CONCLUSIONS & PERSPECTIVES

These results indicate that individual coffee particles do not swell significantly over 5 minutes (the duration of typical brew methods), in contrast to previous reports (e.g. Mateus et al. 2007). The measurements have an estimated error of 2% on the diameter, so it is possible that a smaller amount of swelling takes place during hydration. The well-known expansion of coffee beds (blooming) must be due to gas escaping from the particles and segregation of fines rather than swelling of the individual particles.

References:

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- M.-L. Mateus et al., Agric. Food Chem. 55 (2007) 2979−2984.

S8-PO-02

Spectroscopic methods for discrimination of specialty coffee quality: NIR vs. FTIR

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RATIONALE

A cup of coffee represents a food product of global economic importance and an icon of a lifestyle. Only a small portion of the coffee produced worldwide achieves the highest quality standards, being considered "specialty coffees", and increasingly sought by consumers. The *Specialty Coffee Association* (SCA) sensory analysis protocol is the official methodology employed to classify specialty coffees. However, because sensory analysis is sensitive to the taster's training, cognitive psychology and physiology, among other parameters, the feasibility of instrumental approaches has been recently studied for complementing such analyzes. Spectroscopic methods, mainly near infrared (NIR) and mid infrared (FTIR – Fourier Transform Infrared), have been extensively employed for food quality authentication. Applications in coffee analysis include discrimination between Arabica and Robusta species, between graded and defective beans, evaluation of roasting conditions, separation between decaffeinated and regular roasted coffees, and discrimination between coffee and adulterants, as well as geographic and sensory discrimination. In view of the aforementioned, in the present study we compared NIR and FTIR to distinguish different qualities and sensory characteristics of specialty coffee samples.

METHODS

Samples of specialty coffee were provided by Federação dos Cafeicultores do Cerrado Mineiro and Fazenda Barinas (n=28), roasted in a IKAWA coffee roaster (in duplicate, n=56) and submitted to NIR analysis (two fractions of each sample were analyzed, providing a total of 112 spectra in the range of 900 to 2300 nm). The beverage was prepared in accordance with the SCA protocol, tasted by a group of Q-graders and submitted to FTIR analysis. Two fractions were withdrawn from each sample and analyzed in duplicate, totaling 224 spectra in the range of 4000 to 600 cm-1 (56 beverages × 2 aliquots × 2 analysis).

RESULTS

PLS models were built, and although both NIR and FTIR were able to provide good predictions, the FTIR results were more accurate, providing consistent models for predicting cup quality based on the SCA sensory analysis, with low values of RMSEC and RMSEP (0.23 both for FTIR and 0.50 both for NIRS). Also, the FTIR models showed slightly higher values of calibration (Rc - 0.99) and validation (Rv - 0.97) coefficients in comparison to NIR results (Rc - 0.98 and Rv – 0.96).

CONCLUSIONS & PERSPECTIVES

The results confirmed the feasibility of both techniques for predicting specialty coffee quality. In general, FTIR results were more accurate, but either technique could be implemented by the coffee industry.

S8-PO-03

Predictive Models for Precision Control of Drip Brew Coffee

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RATIONALE

Given that the flavor profile of drip brew coffee is strongly dependent on the brew strength and percent extraction, it is desirable to have a predictive model that assists coffee professionals in achieving desired values. Current and newly proposed versions of the Coffee Brewing Control Chart present sensory attributes in terms of the dependent variables of brew strength and percent extraction, without providing insight on how to achieve specific values. Here we explore how the «water pulsing duty cycle» in commercial drip brewers can be exploited to yield desired values of the brew strength and percent extraction, and we show that a model with three independent parameters - the brew ratio, the total brew time, and a grind-size dependent mass transfer coefficient - can be used to predict the brew strength and percent extraction.

METHODS

The total dissolved solids (TDS) was measured across a wide range of different drip brews, where the total brew time, brewing ratio, water pulsing duty cycle, and grind size were systematically varied. Core sampling of the spent coffee bed from a commercial drip brewer was performed to see how the percent extraction of coffee varies with position and to inform a mass transfer model based on the findings. Under the assumption of "perfect mixing", a mass transfer model was developed to predict the brew strength and percent extraction using the brew ratio, total brew time, and a grind dependent mass transfer coefficient. The TDS data were fit to the mass transfer model via non-linear regression.

RESULTS

The core sampling data indicated that to good approximation the percent extraction varies little with depth in the coffee bed. The mass transfer model was found to accord with the experimental brew data over a wide range of brewing parameters.

CONCLUSIONS & PERSPECTIVES

The model yields a predictive chart that provides insight on how practitioners can adjust their drip brew conditions to yield desired brew strengths and extraction yields, which in turn will help provide insight on how to better target desired sensory profiles.

S8-PO-04

Effect of storage condition on the shelf life of Liquid Coffee Concentrates and their relation with oxygen contents

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RATIONALE

The causes of the loss of sensory quality of Concentrated Liquid Coffee (CLC) during storage have not been yet clarified. This study consisted of identifying critical factors related to the deterioration of the product's sensory profile over time. The acceptability was modeled with the results of the hedonic tests. Simultaneously, multivariate statistics were implemented to identify quality markers from the descriptive-quantitative sensory attributes, pH, titratable acidity, oxygen, carbon dioxide, and chlorogenic acids contents obtained from the samples.

METHODS

A hedonic analysis was used to model the acceptability, and sensory quantitative-descriptive analysis (QDA) was included to evaluate six attributes: aroma, acidity, body, bitterness, sweetness, winey and overall. The acceptability's survival response was modeled with a mixed-effects statistic combined with logistic regression, with storage time as the fixed factor and the judges as to the random factor. The physicochemical analysis consisted of evaluating pH, titratable acidity, oxygen, carbon dioxide, color CIE-L*a*b*, chroma, and hue. Additionally, chlorogenic acids (5CQA, 3CQA, 4CQA, 3,4 diCQA, 3,5 diCQA and 4,5 diCQA) were also studied. A single numerical and graphical representation model, a bootstrapped-feature selection version of PLS condensed sensory analysis and chemical analysis.

RESULTS

The results showed that the survival analysis for CLCs presented an acceptance limit of 45 days (once the survival coefficient is below 0.5). The attributes of aroma and wine were significantly different over time and explained by the sensory acceptability. PLS analysis showed that the chlorogenic acids, color, titratable acidity and oxygen, were relevant to explain the aroma changes, acidity, and winey notes.

CONCLUSIONS & PERSPECTIVES

Deterioration mechanisms in CLCs are mainly correlated with oxygen availability. Oxygen is present in CLCs during their production, remained dissolved during storage. It reacts with chlorogenic acids 3CQA and 5 CQA; decreasing the aroma as the main sensory attribute; consequently, the product's acceptability is affected.

The Winey attribute increased with time and when the extracts were rejected. Color changes were correlated with the occurrence of winey notes, probably due to non-enzymatic browning reactions during storage. Nevertheless, in order to understand these mechanisms, specific carbohydrate analyses will be performed in future studies.

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S8-PO-05

Brew Temperature Strongly Affects the Color of Brewed Coffee

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RATIONALE
The color of a beverage is an important sensory characteristic quickly noticed by consumers, and it is well known that changes in the color strongly affect perceived sensory quality and consumer preference. Although roast level clearly changes the color of coffee beans, little work has examined how brew temperature affects the color of the final beverage.
METHODS
Coffees from three different origins were roasted to three different levels and then brewed at three different temperatures (4°C, 22°C, and 94°C). Four trial replicates were performed at each condition, yielding 3x3x3x4 = 108 unique brews. Each sample was brewed towards full extraction and then diluted to precisely 2% total dissolved solids (TDS). Each brew was then photographed with a stereomicroscope and analyzed with a colorimeter and UV-vis spectrophotometer. Absorbance spectra and color tristimulus values were then compared and analyzed to test for statistically significant differences.
RESULTS
The data indicate that brew temperature strongly affects the color of the coffee brew. Qualitatively, cold brew coffees tended to be more red, while hot brewed coffees were more black. Different origins and roast levels yielded varying degrees of redness or blackness in the brew. Precise quantification of the color differences was reflected in absorbance spectra, absorbance values, and L*a*b* values. Under some conditions, the brew temperature affected the brew color more strongly than the roast level.
CONCLUSIONS & PERSPECTIVES
Brewing at different temperatures, even with the same origin, roast level, and TDS, yields drastically different colors in the final brew, suggesting brew color is an overlooked variable when it comes to sensory perception of coffee.

S8-PO-07

Cupping quality evaluation of hybrids of *Coffea arabica*

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RATIONALE Cupping quality represents one of the most important factors for purchasing decisions, commodity price, and thus a breeding goal. Varieties with high cupping scores are not always associated with other important traits, like yield and disease resistance. A fast solution is the development of hybrid varieties that could achieve high levels of productivity and quality.

METHODS

Thirty hybrids and Marsellesa were evaluated by 8 professional cuppers. The hybrids consist in combinations of four female lines (Geisha, IAPAR 59, Marsellesa and Obata) crossed to accessions originating from Ethiopia, conserved at CATIE genebank. The hybrids were grown in the Aquiares farm (1,100 masl) in Costa Rica, grown in a non-shaded system. The cherries were wet processed, and the green coffee was all centrally roasted and frozen, shipped in 4 consecutive weeks. Two samples of three commercial coffees were included in each week for each cupper as controls to help assess the variation and repeatability.The SCA cupping protocol was used, with the addition of a standardized list of sensory descriptors provided by the Tastify cupping app. Also included was a survey question about meeting quality specifications (purchase intent).

RESULTS

The coefficient of variation and repeatability for total cupping score were 0.12 and 0.84, respectively, suggesting the evaluation was well designed and the results were consistent. Tthe effect of genotype, cupper and its interaction were significant. Although the interaction explained a relatively small percentage of the variation (9.1%). The overall range of cupping was from 75.3 to 85.8, with a mean of 83.3. As a reference, Marsellesa had a score of 84. Cluster analysis revealed 6 clusters of the hybrids and 3 between cuppers. The purchase intent was highly associated with total cupping score. Among the categories that are a component of the total score, flavor and body were the best predictors of purchase intent. Furthermore, the best predictors of flavor score were the descriptors floral, sweet, fruit and chocolate.

CONCLUSIONS & PERSPECTIVES

The evaluation helped identify the hybrids with high cupping scores and flavor profiles, as well promising parents to include in breeding programs to improve the quality. Furthermore, the results underlined the need for the use of a relatively high number of cuppers and the utility of collecting and analyzing descriptive data.

S7-PO-01

Study of the lipid fraction of two hybrid varieties of coffee at different roasting temperature profiles

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The main objective of the present work was to evaluate the impact of 9 roasting heat treatments on the lipid profile of the hybrid varieties H15 and H16.

METHODS

The methodology applied to obtain it consisted of three stages: 1) harvest and wet coffee cherry benefit to green coffee of the H15, H16 and Costa Rica varieties as a control; 2) Study of the lipid fraction, (content of total lipids, fatty acids and cafestol) in green coffee and; 3) Application of 9 heat treatments to green coffee, considering preheating (Tp), flame change (Tc) and final (Tf) temperatures, in the rotary drum roasting process.

RESULTS

The results obtained in green coffee from the study varieties, showed that the total lipid content was: H15 (7.34%), H16 (7.8%,) and Costa Rica (9.42%); The fatty acid profile was palmitic (C16: 0 = 33.5%), stearic (C18: 0 = 6%), oleic (C18: 1 = 7%), arachidic (C20: 0 = 2.3%), linolenic (C18: 3 = 1%), behenic (C22: 0 = 0.7%) and lignoceric (C24: 0 = 0.32%). The relative fraction of linoleic acid was 47% for H15, 49% for Costa Rica and 50% for H16, the content of cafestol in H15 (1066.64 mg / 100 g), H16 (809.32 mg / 100 g) and Costa Rica (1023.2 mg / 100 g).

CONCLUSION

An increase in the relative fraction corresponding to C16: 0 (33 to 36%) and decrease in C18: 2 (49 to 45%) from green coffee to roasted coffee were observed, while the fatty acid content was mainly influenced by high flame intensity The decrease in cafestol content was greater in dark roasts (966 to 292 mg / 100 g) than in light roasts (966 to 461 mg / 100 g). According to the above, it is concluded that the variety and heat treatment have an effect on the total lipid, fatty acid and coffee bean coffee content.

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S8-PO-08

Consumer preferences for black coffee are spread over a wide range of brew strengths and extraction yields

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RATIONALE

Brewing is the final step in the production of the coffee beverage. Extraction related metrics such as total dissolved solids (TDS), percentage extraction yield (PE) of solutes, and brew temperature (BT) are believed to govern the flavor and consumer acceptance of the resulting brew, as summarized in the industry standard "Coffee Brewing Control Chart." In this study, we investigated how the three factors of TDS, PE, and BT affected consumer acceptance of a medium roast, single□origin coffee and whether consumer preference segmentation would be observed based on these variables.

METHODS

One hundred and eighteen consumers of black coffee evaluated 27 coffees that varied in TDS, PE and BT over 3 tasting sessions. Consumers rated overall liking using the 9-point hedonic scale; evaluated the adequacy of temperature, flavor intensity, acidity and mouthfeel using 5-point just-about-right scales; and described the flavor of the coffee using a list of 17 attributes. Consumer preference clustering was conducted and mean cluster liking scores were mapped to the BCC using response surface methodology. External preference mapping and penalty analysis were used to find drivers of liking for the preference clusters.

RESULTS

Cluster analysis revealed 2 preference clusters that varied in their liking of different extractions, mostly as a function of TDS. Response surface methodology produced dome and saddle-shaped curves, respectively. External preference mapping and penalty analysis indicated that opinions regarding overall flavor intensity as well as acidity influenced the preferences of the two clusters.

CONCLUSIONS & PERSPECTIVES

This study showed that TDS and PE were drivers of Northern California consumer preferences for black coffee. This study also corroborated findings from prior studies which found that brewing temperature does not have a significant impact on coffee flavor nor acceptability if extraction profile is controlled for. Our findings will be used to revisit the concept of 'ideality' in a redesign of the BCC.

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S9-PO-01

α-Glucosidase enzyme inhibition effects, and antioxidant capacity in extracts of *Coffea arabica* leaves

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RATIONALE

Type 2 diabetes mellitus is an age-related metabolic syndrome characterized by hyperglycemia, which alters carbohydrate metabolism. In general, synthetic oral antidiabetics are used to suppress blood glucose levels, but they are associated with unpleasant side effects. The search for natural therapeutic alternatives, such as for example extracts of *C. arabica* leaves, appears to be a promising future alternative strategy, because they contain a range of polyphenolic compounds (Almeida *et al.*, 2018) at appreciable levels, possessing a breadth of health promoting properties.

METHODS

Individual polyphenolic compounds were identified and quantitated by high-performance liquid chromatography (HPLC) in coffee leaf tea extracts; total polyphenolic compounds evaluated according to the Folin-Ciocalteu method (Gülçin *et al.*, 2012); antioxidant capacity by the DPPH assay (Gülçin *et al.*, 2012) and α-glucosidase capacity by the *Saccharomyces cerevisiae* (Shinde *et al.*, 2008) enzyme inhibition assay, relative to the positive control acarbose.

RESULTS

The HPLC analyses specifically revealed that aqueous extracts of the leaves of *C. arabica* contain, on average, 20.37 g of chlorogenic acid, 7.89 g of mangiferin, 8.53 g of rutin and 10.78 g of caffeine per kg of dry material. In general, coffee leaf extracts presented a total polyphenol level of 53.813 ± 0.02 mg per GAE/g and the antioxidant capacity showed satisfying results with an IC50 equal to 1.23 ± 0.003 mg/mL. High antioxidant capacity correlated with high total polyphenol concentration in the aqueous extracts. On average, aqueous extracts inhibited α-glucosidase by 85.92%, equating to an IC50 of 85.5 µg/mL, which was far lower than the positive control acarbose (IC50 = 5353 µg/mL). The high capacity to inhibit α-glucosidase can be attributed to the levels of polyphenolic compounds present in the coffee leaf extracts, such as chlorogenic acid, rutin and mangiferin, the latter of which has proven antidiabetic effects. Because these compounds are derived from a natural plant product, the likelihood of unpleasant side effects are reduced.

CONCLUSIONS & PERSPECTIVES

The results indicate that the high content of relevant polyphenols such as chlorogenic acid, mangiferin and rutin, present in aqueous extracts of the leaves of *C. arabica* may be responsible, possibly through synergism, for the inhibition of α-glucosidase. Therefor extracts of coffee leaves, may be an useful therapeutic alternative to synthetic drugs in the control of Type 2 diabetes and its complications.

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POSTER PRESENTATIONS

Session 2: Plant pathology & protection

S2-PO-01

Estimating coffee pest and disease attacks embedded application

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RATIONALE

Producers confronted with attacks by coffee pests and diseases tend to overestimate the level of attack on their plots and therefore over-treat their coffee trees (Rémond, 1996). In order to reduce chemical treatments on plants, we are developing an embedded decision-support tool to help estimate the health status of their plots, for use in South America and Africa. Embedded application are promising tools in view of the importance of mobile phones in people's daily lives (Berrou and Mellet, 2020). One disease and two pests are specifically targeted: leaf rust, Black twig borer and coffee berry borer.

METHODS

The condition of the plot is evaluated using a sequential statistical procedure. The embedded application determines the number of plants to be sampled and guides the grower in entering the plant information. The various sensors in the phone are used for this guidance by mobilizing the GPS chip and the accelerometer to help determine distance travelled and direction. The architecture of the application follows a classical scheme with an initialization phase (description of the plot, targeted pests and diseases, etc.), a data entry phase using intuitive picture based selections, and a restitution phase, synthetizing the statistical analyses and proposing treatments from predefined tables.

EXPECTED RESULTS

We expect an Android application that is easy to use, tested and approved by growers. As a first outcome, the application will provide information on the general health status of the plot and an index of heterogeneity. As a second output, the tool will propose an advice for a recommended action (treatment ...).

CONCLUSIONS & PERSPECTIVES

The modularity of the application allows numerous upgrades. At a later stage, the integrated tool could include automatic data acquisition in the field by processing image captures. Statistical procedures may evolve and a production and attack development model could be included to refine recommendations for action. The ability to send analyses to a server could also feed models for the evaluation of attacks on a regional scale.

References:

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  URL: https://www.cairn.info/revue-reseaux-2020-1-page-11.htm

S2-PO-02

Small-RNA characterization of Coffee Leaf Rust races having different virulence profiles

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RATIONALE

The obligate biotrophic rust fungus *Hemileia vastatrix* (causal agent of Coffee Leaf Rust), which is the most widespread pathogen of Arabica coffee, causes severe yield losses. More than 50 rust physiological races have been detected resorting to 27 coffee differentials, since their molecular identification has not yet been possible. In the present study we have identified small-RNAs from urediniospores of three rust races with different spectra for virulence: race VI (v?-unknown) non-pathogenic to all known *C. arabica* genotypes and races II (v5) and XXIV (v2,4,5) pathogenic to the majority of *C. arabica* genotypes.

METHODS

Urediniospores of the three races were collected in triplicate samples that were immediately frozen in liquid nitrogen. Total RNA was isolated using the NOGEN KIT Plant/Fungi Total RNA Purification Kit and then sent to small-RNA-sequencing. The genome of *H. vastatrix* race XXXIII (Porto *et al.* 2019) and miRBase v22.0 were used to analyze the small-RNA-seq data by means of the miRPursuit pipeline.

RESULTS

The three races of *H. vastatrix* have a predominance of reads with 22 and 29 nt length, but only in race XXIV the relative abundance of these small-RNA species is highly differentiated. The PCA analysis revealed that the profiles of small-RNAs among the three races are completely different with the first two components explaining 90 % of the variance. The majority of the miRNAs present in *H. vastatrix* was not yet reported in miRBase and so the number of sequences annotated as conserved miRNAs is low, with only about 10 conserved miRNAs being found.

CONCLUSION AND PERSPECTIVES

Unlike what has been described for the small-RNA profile in plants, in which the main species abundance has 21 or 24 nt length, *H. vastatrix* small-RNA profile revealed a higher abundance of species with 22 and 29 nt. With this approach we have identified differences between the three races that will potentially contribute to unveil the virulence divergence observed among *H. vastatrix* races.

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S2-PO-03

New technologies using volatiles for the control of coffee berry borers (*Hypothenemus hampei*)

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RATIONALE

The coffee berry borer (CBB) is the main coffee pest in Colombia, and it is difficult to manage due to its cryptic habits and the constant availability of fruits. The IPM strategy to control CBB is based on cultural control and the use of chemical insecticides and entomopathogens as a complement. So far, the use of attractants and repellent volatiles arrived as a new chemical ecological strategy to be added to the control strategies.

METHODS

The olfactory preference of CBB was evaluated using a Y-tube olfactometer, in which ripe coffee fruits were accompanied by terpenes identified in the CBB-repellent plant *Lantana camara*, such as: a-terpinene, limonene, farnesene and b-caryophyllene. The volatiles were evaluated in concentrations between 25 to 200 ppm using 50 CBB independent females and then replicated four times. The number of insects reaching each branch of the Y-olphactometer was registered, so the repellent effect was recorded as the proportion of females entering the branch containing only the ripe coffee berries. Furthermore, the protection of coffee fruits in coffee trees was evaluated in the field using two devices produced by Russell IPM containing b-caryophyllene. They were tested at a concentration of 1.5 g in liquid formulation (PTC magipal-50) emitting 450 ppm and solid formulation (PCT predipal-50) emitting 900 ppm. Infestations were artificially induced using CBB-bored raisin fruits left on the ground.

RESULTS

As a result, the olfactory preference showed that only b-caryophyllene induced a significant and consistent CBB repelling effect at all examined doses, repelling at 25 ppm and higher showing up to 78% repellency at 50 ppm. The results with the devices under field conditions showed that after 20 days, the trees that contained the two types of devices had a reduction between 32 and 42% of fruits attacked by CBB compared with the unprotected trees. Greater volatilization of the compound was observed in the liquid devices when compared to the solids, where the release of the repellent was slower, ensuring greater volatile permanence in the field over time.

CONCLUSIONS & PERSPECTIVES

b-caryophyllene is a promising compound for an integrated pest management program in commercial coffee plantations.

S2-PO-04

Assessing the involvement of retrotransposons in the genetic and genomic variability of *Hemileia vastatrix*

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RATIONALE

Coffee leaf rust is a devastating disease caused by *Hemileia vastatrix* (Hv), leading to huge field losses and economic consequences. Understanding the genetic variation and mechanisms underlying pathogen virulence is thus crucial to address disease control. Retrotransposons have been reported to play a role in genome shaping and expansion events, in association with the ability of plant pathogens to rapidly evolve virulence. In this study, the genome size, copy number and sequence polymorphism of three retrotransposons were investigated in a set of a Hv isolates comprising different pathotypes to assess diversity and differentiation patterns as well as putative associations with virulence profiles.

METHODS

Retrotransposons were selected from an annotated EST database of three Hv differentiation/ infection stages [1] upon alignment with diagnostic loci differentiating Hv genetic lineages [2]. Three selected retrotransposons were cloned and sequenced for 10 Hv isolates with contrasting pathotypes. Medium-joining haplotype networks were obtained with PopART. Genome size was estimated by flow cytometry for 21 Hv isolates. Retrotransposons copy number per genome was estimated by qPCR for the same 21 isolates.

RESULTS

Sequence data analysis for all retrotransposons showed high levels of variability, both among and within Hv pathotypes, revealing very divergent and singleton haplotypes/copies which could differ in more than 30 SNPs and up to 84 bp indels. Haplotype networks revealed a complex diversification pattern, suggesting rapid evolution, but no apparent relation with isolate/pathotype. Genome size estimation revealed a wide variation among Hv isolates ranging from 713Mbp to 879Mbp. In addition, copy number was also quite variable and depending on the retrotransposon, the presence of 1-12 copies or more than 100 copies per genome could be detected. Although the differences in genomic content did not seem directly related with copy number for the retrotransposons under study, our results indicate a high level of proliferation and an active role of retrotransposons in generating variability in Hv.

CONCLUSIONS & PERSPECTIVES

Our study provides a novel approach to address coffee rust adaptive evolution and offers a first insight on the increased genetic variability provided by the presence of retrotransposons on the genome of Hv. Further analyses are being carried out to characterize these retrotransposons and ascertain putative causal relations with virulence profiles.

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S2-PO-05

Selection of coffee progenies with multiple resistance to biotic agents

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RATIONALE

The seminal propagation and the perennial nature of *Coffea arabica* specie are factors that make a time consuming for superior individuals selection, since the hybridizations up to the cultivar registration. Therefore, the breeding programs of *C. arabica* seek the resistance genes pyramidation in highly productive cultivars. The choice of parents should favor the pyramidation of genes in order to have simultaneous resistance to different biotic agents and allow a greater selection gain. Thus, the aim of this work was to select coffee trees with multiple resistance to root knot nematodes (*Meloidogyne* spp.), coffee rust (*Hemileia vastatrix*) and bacterial halo blight (*Pseudomonas syringae* pv. *garcae*), through recombination and selection of multiple resistance to pathogens in support of the breeding program conducted by the Instituto Agronómico de Campinas (IAC).

METHODS

Coffee trees in F2 generation, obtained from hybridization between Sarchimor IAC 4933 and wild access IAC 2036-6 of *C. arabica* (FAO, 1968), were evaluated for resistance to *P. syringae* pv. *garcae*, *H. vastatrix* and simultaneously to *M. exigua*, *M. incognita* and *M. paranaensis*. In all experiments, the *C. arabica* cultivar Mundo Novo IAC 515-20 was adopted as a susceptibility standard.

RESULTS

Of 597 F2 coffee plants inoculated with *P. syryngae* pv. *garcae*, 372 proved to be resistant to the pathogen. As for the coffee rust, only 18 plants out of 355 tested were susceptible to *H. vastatrix* race II. Plants with resistance to coffee rust and bacterial halo blight were further evaluated for resistance to root knot nematodes. At the end of the experimental period it was possible to select 35 plants with high levels of root knot resistance and, consequently, with simultaneous resistance to all biotic agents studied.

CONCLUSIONS & PERSPECTIVES

The results showed important aspects of coffee - pathogen interactions: (i) the resistance to bacterial halo blight seems to be of qualitative character, and probably with allelic interactions; (ii) matrix H 20393-2 showed no susceptible plants to *H. vastatrix* race II, showing their homozygous nature; (iii) plant inoculation with *Meloidogyne* species was efficient in selecting coffee plants with high levels of multiple resistance.

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S2-PO-07

Poster presentations

Crossings compatibility on *Coffea canephora* aiming multiple resistance in clones and full-sib progenies to *Meloidogyne exigua, M. incognita* and *M. paranaensis*

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RATIONALE

For coffee trees in Brazil, root knot nematodes (RKN), belong in the genus *Meloidogyne*, are of great importance due to the geographical distribution and damage for the crop. In São Paulo State, four species of RKN were found parasitizing coffee plants, being *M. incognita* the most widespread, followed by *M. exigua, M. paranaensis* and *M. coffeicola*. Among the different strategies adopted on coffee production for management of diseases caused by these pathogens, the use of resistant crops has high cost / benefit and can be an efficient option. For the other hand, genetic resistance of nematodes has some particularities that deserve attention, such the overcoming of the resistance by of pathogen and mixture of inter and intra-specific populations in the same location of crop production, that may block the use of specific resistance of crop cultivars. Considering the above, cultivars with multiple resistance to different populations of nematodes may be determinants in the use of this technology. Therefore, the objective of this study was to select resistant coffee clones based on the compatibility of crossings and on the multiple resistance of progenies of germanic siblings to *M. paranaensis*, *M. incognita* and *M. exigua* to obtain rootstock cultivars of *Coffea canephora*.

METHODS

The study involved the crossings in diallel scheme of 10 resistant clones to *M. exigua*, *M. incognita* and *M. paranaensis*. The reaction of progenies to nematodes was evaluated aiming the identification of combinations with high levels of resistance.

RESULTS

The results have evidenced the male sterility of clone IAC 263; the incompatibility in only one of the possible interclonal combinations; the absence of a maternal effect on the expression of resistance to the three species of *Meloidogyne* and the multiple resistance of 25 progenies of germanic siblings to *M. paranaensis, M. incognita* and *M. exigua.*

CONCLUSIONS & PERSPECTIVES

Based on FR and RFR analysis, it was possible to identify 21 hybrid combinations between two clones, 16 combinations between three clones and five combinations between four clones with multiple resistance to *Meloidogyne* spp., which may constitute new resistant cultivars.

References:

- Bertrand et al. 2000 Euphytica, v.113, p.79-86.
- Fatobeneet al. 2018 Experimental Agriculture, v.55, p.443-451.
- Gonçalves W, Silvarolla MB. 2007 O Agronômico, v.59, p.54-56.

S2-PO-08

First steps on the resistance profiling of Kawisari coffee hybrid through cytological and gene expression analyses

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RATIONALE

Coffee leaf rust (CLR), caused by *Hemileia vastatrix* (*Hv*) has been a permanent threat to Arabica coffee production. Breeding for CLR resistance has been the most appropriate and sustainable strategy for disease management. The most widely used sources of resistance to CLR were Timor hybrids – HDTs (*C. arabica* x *C. canephora*). The recent breakdown of resistance in some HDT-derived varieties, due to the occurrence of more virulent *Hv* races, as well as the current CLR epidemics in Central America, highlights the importance and obvious need for the discovery and characterization of new sources of resistance. This work aims to start unveiling the cellular and molecular resistance profile of the Kawisari hybrid (*C. arabica* x *C. liberica*) derivative, recently used as donor for resistance in Arabica breeding programs in India.

METHODS

The Kawisari hybrid was challenged with two distinct *Hv* races in order to establish an incompatible (resistance) and a compatible (susceptibility) interaction for a comparative analysis. Samples were collected during infection time-course, simultaneously for evaluation of fungal growth and plant responses by light microscopy and qPCR gene expression analysis [1]. Whole genome sequencing data previously obtained for Kawisari hybrid was aligned against the *C. arabica* reference genome using BWA and variants were called using bcftools. The resulting variants were used to identify and select polymorphic target genes putatively involved in defense/resistance responses. Expression analysis of selected candidate genes was initiated.

RESULTS

In the incompatible interaction, *Hv* ceased its growth more frequently after the formation of the first haustorium (post-haustorial resistance) inducing a hypersensitive-like reaction, accumulation of phenolic-like compounds and haustorium encasement with callose. SNPs retrieved from Kawisari´s data analysis were mapped back to the annotated genome to identify the variant loci. From these, 50 genes with a functional category assigned were identified, including specifically pathogen defense-related. Assessment of the candidate genes' differential expression profiles throughout the infection process is in progress.

CONCLUSIONS & PERSPECTIVES

Our study provides the first insights on the characterization of the resistance response of a coffee hybrid with a high potential to be explored as a new source of resistance.

Acknowledgments: Funding from national funds through Foundation for Science and Technology (FCT) and FEDER funds through PORNorte under the projects CoffeeRES ref. PTDC/ASP-PLA/29779/2017 and HDT-Coffee ref. PTDC/ASP-PLA/32429/2017, and FCT UNIT LEAF (UID/AGR/04129/2020).

References:

- 1- Diniz, I. et al. European Journal of Plant Pathology. 2012. DOI:10.1007/s10658-011-9925-9.

POSTER PRESENTATIONS

Session 1: Plant science

S1-PO-02

Microscopy detection of cytoplasmic lipid droplets (LDs) in leaves of different *Coffea* species

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RATIONALE

Cytoplasmic lipid droplets (LDs), also known as oil bodies, are the common storage form of lipids that mainly occur in seeds of some Angiosperms, comprising also several coffee species (Huang 2018). However, LDs were also detected in leaf mesophyll cells of many plant families as reported by Lersten et al. (2006). Unfortunately *Rubiaceae,* the family of coffee, was not taken into consideration in this screening. The great majority of plants has a single LD per mesophyll cell, ranging from 1 to 18 µm in diameter; clusters of smaller LDs per cell can also be observed. The possible functions of these bodies range from adaptation to cold temperatures and intermediate storage products of photosynthesis to plant defense. For the latter, in senescent leaves, a plant defense role against fungal infection has been highlighted. Surprisingly, very little attention has been given so far to oil reserves in coffee leaf cells. This preliminary study is aimed at ascertaining the presence of LDs in coffee leaves, in order to deepen the knowledge about coffee leaf anatomy and cell behavior.

METHODS

Mature fresh leaves of six different *Coffea* species (*C. arabica* L., *C. canephora Pierre ex-Froehner*, *C. myrtifolia* (A.Rich. ex DC.) J.-F.Leroy, *C. sessiliflora* Bridson, *C. brevipes* Hiern, *C. eugenioides* S. Moore), coming from plants kept under the same greenhouse conditions (Italy), were sampled. Leaf sections made by a razor blade were observed by an optical microscope (Leica DMRXE) in their native aspect and after staining with a saturated solution of the Sudan IV dye, a test used to detect oils. LD diameters and leaf tissue thickness were measured by the Leica LAS X software (Leica microsystems).

RESULTS

LDs were present in all leaves of the six investigated *Coffea* species. However different quantity, size and presence in the mesophyll and/or epidermal tissue were observed among species. LDs were mainly present as single body (1-10 µm) in both palisade and spongy parenchyma cells. A maximum size of 10 µm was detected in *C. arabica* leaves. *C. myrtifolia* leaf is characterized by a different organization, having both single ones and clusters of small LDs (1-4 µm), the latter observed only in the spongy parenchyma.

CONCLUSIONS & PERSPECTIVES

This preliminary investigation confirms the presence of LDs in *Coffea* leaves, independently of the observed different foliar anatomy. Coffee leaf physiology could certainly be influenced by this type of oil-reserve system revealed for the first time in several coffee species. Further studies are necessary to clarify the role played by LDs in coffee physiology, including the possible role in plant defense mechanisms against pathogens.

References:

- Huang A.H.C., 2018. Plant Physiol. 176: 1894-1918.
- Lersten et al., 2006. Am J Bot, 93: 1731-1739.

Development of decaffeinated clonal cultivars of Arabica coffee

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RATIONALE

Arabica coffee (*Coffea arabica*) has approximately 1.2% caffeine in its beans. Although it offers health benefits, caffeine consumption can cause certain discomfort to some consumers. In the currently available decaffeinated coffees, caffeine is removed from the beans using water or organic solvents. This work aimed to use the classic improvement to make a clonal cultivar of decaffeinated coffee available, without the use of gene editing or genetic manipulation techniques, producing beans with a maximum caffeine content of 0.10% and with adequate productivity. Thus, such grains would spare the use of industrial processes of removing caffeine, benefiting both the environment and human health.

METHODS

After the discovery of three plants with caffeine content below 0.10%, at the Germplasm Bank at the Agronomic Institute of Campinas (IAC) in Brazil (Silvarolla et al., 2004), hybridizations between this germplasm and Brazilian cultivars were carried out. The progenies were subjected to selection by classical genetic improvement and the F3 plants are being evaluated in an experiment installed in randomized blocks, with 35 treatments, four replications and five plants per plot. Agronomic evaluations and determinations of the caffeine content in the beans have been carried out for three years.

RESULTS

700 plants were evaluated, twelve of which had simultaneously maximum levels of caffeine of 0.10% and yields above 1,800 kg/ha. The lowest caffeine content was found by the F3-293688 plant, only 0.01%, with a productivity of 1,986 kg/ha. The F3-293540 plant reached 0.08% caffeine with a productivity of 4,962 kg/ha, the highest in the experiment. The F3-293611 plant produced 3,360 kg/ha and only 0.05% caffeine in the beans. The other plants obtained the following results: F3-293596 = 0.08% caffeine and 3.132 kg/ha; F3-293667 = 0.06% and 2.952 kg/ha; F3-293578 = 0.04% and 2.922 kg/ha; F3-293604 = 0.10% and 2.688 kg/ha; F3-293712 = 0.05% and 2.532 kg/ha; F3-293739 = 0.04% and 2.364 kg/ha; F3-293653 = 0.08% and 2.280 kg/ha; F3-293525 = 0.03 and 2.100 kg/ha and F3-293681 = 0.06% and 1.848 kg/ha.

CONCLUSIONS & PERSPECTIVES

Twelve plants with levels equal to or less than 0.10% of caffeine in the grains and with yields above 30 scs/ha have been selected and will be cloned for validation in regional clonal experiments in order to provide the coffee grower with a decaffeinated clonal cultivar, the production of which serves consumers who have a caffeine intolerance**.**

References:

- Silvarolla et al. 2004 Nature DOI: 10.1038/429826[a]

S1-PO-04

Spatial patterns of apparent fractionation on the subgenome chromosomes in *C. arabica* and gene loss in *C. canephora* and *C eugenioides*

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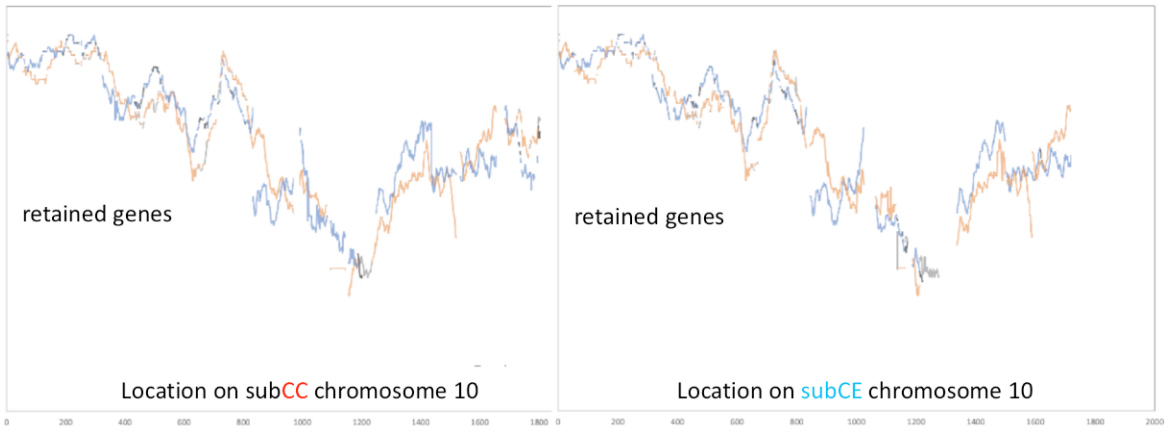
In the context of the genome sequencing and population genetics carried out by the Arabica Coffee Genome Consortium [1], we analyzed all the synteny blocks produced by SynMap [2] in all of the comparisons among the two subgenomes of the allotetraploid *Coffea arabica* and the two diploid "progenitor" genomes *C. canephora* and *C eugenioides*, six comparisons in all.

The density of retained genes after fractionation, and their spatial distribution along homeologous chromosome is almost identical in the two subgenomes, as exemplified by two hoeologous chromosomes in the figure. Indeed these tendencies reflect the same propensities in the corresponding chromosomes of the progenitor genomes. Most of the gene loss from the subgenomes actually consist of pre-existing deletions already affecting *C canephora* and *C. eugenioides*. The parallel distribution in these two diploids reflect in part ongoing fractionation, in part inheritance from their common ancestor 6-10 Mya, but more importantly contrasting propensities for gene loss in pericentromeric regions versus the rest of the chromosome, as can be seen in the figure. This tendency must date to the origins of the genus or earlier.



Proportion of retained duplicates in synteny blocks of the *C. arabica* subgenomes.

References:

- [1] DeKochko, Crouzillat 2015 12th Solanaceae Conference.
- [2] Lyons, Freeling 2008 The Plant Journal 53, 661–673.

S1-PO-05

Warming impact and intraspecific differences in thermoregulation of *Coffea arabica* L. genotypes

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RATIONALE

The elevated temperatures predicted for the next decades will reduce global yields of major crops (Zhao et al., 2017). For coffee, several studies project scenarios with remarkable negative effects (DaMatta et al., 2019), which require more adapted genotypes to ensure coffee production and, thermotolerance intraspecific variation could be a valuable information for breeding programs. Here, we compared the effects of warm temperatures on two commercial coffee genotypes conciliating physiological, global transcription and sugar metabolism data.

METHODS

Coffee genotypes were maintained in growth chambers for four weeks at optimal temperatures with 23/19°C (day/night) and after for 30/26°C (day/night) as a possible future warming scenario in producer regions. Plant physiology was accessed by Infrared Gas Analyser (IRGA), differences on gene expression by RNAseq analysis and sugar contents by enzymatic assays.

RESULTS

Coffee cultivars showed differences in the control of leaf temperatures, whereas slightly or no differences for transpiration rates, photosynthetic activity and stomatal conductance. Leaf transcriptome examined using RNAseq, showed a marked number of differentially-expressed genes (DEGs) under optimal temperature between genotypes, however DEGs strongly decrease in both genotypes as warmer temperature is imposed indicating a transcriptional constraint. The examination of DEGs in response to warmer temperatures revealed shared genes between cultivars, as well as, genotype-specific genes that were mostly related to carbohydrate metabolism. Indeed, sugars analysis showed that elevated temperatures impact sugar contents in a genotype dependent manner in coffee plants.

CONCLUSIONS & PERSPECTIVES

This work provides a first examination of the intraspecific molecular responses of coffee genotypes to warmer temperatures, relating thermotolerance to the carbohydrate homeostasis capacity, which may be useful for crop breeding in face of the expected climate changes.

References:

- Zhao C et al. 2017 Proc Natl Acad Sci U S A. 2017 Aug 29; 114(35): 9326-9331.
- DaMatta, FM et al. 2019. Climatic Change, 152:167.

S1-PO-06

Genetic diversity of cultivated and wild *Coffea canephora* trees in Yangambi (DR Congo) and the risk of introgression

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RATIONALE

As wild *Coffea canephora* is the crop wild relative of worldwide cultivated Robusta coffee, its natural gene pool from rainforests in the Congo basin may contain useful genetic diversity for coffee breeding, and make coffee production more sustainable. Therefore, the conservation of its genetic diversity is of major importance (Laikre et al., 2010). A serious threat to the genetic integrity of the wild *C. canephora* gene pool, is introgression of foreign alleles from cultivated coffee (Kwit et al., 2011). This is especially in the Congo basin, where local residents grow cultivated material, in their backyards near wild populations. The risk of introgression might be exacerbated in secondary forest where there is ample opportunity for mixing following forest regrowth. Here, we aimed to quantify genetic diversity and structure of wild *C. canephora* populations in relation to locally grown cultivars in the Northern region of the Congo basin.

METHODS

Coffee leaf samples were collected from fifteen plots in both primary and secondary rainforest around Yangambi and from backyards of local residents, in which cultivars are grown. Genotyping-by-sequencing was applied on a total of 264 individuals to obtain single-nucleotide polymorphisms (SNPs). Allele frequencies of these SNPs were compared between backyard and wild samples and between primary and secondary rainforest samples. Admixture and hybridization was examined using a Bayesian clustering algorithm in fastSTRUCTURE and a Discriminant Analysis of Principal Components (DAPC).

RESULTS

We identified 7 641 polymorphic SNPs. Wild *C. canephora* samples were genetically different from those cultivated in backyards. fastSTRUCTURE analysis show that the samples from primary rainforest were genetically differentiated and grouped in four different clusters. The samples in secondary rainforest plots displayed signs of genetic admixture with genetic material from the different clusters of primary plots and from the backyard samples.

CONCLUSIONS & PERSPECTIVES

Cultivars were genetically different from wild samples, suggesting that they have a different geographical origin. The different clusters in the population structure of the primary forest samples could be explained by low levels of gene flow. The presence of admixed genotypes in secondary forest samples, provides evidence that wild and cultivated individuals, when in close proximity, are able to exchange genetic material. This suggest that there is ample opportunity for genetic mixture, creating a risk for introgression and potentially threatening the integrity of the wild coffee gene pool.

References:

- Laikre et al. 2010 Conservation Biology, 86-88.
- Kwit et al. 2011 Trends in Biotechnology, 284-293.

S1-PO-07

Genome-wide association study for morphological and yield component traits in C*offea arabica*

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RATIONALE

Genome-wide association study (GWAS) has been successfully applied in several annual and perennial plant species. However, few GWAS works have addressed *Coffea arabica*. The incorporation of this methodology in coffee breeding programs is important to explore the low variability of this species, maximize genetic gains and make the breeding process faster and more efficient.

METHODS

A phenotypic evaluation of 18 agronomic traits were performed in a population of 195 individuals of *C. arabica*, in three consecutive years. The analyzed traits were yield (Y), leaf length (LL), leaf width (LW), branch length (BL), number of reproductive (NRN) and vegetative (NVN) nodes, number of fruits (NF), fruit volume (FV), plant height (PH), diameter of canopy (CD) and stem (SD), ripening fruit size (RFS), maturation uniformity (MU), maturation cycle (MC), incidence of rust (Rus) and cercosporiosis (Cer), leaf miner infestation (LM), and vegetative vigor (VV). The coffee population was also genotyping using Capture-Seq methodology (RAPiD genomics) and 10,000 polymorphic probes. The genetic structure of the population was obtained through the Principal Component Analysis (PCA), using prcomp (software R). PCA were used as covariates in the GWAS model, to detect SNP associated with phenotypic traits.

RESULTS

The phenotypic data were corrected for years, plots, and years × plots interactions, using the REML/BLUP methodology. Coffee trees, besides being phenotyped, were genotyped with SNP markers and after quality analyses, 20,477 SNP widely distributed in the genome were used for GWAS. A total of 110 SNP were significantly associated ($p < 0.05$) with seven of the analyzed traits (PH, BL, NVN, CD, RFS, Rus and Cer). Plant height (PH) showed the highest number of significant associations SNP (56 SNP), and the data suggested a major QTL in the control of this trait, located on chromosome 6. The effects of each associated SNP in the seven target traits were obtained, allowing to identify favorable allele and markers for future use in assisted selection. The genes with the significant SNP were analyzed and 19 candidate genes were identified. Further analysis was performed to screen the potential genes involved in each trait.

CONCLUSIONS & PERSPECTIVES

The efficiency of the GWAS methodology for coffee was confirmed. Molecular markers associated with main agronomic traits, including disease resistance, morphological and yield component traits were identified. Our results provide new insights into the genetic architecture of these traits, and will be helpful in assisted *C. arabica* breeding program.

S1-PO-08

Unravelling the metabolic and hormonal machinery during key steps of somatic embryogenesis: A case study in coffee

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RATIONALE

Somatic embryogenesis (SE) is one of the most promising processes for large-scale dissemination of elite varieties. However, whatever the species considered, SE research still remains essentially empirical resulting in many drawbacks to fulfil market demands, especially due to an overall slow technical progress over the last 20 years. Knowledge about the molecular events involved in the key steps of the SE process is urgently needed to pilot the optimization of SE protocols. In this study, we took advantage of the latest metabolomics technologies and applied them to one of the most advanced and reliable large-scale SE processes, the one developed for coffee.

METHODS

Sampling covered 15 key developmental stages. Five independent leaf introductions were carried out with more than 4,000 leaf explants and a total of 25 independent cell lines. All obtained cell lines were high-yielding and time-synchronized during embryo regeneration enabling a successful sampling. Primary metabolites, secondary metabolites and phytohormones were quantified using GC-MS, HPLC and UPLC- MS/MS respectively. A robust statistical method was used to identify metabolic pathway changes associated with the main developmental phases and phase switches. Histological analysis and cell imaging were also required to characterize developmental stages and associate metabolic profiles with cell structure organization. Lastly, comparing Arabica embryogenic and non-embryogenic calli enabled the identification of metabolic markers of the embryogenic capacity.

RESULTS

Statistical analysis performed on 104 metabolites revealed that massive re-configuration of metabolic pathways induced SE. During initial dedifferentiation, a sharp decrease in phenolic compounds and caffeine levels was observed while auxins, cytokinins and ethylene levels were at their highest. Totipotency reached its highest expression during the callus stages when a shut-off in hormonal and metabolic pathways related to sugar and energetic substance hydrolysis was evidenced. Abscisic acid, leucine, maltotriose, myo-inositol, proline, tricarboxylic acid cycle metabolites and zeatin appeared as key metabolic markers of the embryogenic capacity. Combining metabolomics with multiphoton microscopy led to the identification of chlorogenic acids as markers of embryo redifferentiation.

CONCLUSIONS & PERSPECTIVES

The present analysis shows that metabolite fingerprints are signatures of cell fate and represent a starting point for optimizing SE protocols in a rational way. These findings should be informative and useful to a wide range of plant species, offering unprecedented perspectives in plant micropropagation.

References:

- Awada et al. 2019 International Journal of Molecular Sciences DOI: 10.3390/ijms20194665

S1-PO-09

Vegetative reproduction of *Coffea canephora* with different cutting standards

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RATIONALE

Considered one of the main vegetative reproduction techniques, cutting is a promising method for *Coffea canephora* production, as it promotes more root development, and thus a better agronomic performance for coffee trees (Partelli et al., 2014; Nunes-Gomes and Krinski, 2016). The success in the cutting method depends upon some factors, such as the length of the cutting and the number of stem buds. This work aimed to assess the development of *Coffea canephora* seedlings propagated via cutting with different cutting standards.

METHODS

Four treatments were assessed, which are as follow: T1, composed of one stem bud and with apical cutting; T2, two stem buds and with apical cutting; T3, compose of two stem buds without apical cutting; and T4, three stem buds without apical cutting. The experimental design was completely randomized, with five replicates. The following parameters were assessed in the coffee seedlings after 120 days of the vegetative reproduction via cutting: seedling height, stem diameter, number and height of the plagiotropic branches, number of leaves and roots, total dry mass and IQD (Dickson Quality Index). The resulting data were analyzed with the F test and the means were compared via the Tukey test.

RESULTS

Considering the coffee seedlings produced via cutting, lower mean values were observed in the T1 for the assessed parameters, with the exception of both number of leaves and roots. The steam diameter was lower T1 and T2, with no significant difference between these treatments. The higher mean value for seedling height was found in T2. Overall, the treatments T2, T3 and T4 were very similar for all assessed parameters, with the exception of steam diameter and number of plagiotropic branches.

CONCLUSION & PERSPECTIVES

The development of coffee seedlings was influenced by the number of stem buds as well as apical cutting. The results of this study contribute with information for the production of seedlings with high agronomic quality, considering different cutting standards, which may influence the development of *C. canephora* fields.

References:

- Partelli et al. 2014 Pesquisa Agropecuária Brasileira DOI: 10.1590/S0100-204X2014000500004
- Nunes-Gomes &Krinsk 2016 Scientia Agraria DOI: 10.5380/rsa.v17i3.49695

S1-PO-10

Responses of Arabica coffee (*Coffea arabica* L. var. Catuaí) cell suspensions to chemically induced mutagenesis and salinity stress under in vitro culture conditions

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RATIONALE

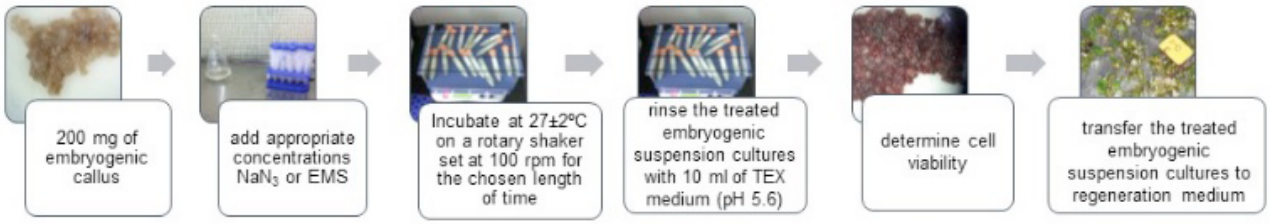
Crop improvement of *Coffea arabica* L. via mutagenesis could accelerate breeding programs; thus, the present study aimed to develop an in vitro protocol using the chemical mutagens sodium azide (NaN3) and ethyl methanesulfonate (EMS) on embryogenic cell suspensions of Arabica coffee variety Catuaí and, subsequently, to evaluate the responses of the resulting mutagenized tissues to salinity stress.

METHODS

Embryogenic suspension cultures were incubated with 0.0, 2.5, 5.0, or 10.0 mM NaN3 or 0.0, 185.2, 370.5, or 741.0 mM EMS.

RESULTS

As the concentration of NaN3 or EMS increased, the survival of embryogenic suspension cultures decreased compared to controls. Embryogenic suspension cultures treated with NaN3 or EMS, cultured on selective medium supplemented with 0, 50, 100, 150, 250, or 300 mM NaCl, showed that 50 mM NaCl could be used as selection pressure. Plantlet growth and total amino acid content were affected by NaCl stress; some mutants had longer shoots and higher amino acid content than controls. Random amplified polymorphic DNA (RAPD) analysis was performed and a total of 22% polymorphism were determined between putative mutant and non-mutant arabica coffee embryogenic cultures.



Bulk mutagenesis process under in vitro conditions of embryogenic calli of coffee (*C. arabica* L. var. Catuaí).

ABSTRACTS TUESDAY 29 JUNE

POSTER PRESENTATIONS

Session 1: Plant science

S1-PO-11

Coffee genetic resources in Yemen, diversity and importance for Arabica coffee improvement

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RATIONALE
Yemeni coffee landraces (YCL) have been a corollary for the heritage of coffee cultivation in diverse conditions for more than a thousand year; nevertheless, the genetic diversity of these landraces is not well studied. The primary objectives of this study were to develop clear morpho-physiological metrics that were repeatable, could be used to differentiate coffee landraces, and could provide scientific evidence of the genetic diversity of traditional coffee landraces still grown by farmers in Yemen. Increasing our understanding of this diversity is the first step to maintain diversity for the benefits of farmers in Yemen and worldwide as well as coffee consumers.

METHODS
Thirty Arabica coffee landraces were collected within Yemen and evaluated for eleven morpho-physiological characteristics under greenhouse conditions and compared to the genetic diversity of cultivars and commercial varieties from worldwide.

RESULTS
Statistical analysis demonstrated significant phenotypic variation observed between these landraces showing the importance of farmer's roles, traditional cultural practices, the geographic conditions in selection and genetic isolation of these landraces. Results demonstrated that these landraces differed by geographic areas, region of cultivation, and genetic components which controlled the variation of these morpho-physiological traits related to adaptation and production characteristics. Correlation analyses illustrated strong degrees of relation between various traits, most interesting were significant correlations between stomata numbers and most of the traits related to plant vigor, such as plant height, diameter of stem, and number of nodes per plant. Chlorophyll content measured by SPAD was also highly significant correlated with plant height, stem diameter, leaf areas, and leaf specific weight. Multivariate analyses for these parameters was conducted using principal component analyses (PCA) which distinguished seven groups of landraces, as well as variation within these groups, which reflects the genetic factors controlling this variation rooted in heterogeneous environmental effects, genetic isolation and the traditional selection process of Yemeni farmers.

CONCLUSIONS & PERSPECTIVES
Comparing variations of Yemeni coffee landraces to other commercial varieties confirmed the potential value of Yemeni coffee landraces for improvement coffee cultivation and the need to protect the genetic diversity of Yemeni Arabica coffee populations to enrich the genetic basis of cultivated *C. arabica* L. germplasm.

S1-PO-12

From the herbarium back to the forest: a successful collection of wild Robusta coffee (*Coffea canephora)* in Guinea

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RATIONALE

Coffea canephora Pierre can be classified in two genetic groups: the Guinean group originating in West Africa and the Congolese group that stretches over a vast area from Atlantic Central Africa to Uganda. A recent genotyping study on old herbarium specimens showed that wild coffee of the Guinean group can be assigned to five sub-groups (sgG1 to sgG5) with distinct geographic distribution (Labouisse et al. 2020). We focused on the sub-group sgG1, which is represented by one population described by Chevalier (1905) as *C. canephora* var. maclaudii. Located in a forest island on the slope of the Bilima Hénéré plateau near Mamou, Guinea, this population is considered vulnerable and a priority target for conservation.

METHODS

The partners of FOGEFO-PLUS, a project of the SEP-2D programme (http://sep2d.org), undertook an inventory of the forest vegetation and a survey on the uses and products of the forest. Leaves were taken from 53 coffee trees and their DNA extracted. For comparison, we used DNA of herbarium specimens of the Guinean group collected between 1905 and 1993, as well as a few samples of the Congolese group. In total, we genotyped 83 coffee samples with 21 nuclear microsatellite markers. A representation of the structure of genetic diversity was obtained by a factorial analysis (PCoA), while genetic relationships between individuals were assessed using NJ method.

RESULTS

The Bilima Hénéré forest has a tree layer composed, inter alia, of *Piptadeniastrum africanum* and *Parkia bicolor*, and a shrub layer of *Maesobotrya barteri* and *C. canephora.* Neighboring villagers gather forest plants for food, pharmacopeia, and handicrafts making. The average density of coffee trees per ha (with Ø> 5 cm) is 177, and some of them can reach more than 10 m in height. Genotyping data analyses showed that all the coffee samples taken from the forest have close relationship with the herbarium samples collected from the same place as early as 1905. Within the Guinean group, the sub-group of *C. canephora* var. maclaudii is characterized by a low level of admixture with other sub-groups due to its geographical isolation and distance from the main areas of coffee cultivation.

CONCLUSIONS & PERSPECTIVES

Located in the forest-savannah transition zone, the forest in vulnerable to drought, fire, and crop extension. Coffee seeds and cuttings were collected and transferred to the gene bank of IRAG Sérédou Research Center for *ex situ* conservation and potential use in a future breeding programme. This should be complemented by measures for *in situ* conservation with the participation of people of neighboring villages to set up sustainable management of the forest resources.

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S1-PO-13

A contribution to the future of Robusta coffee by investing in the INERA coffee collection in Yangambi (the Democratic Republic of the Congo)

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RATIONALE

The Congo Basin contains important genetic resources of coffee with 12 native species, of which 6 are endemic in the region. Moreover, it is the homeland of important diversity of *C. canephora*, *C. eugenioides* and their close relatives. Unfortunately, the diversity is poorly studied and underrepresented in gene core collections. Moreover, the local coffee collections are small and do not meet international standards. In this project we study these genetic resources, rehabilitate the collection and enrich the collection with local diversity, in order to safeguard them for the future.

METHODS

In order to achieve these objectives we work along four axes: rehabilitation of infrastructure, inventorying and collecting new genetic resources, capacity building and multidisciplinary research.

RESULTS

Although it is work in progress, we can already present some results: rehabilitated basic infrastructure, trained local collaborators, inventoried collection, evaluated genetic diversity of the collection, assessment of gene flow between collections, cultivated and wild Robusta coffees in Yangambi, an inventory of wild coffees in the Yangambi Man and Biosphere Reserve and identification of valuable sites within the DRC for future sampling.

CONCLUSIONS & PERSPECTIVES

The project illustrates that in a diversity rich country facing instability, insufficient infrastructure and limited resources, coffee genetic resources can be conserved and local capacity can be built up if the stakeholders collaborate on the basis of sound scientific knowledge, long standing relations and a good network. Training of dedicated local partners is a key to success. We generate a basis for the of coffee in the DR Congo and abroad. In the context of the Convention of Biodiversity it is essential to support and involve countries housing important genetic diversity in research and conservation programs. Long term sustainable funding is essential but remains challenging. Coffee can contribute to the UN Sustainable Development Goals 1, 2, 7, 15 & 17.

These projects create infrastructure and capacities for conservation of and research on these important genetic resources. We contribute to the conservation and study of important but poorly documented coffee genetic resources of the DR Congo. We pave the way to involve the DR Congo, which holds important genetic resources of coffee, again in international programs. Finally we aim to contribute to local development.

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S1-PO-14

Coffea spp. Membrane Responses to Superimposed Elevated [CO_2] and Drought in View of Higher Acclimation Ability

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RATIONALE

Increased air [CO_2] and drought due to climate changes affect the physiological processes of plants and their productivity. Obtaining coffee genotypes with higher tolerance to abiotic stresses represents a major challenge for coffee producing countries. Membranes are crucial selective homeostasis barriers that ensure cell metabolism, also acting as buffer interfaces able to adapt under changing environment. The purpose of the present work was to assess physiological responses regarding protoplasmic integrity and chloroplast membranes lipid composition in leaves of two *Coffea* genotypes (*Coffea arabica* L. cv. Icatu and *C. canephora* cv. Conilon Clone 153), when exposed to conditions of soil water stress and elevated air [CO_2].

METHODS

Seven-year-old plants of *C. canephora* cv. Conilon Clone 153 (CL153) and *C. arabica* cv. Icatu, were grown under controlled conditions of relative humidity (70%), photoperiod (12h), temperature (25/20 ºC, day/night), irradiance (*ca*. 700 µmol m^{-2} s^{-1}) and [CO_2] (380 or 700 µL L^{-1}). Some plants were kept well-watered (WW) while others were subjected to severe water deficit (SWD) by partially withholding irrigation for two weeks. Protoplasmic tolerance was evaluated by electrolyte leakage test and expressed as an injury index (I%). Quantitative and qualitative changes in chloroplast membrane lipids were assessed by GC-FID chromatography, through total fatty acid (TFA) content, fatty acids composition and unsaturation degree (DBI) (for details see refences 1-3).

RESULTS

Under drought, I% values increased in 380-plants of both genotypes and in Icatu-700, while values were stable in CL153-700 plants. As regards lipid content, TFA mainly increased with drought except in CL153-380 plants. Changes in DBI were also observed in response to drought (Icatu) or to high CO_2 (CL153).

CONCLUSIONS & PERSPECTIVES

Stable I% values in CL153-700 under drought suggested that elevated air [CO_2] enhanced protoplasmic tolerance. Genotypes differed in quantitative and qualitative changes of chloroplast lipids in response to water stress. In some cases, effects of high CO_2 were also observed. Results are being analysed in order to better unveil the role of lipid profile changes in coffee plant acclimation response to superimposed drought and high [CO_2] conditions.

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S1-PO-15

Targeted and untargeted metabolomics for the valorisation of *Coffea anthonyi*

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RATIONALE

This study is on three *Coffea* species: *arabica* (arabica coffee), *canephora* (robusta coffee) and *anthonyi*. The two first species are currently used for coffee making while the third one is closely related to *C. arabica.*[1] Previous studies showed that *C. anthonyi* has a great content of chlorogenic acids.[2] Leaves, fruits and phloem sap extracts of the three species were compared in a targeted and an untargeted metabolomic approach to improve the understanding of coffee plant biochemistry. In addition, an expression study of genes involved in the biosynthesis of the main xanthines and polyphenols found in coffee genus was performed.

METHODS

Samples were collected on plants grown with the same environmental conditions at Meise Botanical Garden (Belgium). Extracts of mature leaves from the three species and of fruits from *C. arabica* and *C. anthonyi* were obtained after suspending dried powdered tissues in milliQ water.[3] Phloem sap extracts were obtained using the phloem exudation technique. Semi-polar metabolite fingerprints were monitored using a reverse phase LC-HRMS method with positive and negative ionisation mode, and data treatment was done by the Workflow4Metabolomics infrastructure. Total RNA was extracted from tissues with the Maxwell RSC Plant RNA Kit (Promega) and reverse transcription was done with the GoScript Reverse cDNA Synthesis Kit (Promega).

RESULTS

The leaves, the fruits and the phloem sap of the three coffee species were analyzed. Supervised multivariate analyses were performed on LC-HRMS data chromatograms showing a good inter species discrimination. Caffeine appeared as one of the strongest discriminant metabolites in the leaves and in the phloem sap. Caffeine was abundant in fruits but almost absent in leaves of *C. anthonyi*. The very low content of caffeine in *C. anthonyi* leaves could be explained by the low expression of caffeine synthase genes. Strong differences were also detected between the amount of chlorogenic acids and of xanthone derivatives. *C. canephora* leaves showed the lowest amount of caffeoylquinic acids and a higher degradation of caffeine mirrored by a higher theophylline amount. The highest content of caffeoylquinic acids and xanthones were detected in *C. anthonyi* leaves and fruits.

CONCLUSIONS & PERSPECTIVES

The metabolomic approach allowed us to better describe the chemical content of *C. anthonyi* and the expression study of genes in its organs was helpful to better understand how the main purine alkaloids are synthesized in this species. Additional studies will be necessary to establish whether this coffee species is suitable to be marketed.

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S1-PO-16

Safeguarding the diversity of species of the genus *Coffea* in Réunion Island

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RATIONALE

While wild species of the genus *Coffea* are critical for coffee crop development, a majority of these species are threatened with extinction in their natural environment, due to habitat loss and climate change (Davis et al. 2019). Both *in situ* and *ex situ* conservation programs should be developed for the long-term safeguarding of these resources, heritage of worldwide importance.

METHODS

Several collecting campaigns of *Coffea spp.* germplasm were carried out by IRD in Africa (Cameroon, Côte d'Ivoire, Ethiopia, Guinea, Kenya, Central African Republic, Kenya, Mozambique, Republic of Congo, and Tanzania) with the participation or support of national and international institutions (Bioversity, CIRAD, CNRA, FAO, MNHN, etc.) and over a period going from the 1960s to the 1980s. Other surveys in the Indian Ocean islands (Mauritius, Mayotte, and Reunion) were carried out during the 2000s.

RESULTS

A field genebank of wild *Coffea* species has been established in Réunion between 2008 and 2012. To date, the gene bank contains about 700 accessions belonging to 35 coffee species representing the diversity of the genus *Coffea* throughout its natural range: African species (except Madagascar) with more than 400 genotypes, endemic species of Réunion, Mauritius, and Mayotte (ca. 200 genotypes), and a few species formerly cultivated (18th and 19th centuries) in Réunion (ca. 100 genotypes). This field gene bank is complemented by a cryobank, made up of seeds collected in Réunion and cryopreserved in Montpellier (more than 200 genotypes). The collection is part of the Florilège network, which is the portal for all plant Biological Resources Centres in France (http://florilege.arcad-project.org) and is co-managed by CIRAD and IRD since 2019 in order to enable its maintenance and scientific valorisation.

CONCLUSIONS & PERSPECTIVES

Because of past and current trends in forest degradation, *ex situ* conservation is of primary importance for a few species conserved in the Réunion genebank and considered as endangered or vulnerable according to UICN Red list of threatened plant species, e.g. *C. anthonyi, C. costatifruta, C. macrocarpa*. With the increasing incidence and duration of drought and the emergence or spread of diseases and pests, accessions of wild *C. arabica* and *C. canephora* will also serve as a valuable resource for future genetic improvement of both cultivated species.

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S1-PO-17

Functional characterization of caffeine synthase genes from wild coffee, and discussion of those molecular evolutions

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RATIONALE

Coffea genus, including three cultivated coffee species (*Coffea arabica*, *C. canephora*, and *C. liberica*), have different caffeine concentrations respectively. Examining those caffeine biosynthetic enzymes and exploring their molecular evolution, we reveal how has *Coffea* genus obtained and developed caffeine synthetic ability.

METHODS

Total RNA was extracted from young leaves of wild coffee species, and then cDNAs were synthesized. PCR was performed with those cDNAs as templates and oligonucleotides synthesized from the sequence information of caffeine synthetic enzymes from *C. arabica* as primers. Isolated genes were compared with corresponding *C. arabica* genes and classified into each other. Subsequently, recombinant enzymes for the identified genes were obtained using the *Escherichia coli* expression system. To determine the substrate specificity, those recombinant enzymes were used to react with xanthine derivatives, which are a caffeine precursor, and nicotinic acid as substrates. Then, the products were detected by thin layer chromatography (TLC).

RESULTS

We isolated five, two, five, six and four caffeine synthetic genes from *C. stenophylla*, *C. millotii*, *C. racemosa*, *C. humilis*, and *C. pseudozanguebariae*, respectively, that are highly homologous to those corresponding genes from *C. arabica*. Each of these genes had a full length of approximately 1200 bp and presented over 94% of homology with the corresponding genes from *C. arabica* at nucleotide level. However, since the enzyme gene corresponding to CmXRS (coffee 7-methylxanthosine synthase), CTS (coffee theobromine synthase) and CCS (coffee caffeine synthase) were not isolated from *C. millotii*, it was considered that the low or no caffeine content species had extremely low or no expression of those genes. Furthermore, when a molecular phylogenetic tree covering the caffeine biosynthetic genes from the *Coffea* genus was constructed, it was classified into four clades depending on the substrate specificity.

CONCLUSIONS & PERSPECTIVES

Recombinant enzymes of the genes with high homology to CCS isolated from *C. racemosa* and *C. pseudozanguebariae* did not have the same activity as CCS but only as CTS activity. It was revealed that there is a novel caffeine synthase gene class, “highly homologous to CCS but the activity is CTS.”

S1-PO-18

Studies of the *Baracoffea*: Malagasy coffee trees growing on the West Coast of Madagascar

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RATIONALE

In Madagascar, the deforestation and other anthropogenic activities have caused a strong fragmentation of the forest and have considerably modified the natural forest ecosystems. One of the direct consequences is that nearly 75% of Malagasy coffee species are classified as vulnerable, threatened or highly endangered according to the list of the International Union for Conservation of Nature (IUCN), among these coffee species is the *Baracoffea* group (*Coffea* subgenus). Baracoffea species are known to present remarkable adaptation to drought and spectacular large seed sizes.

METHODS

An ecological study was carried out: floristic inventory; analysis of vegetation cover; numerical abundance; natural regeneration rates, associated species and study of the distribution of these species.

RESULTS

The objective of this work is to characterize the species diversity of the *Baracoffea* group in the western region of Madagascar in view of its IUCN status, particularly in the city of Mahajanga and to characterize their ecological requirement in order to be able to give recommendations for its conservation. It was revealed from this study that 3 *Baracoffea* species are present near the town of Mahajanga, such as: *Coffea ambongensis*, (in the forest of Antsanitia), *C. boinensis* (in the forest of the National Park Ankarafantsika) and *C. bissetiae* (in the forest of Antsanitia and in the National Park Ankarafantsika). The most favorable habitat for these species appears to be the dense deciduous semi-deciduous forest with a semi-open cover, resting on a ground of sandy nature with orange sand. The associated families are Annonaceae (25.95%), Fabaceae (16.79%) and Rubiaceae (16.03%).

CONCLUSIONS & PERSPECTIVES

The population of this group of *Baracoffea* is very restricted in its natural environment which implies a real threat of extinction. In order to preserve these coffee trees, an ex-situ conservation must be implemented urgently in Mahajanga. Finally, phylogenetic analyses will have to be carried out in order to compare the evolutionary relations of the coffee bushes with the other coffee trees.

S1-PO-19

Chromosome-level assembly of allotetraploid *Coffea Arabica* reveals the complex history of a recent allopolyploid

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RATIONALE

Coffea arabica formed as an allotetraploid hybrid of *C. eugenioides* and *C. canephora* approximately 2000-4000 generations ago. The quality of arabica coffee surpasses the other *Coffea* species, but the species is susceptible to many plant pathogens. To increase pathogen resistance, currently bred *C. arabica* varieties are introgressed with diploid *C. canephora*. Unfortunately this breeding strategy, which is commonly pursued for many monocultured crop plants, introduces unwanted side-effects to the novel hybrids, such as decreased gustatory quality of the coffee beverage. To address this problem and facilitate enhanced breeding and bioengineering strategies, modern genomic tools are needed.

METHODS

We, the Arabica Coffee Genome Consortium, sequenced the genomes of a di-haploid *C. arabica* accession as well as modern representatives of its diploid progenitors *C. eugenioides* and *C. canephora* using a combination of Pacbio long read sequencing and chromosome conformation capture technology. Genome annotation was carried out using a comprehensive library of RNAseq evidence from different tissues and protein homology.

We further resequenced the genomes of 18 wild accessions, including the lectotype used by Carl von Linné, 15 commonly cultivated accessions as well as six modern cultivars from a spontaneous hybrid originating from Timor containing introgression from *C. canephora*.

RESULTS

The assembly yielded 2 x 11 high quality pseudo-chromosomes for *C. arabica,* with 89% of the total of 54,562 predicted genes placed on the chromosomes. Since *C. arabica* hybridization is a recent event, the chromosome-level assemblies made it possible to study the processes of genome evolution in a newly formed tetraploid hybrid. Genomic excisions were found to be the dominant process. Even though the diversity of subgenome *C. eugenioides* was higher, we did not detect subgenome dominance within the species. Using molecular data, we followed the breeding history and resolved the relationships between coffee cultivars. We were able to shed light to the geographic origins of domesticated *C. arabica*. Furthermore, the study of Timor hybrid lines illustrated how introgression shaped the genomes of these cultivars and suggested regions underlying their resistance to coffee leaf rust disease.

CONCLUSIONS & PERSPECTIVES

The chromosome level assembly of *C. arabica* reveals the evolution and cultivation history of the species and provides molecular tools for accelerating coffee breeding in future.

S1-PO-20

The transcriptomic basis for understanding the mitigation of heat impact by elevated [CO_2] in the photosynthetic response of *Coffea arabica* and *C. canephora*

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RATIONALE

Several works revealed unexpected tolerance to high temperature of *C. arabica* and *C. canephora* genotypes without a noticeable impact on the photosynthetic metabolism. Also, elevated [CO_2] promoted vigour and heat tolerance of coffee plants, while modifying and mitigating the heat impact on physical and chemical traits of coffee beans1-3. Here, we explored the transcriptome of *C. arabica* and *C. canephora* leaves to unveil how plants regulate responses to high temperature and [CO_2].

METHODS

1.5 year-old plants of *C. canephora* cv. Conilon Clone 153 (CL153) and *C. arabica* cv. Icatu were grown for 10 months as previously described [1-3]. Leaf material was collected at 25/20ºC, 37/30ºC, and 42/34ºC. RNA was extracted and sequenced on Illumina HiSeq 2000. Gene expression of significant pathways was explored with a focus on responsive genes linked to photosynthesis.

RESULTS

Genes involved in repair processes of photosystems were found to be up-regulated in Icatu. In contrast, CL153 plants seemed to be less affected by the heat increase, with a high up-regulation of genes involved in binding and transport. Overall, results are in line with previous experiments, where photosynthesis seemed to be more affected in Icatu than in CL153, with elevated [CO_2] helping to mitigate the impact of heat.

CONCLUSIONS & PERSPECTIVES

We provide new transcriptomic clues regarding the acclimation capabilities of genotypes from the two most important cropped *Coffea* species to projected climate changes, under the combined exposure to heat and elevated [CO_2].

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POSTER PRESENTATIONS

Session 2: Plant pathology & protection

S2-PO-10

Survey of *Hemileia vastatrix* races from Peru to identify potential coffee mutants with disease resistance

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RATIONALE

Coffee leaf rust (CLR), a disease caused by the fungus *Hemileia vastatrix* (*Hv*), is the main limiting factor of coffee production in Peru. According to Julca *et al.* (2019), crop losses caused by CLR were evaluated in 290 million dollars, leading to the implementation of an emergency plan, with a financial fund of around 30 million dollars, managed by the "Servicio Nacional de Sanidad Agraria". This situation led to the renewal of coffee plantations with resistant varieties from Timor Hybrid (HDT) derivatives, like Catimors. However, the appearance of new and more virulent *Hv* races has resulted in the gradual loss of resistance of these varieties (Talhinhas *et al*. 2017, CIFC Records).

Since 2016, the Universidad La Molina participates in a research project together with CIFC (Portugal) and other Institutions from China and Costa Rica. This Project, coordinated by the Joint FAO/IAEA Plant Breeding and Genetics Laboratory, International Atomic Energy Agency, aims to produce coffee mutants using gamma-ray irradiation with potential resistance to CLR. Irradiation treatments of 0, 50, 100, and 150 Gy on seeds of *Coffea arabica* L. var. Typica performed in Peru resulted in several mutants (Quintana *et al*. 2019), which are now being screened for resistance to local *Hv* isolates, collected from the same coffee plants of the rust samples sent to CIFC.

METHODS

A total of 57 rust samples from different coffee genotypes and different regions in Peru were sent to CIFC for the assessment of their virulence spectra on a set of 27 coffee differentials.

RESULTS

The virulence spectra of rust samples and their correspondent physiologic races were characterized as follows: races I (v2,5), race XXIII (v1,2,4,5), race XXIV (v2,4,5), race XXXIV (v2,5,7 or v2,5,7,9), race XXXV (v2,4,5,7 or v2,4,5,7,9) and 2 new rust races, not characterized before at CIFC, with the following genotypes of virulence; v2,4,5,7,8 or v2,4,5,7,8,9 and v1,2,4,5,7,8 or v1,2,4,5,7,8,9.

CONCLUSIONS & PERSPECTIVES

Hv race genotypes comprise 9 virulence genes (v1 -v9) and in this survey, complex races from 2 to 7 virulent genes were identified. The Peruvian coffee growers must be aware about the introduction of new resistant varieties without knowing their spectra of resistance. The majority of lines of the resistant population Catimor (Caturra x HDT 832/1), widespread in the vast majority of coffee-growing countries, are susceptible to the 2 new races, as well as to races XXXIV and XXXV identified in this study.

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S2-PO-13

Identification of distinctive transcriptomic profiles among *Hemileia vastatrix* pathotypes throughout key stages of the infection process

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RATIONALE

Hemileia vastatrix (Hv), the pathogen responsible for Coffee Leaf Rust, has been spreading across the globe and causing devastating socio-economic consequences within coffee production. Nowadays, more than 50 races of Hv have been identified, but its virulence mechanisms are still poorly understood. To achieve a sustainable disease control, it is crucial to unveil the evolutionary adaptation behind this host-pathogen interaction. In this study, we applied a transcriptomic approach to identify candidate virulence genes harbouring differential expression patterns, related to rust pathotypes during its compatible interaction.

METHODS

RNA-seq data was obtained from five pathotypes during compatible interactions, at three key steps of the infection process. A *de novo* assembly of the fungus transcriptome was performed with Trinity and the transcript expression quantification was assessed by Salmon. Differentially expressed genes (DEGs) were identified using EdgeR. Functional annotation was done using Blastx and Blastp searches against the Uniprot database and a HMMER search against the Pfam database. Secreted proteins were searched using SignalP and TMHMM.

RESULTS

Data analysis enabled the identification of 27.679 unigenes and a total of 50.380 isoforms. Within these, 1596 transcripts were exclusively expressed in one of the five pathotypes across all infection time-points. We identified 3.095 DEGs in all sample comparisons between Hv pathotypes and infection time-points. Three distinct clusters of gene expression profiles were recognized in all pathotypes, while five expression profiles were specifically associated to pathotype groups, providing the information to distinguish pathotype-specific expression patterns. Functional annotation assigned protein functions to 9174 unigenes, with a predominance of catalytic activity and binding categories. In addition, 2347 potential secreted proteins with signal peptides were identified that may represent putative effectors. Our results show clear distinct gene expression profiles between rust pathotypes and/or infection stages, and pathotype-specific differential expression.

CONCLUSIONS & PERSPECTIVES

Our study provides a deeper insight on the virulence mechanisms of Hv, unveiling vital information about candidate genes and differential expression patterns linked to rust pathotypes, which will allow future functional studies and to exploit diagnostic markers for Hv pathotypes.

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S2-PO-14

Poster presentations

Agro-climatic constraints to integrated Coffee Berry Borer Management

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RATIONALE

Because it is effective, flexible and environmentally friendly, Integrated Pest Management (IPM) against the coffee berry borer (CBB) is a method that tends to fit sustainably into the agronomic practices of *arabica* coffee producers. However, IPM must adapt to local agro- climatic conditions to be effective. Thus, we compared this method in two contrasting situations, one in Central America with a tropical climate and the other in North Sumatra with an equatorial influence.

METHODS

We adapted the main IPM components, i.e. CBB trapping on plantations and near post- harvesting areas, sanitation harvesting, and pruning and/or plot maintenance operations, according to CBB dynamics. These dynamics depend on the phenology of the *arabica* coffee tree, which is characterized by limited duration of fruit production in Central America and almost permanent fruiting in North Sumatra.

RESULTS

In Central America, trapping used 18 Brocap© traps/ha for four months during the post-harvest period (1). When trapping was combined with sanitation harvesting applied to the branches, infestations were reduced by more than 70% compared with control plots. Adding pruning and maintenance of the plots, infestations decreased by more than 90% (2). In North Sumatra, trapping with 25 Brocap© traps/ha for ten months per year reduced infestation levels by 50% on average in plots affected by CBB. When combined with sanitation harvesting from the ground and on the branches, less than three months after the two main flowering periods, the infestation rate dropped to less than 10%. In this area, pruning had no particular effect on infestations, but it helped to double production the following year (3). In addition, given the dispersion of pulping and drying areas in this region, setting traps near these areas allowed to capture emerging CBBs in order to prevent their return to plots.

CONCLUSIONS & PERSPECTIVES

The control strategies proposed for Central America and North Sumatra have been developed to optimize the use of the different IPM component with a concern for efficiency, environmental friendliness and economy of means. However, they could be reinforced by other measures such as spraying with *Beauveria bassiana* spores at appropriate times and monitoring infestation levels. In other agro-climatic sites dedicated to coffee cultivation, such as those in Africa, other strategies can be imagined and exploit the «parasitoid» component naturally present on this continent.

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S2-PO-16

Agrobacterium tumefaciens-mediated transformation revealed an alkaline phytoceramidase that is required in pathogenicity of *Colletotrichum kahawae* to *Coffea arabica*

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RATIONALE

The hemibiotrophic fungus *Colletotrichum kahawae* (Ck) is the causal agent of Coffee Berry Disease (CBD), the major limiting factor to the *Coffea arabica* (Ca) production in Africa, especially at high altitudes. CBD can cause losses up to 80% if no control measures are applied and its dispersal to other continents like America and Asia represents a serious concern. *Agrobacterium tumefaciens*-mediated transformation (ATMT) were applied to generated random mutagenesis to a Ck isolate. One transformant (Que2-441) was identified as non-pathogenic, as it was unable to produce symptoms on coffee green berries, even on wounded fruits (Cabral et al. 2016).

METHODS

To obtain the entire sequence of the gene disrupted by the ATMT in Que2-441, a bidirectional chromosome walking strategy was followed, according to the DNA Walking SpeedUpTM Premix Kit protocol (Seegene, USA). Amplified fragments in the third step of chromosome walking protocol were excised from the gel, purified and sequenced. A gene expression study was performed in other to characterize the expression of the identify disrupted gene along the infection process of the interaction Ck-Ca, on saprophyte mycelium, ungerminated conidia, conidia with melanized appressoria formed *in vivo* and *in vitro* (Vieira et al. 2016).

RESULTS

The chromosome walking protocol led to the identification of putative alkaline phytoceramidase 94.7% of identity and an e-value of 1e-140 to the accession KAF0322308 of *C. asianum* in the BlastX search. The KEGG Orthology (KO) of the predicted protein is the entry K04711, with definition dihydroceramidase [EC:3.5.1.-] that is involved in two pathways: sphingolipid metabolism (ko00600) and metabolic pathways (ko01100). The gene expression study revealed that the highest relative expression value was observed at 1 day after inoculation (dai), decreasing throughout the infection process (2, 5, 7 and 10 dai), although it is always up-regulated and with significant expression differences (t-Student $p<0,05$) compared to the control (saprophytic mycelium). The relative expression of the *in vitro* and *in vivo* appressoria and ungerminated conidia did not differ statistically from the control.

CONCLUSIONS & PERSPECTIVES

This study showed that the gene alkaline phytoceramidase disrupted by the ATMT is up-regulated along the infection process and is a good candidate for gene knock-out, to validate his involvement in the pathogenicity of Ck to Ca. A better understanding of plant-pathogen interaction mechanisms will open new routes for the deployment of more informed plant protection strategies.

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S2-PO-17

Characterising tolerance to root knot nematodes in *Coffea canephora* varieties

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RATIONALE

A detriment to coffee production is the damage caused by plant parasitic nematodes, which reduces yields by up to 15% although individual growers may experience much higher reductions. The most damaging species include root-knot (*Meloidogyne* spp.) and root lesion nematodes (*Pratylenchus* spp.). The endoparasitic lifestyle of root-knot and lesion nematodes requires the recognition of, and movement towards, host roots followed by invasion for the establishment of a food source. We aim to characterise the tolerance of new coffee varieties against root-knot nematodes and investigate how root exudates mediate the interactions between nematodes and host roots.

METHODS

We have established an assay system to characterise the physiological and growth response of coffee varieties to root-knot nematode infection. Behavioural responses, e.g. chemotaxis, of root-knot and root lesion nematodes in response to the root exudate of different coffee varieties was also analysed. RNA-Seq will be used to analyse differential gene expression between a tolerant and intolerant Robusta variety, in the presence and absence of root-knot nematode infection, revealing molecular components that may contribute to tolerance.

RESULTS

A detrimental effect on photosynthesis, as measured by chlorophyll fluorescence, and a reduction in growth parameters was seen in Robusta plants following root-knot nematode infection, with variation between varieties. We observed differential chemotactic responses of *Meloidogyne* and *Pratylenchus* species to root exudate of resistant and susceptible coffee varieties. In addition, nematodes differentially performed stylet thrusts, a behaviour essential for penetration into and feeding on host tissue, in response to root exudates of the different coffee varieties.

CONCLUSIONS & PERSPECTIVES

Different coffee varieties showed a differential physiological response to infection by plant parasitic nematodes. Genes which showed differential expression following root knot nematode infection were explored to provide insight into the molecular response of *Coffea canephora*. The differential response of root knot and root lesion nematodes to the exudate of susceptible and resistant coffee varieties also provides evidence for a relationship between host-status of a plant variety and the behavioural response of the plant parasitic nematode. In summary, these findings will better inform growers on the best attributes for new coffee varieties, minimising damage caused by the interaction between plant parasitic nematodes and coffee plants.

S2-PO-18

Poster presentations

Identification of bacterial endophytes of interest for coffee crop in Vietnam[1]

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RATIONALE

This project aimed to identify some plant growth promoting agents, as well as some biocontrol agents of coffee parasitic nematodes and fungal pathogens based on bacterial endophytes naturally associated with coffee roots and seeds in Vietnam. Bacteria were identified and selected with *in vitro* screenings for some potential plant growth promoting and biocontrol capacities. Subsequently, direct confrontations with the plant parasitic nematodes *Radopholus duriophilus* and *Pratylenchus coffeae*, as well as the phytopathogenic fungus *Fusarium oxysporum* were performed in order to highlight the bacterial endophytes nematicidal and antifungal activities.

METHODS

Bacterial endophytes were identified by sequencing of the 16S rRNA coding gene and preliminary screenings for phosphate solubilization, as well as the production of siderophores, hydrogen cyanide, gelatinases, chitinases, lipases and esterases were performed *in vitro*. Then, 50 bacterial isolates were selected for *in vitro* direct confrontations with the nematodes and the phytopathogenic fungus. After 48 h, *R. duriophilus* mortality risk ratios (percentage of mortality in treatment divided by percentage of mortality in control) were calculated. Then, isolates with risk ratios higher than 2 were further characterized with different concentrations in order to calculate lethal doses on *R. duriophilus* and *P. coffeae* at 24 h. Finally, dual cultures were used to highlight the antifungal activity of the isolate on *F. oxysporum* (expressed in percentage of fungal growth inhibition).

RESULTS

Fifty isolates were selected after the *in vitro* preliminary screenings for direct confrontation plant parasitic nematodes and the fungal phytopathogen. Seventeen isolates displayed *R. duriophilus* mortality risk ratios higher than 2 after 48 h of which 11 were tested at different concentrations at 24 h on both *R. duriophilus* and *P. coffeae*. Finally, 17 isolates displayed an antifungal activity on F. oxysporum with a growth inhibition comprised between 8 % and 50 %. The results for all the screenings are presented.

CONCLUSIONS & PERSPECTIVES

Several have displayed some nematicidal and/or some antifungal activities. The nematode biocontrol potential of one of the most efficient isolates on the nematode were confirmed *in planta*. We used the isolate CCBLR15 to control the parasitic nematode *R. duriophilus* on *C. arabica* and the result are currently under submission for publication.

References:

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POSTER PRESENTATIONS

Session 6: Biochemistry & biotechnology & composition of green coffee

S6-PO-02

Biochemical characterization of the genetic resources of wild coffee trees collection in Réunion using near infrared spectroscopy

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RATIONALE

Near infrared spectroscopy (NIRS) has been widely used for green coffees characterization and especially for the quantification of the main chemical constituents. The CIRAD database contains more than 2210 spectra and efficient calibrations for major constituents. However, this database is mostly constituted of *Coffea arabica* and *C. canephora*, which limits its representativeness. In order to enhance the database robustness, the analysis of wild coffee species has been undertaken.

METHODS

Over 4 years, 462 seed samples, from 32 species, were collected on individual trees from the *Coffea* Biological Resources Centre in Réunion. Ground dried samples were analyzed for their absorbance spectrum using a FOSS 6500 monochromator (FOSS, Hillerod, Denmark). A selection, based on spectra, of the 90 most representatives samples was done using PCA and Mahalanobis neighborhood distances. The selected samples were analyzed for their caffeine, trigonelline, fat and chlorogenic acids (CGA) contents using standard analytical chemistry protocols.

RESULTS

The distance threshold corresponding to the neighborhood was 0.55. The 90 samples came from 22 different coffee species. The caffeine content ranged from 0 to 3.3%, trigonelline from 0.25 to 1.52%, CGA from 0,22% to 10,53% and fat from 7.12% to 34,45% (dmb). The enhanced predicted models using this new data set present standard errors of 0.08%, 0.07%, 0.58% and 0.48% for respectively caffeine, trigonelline, CGA and fat. These performances are close to original models ones, with an increase of the content ranges, especially for fat.

CONCLUSIONS & PERSPECTIVES

This study demonstrated the efficiency of the use of genetic diversity to enhance the robustness of the database. This approach lead to a real increase of the range for caffeine and fat contents. Thus, the resulting calibrations cover a larger range of values without significant losses of accuracy. The use of Mahalanobis distances permitted an efficient improvement of the calibrations representativeness with a limited number of samples. The new calibrations will be applied to the whole database in order to describe biochemical diversity in wild coffee species. The study will also be pushed forward to other chemicals such as fatty acid composition and diterpenes profiles.

S6-PO-03

Isolation of Mycotoxigenic Fungi and Quantification of Ochratoxin A from Coffee in Ethiopia

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RATIONALE

Globally, coffee is the second most traded and wealth generating commodity after oil. Ethiopia is known for its diverse and unique Arabica coffee flavors**.** Sixteen percent of the country's population depend on coffee Production, Processing and Marketing. However, coffee is naturally associated with several mycoflora and some of them may produce Ochratoxin A unless careful handling measures taken place.

METHODS

A total of 75 coffee samples were collected from three districts namely, Haru, Homa and Nedjo of West Wollega Zone, Oromia regional state of Ethiopia. Malt Extract Agar (MEA) was used for isolation and identification of mycoflora associated with coffee and ELISA kit was used to detect and quantify Ochratoxin A from green coffee bean.

RESULTS

The result showed that numbers of mycoflora associated with coffee were observed and five of them become the major. *Aspergillus niger* was the most dominant (73.37%) species detected from most coffee samples, followed by *Aspergilus ochracious* (11.30%), *Fusarium* spp. (7.37%), *Penicillium* spp. (6.74%), and *Rhyzopus* spp.(1.50%), respectively. The result of the correlation matrix revealed that there was high positive correlation among *Aspergillus ochraceus* and ochratoxin A (r = 0.31), *Aspergillus ochraceus* and moisture content (r=0.25), *Aspergillus niger* and mold condition(r=0.27), ochratoxin A and moisture content(r=0.23), moisture content and mold condition (r=0.44). Average ochratoxinA recorded was 0 (ND) ppb, 1.24 ppb and 2.02 ppb from Haru, Homa and Nedjo. The average detected ochratoxin A from total of tested 75 coffee samples was 1.09µg/kg (1.09 ppb).

CONCLUSIONS & PERSPECTIVES

In conclusion, 75 coffee cherry samples has been analyzed for coffee bean associated fungi and for Ochratoxin A content of green coffee beans. The quantity of Ochratoxin A produced by mycoflora associated with Ethiopian coffee was 1.09 µg/kg ppb(1.09 ppb). Therefore, pre-harvest, harvest and post-harvest management practices should be applied for sustainable quality coffee production.

References:

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S6-PO-04

Production and transfer kinetics of three aroma compounds into the coffee beans during simulated wet processing and their fate after the transfer

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For the consumer, the flavor is arguably the most important aspect of coffee. Thereby, the coffee industry has dedicated efforts in improving and controlling the final beverage quality using roasting and brewing steps (de Carvalho Neto et al., 2018). Moreover, recent research studies have highlighted that the postharvest processing can have a direct impact on the quality and value of the final product, and they showed that wet processing offers a coffee with higher acidity and more aroma than dry and semi-dry processing (Pereira et al., 2017). Furthermore, Lee et al., (2017) assumed that there might exist a diffusion process of microbial metabolites into the coffee beans during the fermentation, enhancing the final coffee quality.

However, one question remains: are aroma molecules able to be produced by yeast and then to cross the different layers (mucilage and parchment) surrounding the coffee beans during coffee fermentation and what happens to them in the bean once they are transferred?

To answer this question, three labelled compounds with deuterium (butanal, 2-phenyethanol, isoamyl acetate) were used to study their transfer kinetics from a liquid medium into the coffee beans during simulated wet processing and to follow their fate in the beans. Ten grams of coffee samples were submerged in distilled water concentrated with marked compounds; they were maintained at 25°C and under agitation (120 rpm) for five time periods (0, 6, 12, 24 and 48 hours), then the transfer was stopped by washing the coffee beans with distilled water and the fate of the transferred volatiles in the coffee beans was studied during four time periods (3, 6, 9 and 15 hours) at 25 °C. For all trials, the labelled molecules were analyzed by SPME-GC-MS. Then the production kinetics of those three aroma compounds by three *Saccharomyces cerevisiae* strains were studied using a simulated coffee pulp media for 48 hours.

Results showed that the three labelled molecules were transferred into the coffee beans with different mass transfer rates, reaching at 12hrs, 0.2±0.03 ,11.2±0.66 and 1.3±0.04µg/g of coffee respectively for butanal, 2-phenyethanol and isoamyl acetate. After their transfer into the beans, the level of 2-phenylethanol remained stable for 15 hours, whereas butanal and isoamyl acetate underwent a first-order degradation reaction that could be linked to the metabolic germination reaction. The three yeast strains were able to produce those three volatiles during the fermentation of the simulated coffee pulp at different rate.

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S6-PO-05

Isolation and characterization of linalool UDP-Glc glycosyltransferases from *Coffea arabica*

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RATIONALE

The aroma is principal to decide the value of coffee beans. Linalool, which is one of the volatile terpene compounds, is constitutive in the coffee aroma. Since terpenoids are generally accumulated as glycosides in plants, the glycosylation is catalyzed by the UDP-glucose glycosyltransferases (UGTs). To reveal the mechanism of accumulation of those volatile compounds in coffee, the functional analysis of the UGT genes that work terpenoids such as linalool was performed. We expect to develop coffees with a rich aroma using this information. In this study, we identified those UGT genes from *Coffea arabica* and analyzed them with recombinant enzymes. Here, we report the isolation and characterization of UGT genes that have activity towards linalool.

METHODS

UGT85K11, which catalyzes the glycosylation of geraniol and linalool, was isolated from *Camellia sinensis* in a previous study[1]. We performed *in silico* screening of the genes from *C. arabica* based on the nucleotide sequence of *UGT85K11*. Then, six genes (termed *Ca4*, *Ca5*, *Ca10*, *Ca14*, *Ca15* and *Ca20*) were candidates to identify and analyze. To prepare the cDNAs, total RNAs were extracted from young leaves and flower buds. Those six genes were amplified by RT-PCR using specific primers and those cDNAs as a template. After sequence analysis, those recombinant enzymes were produced using a pET-system. Those enzyme activities were measured by radioisotope mediated TLC assay.

RESULTS

Five genes (*Ca4*, *Ca5*, *Ca10*, *Ca14* and *Ca20*) were isolated from young leaves, and *Ca15* was isolated from flower buds. Ca4, Ca10 and Ca15 can catalyze glycosylation of linalool, citronellol, perillyl alcohol, terpineol, and geraniol. Ca15 also showed activity in the glycosylation of menthol. In comparison with the results of the TLC assay of Ca4, Ca10 and Ca15, a clear signal of the linalool glucoside was observed in Ca4.

CONCLUSIONS & PERSPECTIVES

We identified six genes, *Ca4*, *Ca5*, *Ca10*, *Ca14*, *Ca15* and *Ca20*, encode polypeptide of 483, 479, 489, 483, 494 and 503 amino acid residues respectively. The amino acid sequence identities among these enzymes with UGT85K11 were 60.8, 60.1, 59.3, 59.0, 58.5 and 58.1%, respectively. Although Ca4, Ca10 and Ca15 can catalyze glycosylation of linalool, the activity was not the dominant activity. Now, we proceed to produce recombinant enzymes of Ca14 and Ca20. These recombinant enzymes will be used for enzyme assay too. The structure-function relationship of these isolated UGT genes would be clarified. Hereafter, to develop high-quality coffee with a rich aroma, we will utilize that information.

References:

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S6-PO-06

Substances with physiological effects in several tissues of different coffee species - Part 1 diterpenes

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RATIONALE

The two economically most important coffee species *Coffea arabica* and *Coffea canephora* differ in their diterpenes: while arabica beans contain kahweol and cafestol, Robusta beans contain cafestol, only small amounts of kahweol (usually <100 mg/kg) but 16-O-methylcafestol in addition to traces of 16-O-methylkahweol. The 16-O-methylcafestol is the indicator substance for admixtures of Robusta coffee beans to Arabica coffees. Of the diterpenes mentioned, the physiological effect of the cafestol has so far been examined almost exclusively. An increased glutathione-S-transferase activity was found, which proved to be advantageous in the course of detoxification compared to aflatoxin B1. The cholesterol-increasing effect is to be regarded as a disadvantage. First research results on the diterpenes in leaves (Kölling-Speer, ASIC, Nairobi 1997) showed that the occurrence in leaves was surprisingly contrary to that in beans. For example, 16-O-methylcafestol could be detected in the leaves of Arabica plants, but not in the leaves of Robusta plants. The aim of the present study was to obtain further information about the respective occurrence of the diterpenes by analyzing different parts of plants such as leaves, roots, branches, pulps, blossoms, and beans of different Arabica varieties and other *Coffea* species. It could also be deduced which parts of the plant might be used for commercial utilization of the physiologically active substances, since the chlorogenic acids and alkaloids were also analyzed in the same samples.

METHODS

The plant material was made available by the Coffee Research Foundation Ruiru, Kenya, the greenhouse for tropical crops Witzenhausen, University of Kassel and the Dicafé company, Mexico City. The diterpenes were analyzed using a modified DIN 10779 method.

RESULTS

The diterpenes cafestol, kahweol, and 16-O-methylcafestol were determined at very different levels in the various parts of the plant. High levels could be analyzed in the beans and roots. The highest cafestol and kahweol contents were determined to be approx. 10 g/kg each in Arabica beans and some roots. Leaves and branches contained cafestol in amounts below 2 g/kg, but kahweol only in amounts below 0.2 g/kg. As expected, 16-O-methylcafestol was determined in Robusta beans, and in the roots and branches of Robusta plants and also in Arabica leaves and blossoms, but not in Robusta leaves and blossoms. The high kahweol contents in Robusta roots were noticeable.

For the contents of chlorogenic acids and alkaloids, see Poster part 2 and 3.

S6-PO-07

Application of spent coffee grounds in water treatment for hemodialysis by adsorption of residual chlorine

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RATIONALE

Coffee grounds are one of the most regularly produced food wastes. Thus, reusing coffee residue as biomass is desirable in order to alleviate environmental problems. In this study, we evaluated the use of spent coffee grounds, which are otherwise difficult to dispose, for treating water used for hemodialysis (HD) in the medical field. HD is performed thrice a week per patient, and one treatment requires a large amount of dialysate (approximately 120–150 liters). The water used for preparing the dialysate is obtained from tap water or wells and requires the prior removal of various contaminants to generate high-purity water. In particular, chloramine and residual chlorine present in tap water cause hemolysis when mixed into the blood via the dialysate. Chloramine and residual chlorine in tap water are generally adsorbed and removed using a coconut shell-activated carbon filter. Activated carbon is usually produced by the thermal decomposition of raw materials and subsequent activation using acid gas, which is a multi-step and costly process. We investigated whether the carbides of coffee residue could be used as an alternative to the coconut shell-activated carbon filter for treating water used for HD.

METHODS

Coffee grounds were heated at about 400-500°C to produce carbide in an electric furnace. The carbide (10−50 g) was mixed in 200 mL of a 400–1000 ppm (0.04–0.1%) sodium hypochlorite (NaOCl) solution for 5−30 min, and the concentrations of bound and free residual chlorine were measured using the N,N-diethyl-p-phenylenediamine method.

RESULTS

The concentration of bound and free residual chlorine in the NaOCl solution was 0 mg/dL in all conditions.

CONCLUSIONS & PERSPECTIVES

This study showed that carbonized coffee grounds adsorbed bound chlorine (chloramine) and free chlorine. Coffee grounds can be potentially used for the treatment of tap water in HD. Coffee grounds can also be used in times of disaster or in areas with inadequate means of water treatment for preparing dialysis pipe disinfectant solutions containing NaOCl. In terms of functional properties, coffee grounds exhibit good adsorption ability. Ease of availability and a simple carbonization process make coffee grounds a promising candidate for use in HD treatment. Nevertheless, a detailed engineering analysis of the adsorption process is required to realize the practical application of coffee grounds for HD.

References:

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S6-PO-08

Profiling of Robusta coffee (*Coffea canephora)* genotypes in the Democratic Republic of the Congo using untargeted metabolomic analysis on green and roasted coffee

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RATIONALE

The share of Robusta coffee in the global market increases, yet research lags behind. This study aims to characterize green and roasted coffee beans of *Coffea canephora* genotypes and their potential for valorization. We determine metabolic fingerprints of different genotypes using metabolomics on green and roasted coffee. Discrimination between the genotypes, using key metabolites, is analyzed. Correlations between coffee bean profile and sensory attributes are further explored.

METHODS

About 95 genotypes of *Coffea canephora* from the Yangambi coffee collection (DRC) were genotyped with GBS. Green coffee beans are sampled from the accessions using the natural process method and roasted to a medium degree. The metabolomics study uses a liquid chromatography coupled to high resolution mass spectrometry (Souard et al., 2018). The Fine Robusta Standards and Protocols is used (Coffee Quality Institue, 2019).

RESULTS

To start, a standardized sampling protocol is drawn up to control for the impact of postharvest treatment on the chemical profile of the coffee (De Bruyn et al., 2017). Secondly, a protocol for the physiochemical and organoleptic evaluation is developed. The protocols will be used to research correlations between genotypic, chemical and organoleptic profiles of the genotypes (Farah A. et al., 2006). Metabolomics will be a complementary approach to evaluate coffee quality.

CONCLUSIONS & PERSPECTIVES

The metabolomic analysis will help to identify differences between Robusta coffee genotypes that allow for discrimination. Furthermore, we contribute to the existing research on the correlations between coffee bean composition and quality by exploring the datasets of the untargeted metabolomic analysis.

References:

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PLENARY SESSION - KEYNOTE LECTURERS

Session 1: Plant science

Session 8: Coffee chemistry & sensory sciences

Genomics of Coffee Quality

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The quality of coffee is determined by many factors in the processing of the green bean to produce coffee in the cup. The impact of the green bean on coffee quality is in turn determined by the genotype and environment in which the coffee is grown and interactions between genotype and environment. Sequencing of the whole chloroplast genomes of *Coffea* species has defined relationships within this group of plants. Options for selection of maternal genomes to support environmental adaptation might be considered. Advances in long read sequencing has allowed the full length sequences of gene transcripts in coffee beans to be determined directly. The nuclear genome of Arabica coffee, a tetraploid species is more complex than that of robusta coffee, a diploid species. Analysis of the genome has revealed many of the genes associated with the biochemical pathways leading to the major components of the bean. Comparison of the beans of more than 200 Arabica varieties with contrasting composition has allowed identification of genes that vary in a way that suggests that they determine the content of caffeine and trigonelline. Analysis of the patterns of expression of genes during bean growth and development illustrate the processes involved in accumulation of the main bean components including sucrose, caffeine and trigonelline. Transcriptome analysis shows that beans lower in the canopy may develop more slowly producing beans of higher quality. Genome and transcriptome sequences have delivered a platform for research on the genetic and environmental control of bean composition and associated coffee quality.

S8-KN

The molecular code of coffee aroma and taste

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RATIONALE
The delicious and delicate flavor of coffee is thought to be due to a subtle ratio of volatile and non-volatile constituents generated during the roasting process. Generations of scientists and engineers have contributed to better understanding the relationship between coffee composition and flavor quality, with remarkable success. However, still today, the empirical knowledge of an experienced barista is very valuable in optimizing conditions of roasting and preparation of the coffee beverage.
METHODS
In the last 3 decades, a tremendous effort has been undertaken to identify and quantify coffee constituents relevant to the cup quality using the "sensory-directed chemical analysis" approach. This includes time-resolved analytical methods monitoring on-line aroma release from the cup and in-mouth. Moreover, new formation mechanisms have been unraveled using labeled precursors based on in-bean experiments.
RESULTS
Despite all these efforts, coffee flavor keeps quite some mystery, most likely due to interactions that are not yet sufficiently characterized. This, by the way, is also valid for many other beverages. In this talk, the aim is to review the main achievements and address topics that may lead to a more advanced understanding of coffee flavor at a molecular level. Focus will be given to aroma and taste components as well as flavor formation and stability. In addition, recent omics-type analytical techniques known in biochemical domains and other untargeted methods will be discussed.
CONCLUSIONS & PERSPECTIVES
The temporal changes in coffee flavor represent a major challenge as they are associated with the change in the volatile as well as non-volatile composition. In both cases, the ratios of molecules change leading to loss of aroma freshness and taste modifications. These changes can partially be explained by the physical and chemical properties of the flavor molecules concerned, such as volatility, polarity, and reactivity in the presence of oxygen and water. Moreover, interactions in the beverage and at the receptor level may play a role which need more attention in the future using targeted and untargeted approaches combined with adequate sensory and receptor-based methods. Coffee flavor will remain a fascinating interdisciplinary research field.

ORAL PARALLEL SESSIONS

Session 1: Plant science

Session 2: Plant pathology & protection

S1-O-03

A single polyploidization event at the origin of the tetraploid genome of *Coffea arabica* is responsible for extremely low genetic variation in wild & cultivated germplasm

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RATIONALE

The genome of the allotetraploid species *Coffea arabica* L. was sequenced to assemble independently the two component subgenomes (putatively deriving from *C. canephora* and *C. eugenioides*) and to perform a genome-wide analysis of the genetic diversity in cultivated coffee germplasm and in wild populations growing in the center of origin of the species.

METHODS

We studied an individual of *C. arabica* 'Bourbon Vermelho'. A BAC library of 175,872 BAC clones was constructed and sequenced using an Illumina HiSeq2000. Each BAC pool was assembled independently with the tool ABySS and scaffolded with SSPACE. Genotyping by sequencing (GBS) was conducted using the restriction enzyme *Pst*I followed by single-end sequencing on an Illumina HiSeq2000. SNP calling was performed using Stacks. Principal Component Analysis was performed using the R package ade4. A hierarchical study of the diversity has been conducted using a model-based clustering procedure with admixture as implemented in STRUCTURE.

RESULTS

We assembled a total length of 1.536 Gbp, 444 Mb and 527 Mb of which were assigned to the canephora and eugenioides subgenomes, respectively, and predicted 46,562 gene models, 21,254 and 22,888 of which were assigned to the canephora and to the eugenioides subgenome, respectively. Through a genome-wide SNP genotyping of 736 *C. arabica* accessions, we analyzed the genetic diversity in the species and its relationship with geographic distribution and historical records.

CONCLUSIONS & PERSPECTIVES

We observed a weak population structure due to low-frequency derived alleles and highly negative values of Taijma's *D*, suggesting a recent and severe bottleneck, most likely resulting from a single event of polyploidization, not only for the cultivated germplasm but also for the entire species. This conclusion is strongly supported by forward simulations of mutation accumulation. However, PCA revealed a cline of genetic diversity reflecting a west-to-east geographical distribution from the center of origin in East Africa to the Arabian Peninsula. The extremely low levels of variation observed in the species, as a consequence of the polyploidization event, make the exploitation of diversity within the species for breeding purposes less interesting than in most crop species and stress the need for introgression of new variability from the diploid progenitors.

S2-O-05

The quest for sustainable management of coffee leaf rust with endophyte bodyguards from coffee and mycoparasites of *Hemileia vastatrix*

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RATIONALE

Major outbreaks of coffee leaf rust (CLR) – *Hemileia vastatrix* (Hv) – hit northern South America and Central America since the early 2010s causing major losses in coffee production. They contributed to the well documented ongoing refugee crisis. Firstly, it was recognized that escaping the disease through highland cultivation was failing to deter CLR and later it was also observed that some CLR resistant cultivars had become ineffective. Among the emergency actions taken by the World Coffee Research, it invested on the development of novel management strategies to mitigate the impact of CLR.

METHODS

Biological control of Hv has been investigated in the past but it only involved microbial natural enemies of Hv obtained in the Neotropics. Although both cultivated coffee species and Hv are native from Africa, coevolved natural enemies of the Hv from Africa had never been investigated by biocontrol scientists. A pioneering combined effort of scientists based in Brazil, Cameroon and Ethiopia revealed a large diversity of mycoparasitic fungi (fungi that attack CLR pustules) and endophytic "bodyguard" fungi (fungi growing inside plant tissues and protecting them against pests, diseases and abiotic stress), including several species which are being described as new to science.

RESULTS

Publications describing species that were found as endophytes belonging to the genus *Trichoderma* and as mycoparasites of Hv belonging to *Digitopodium*, *Calonectria* and *Fusarium* are now becoming available. These will be commented in more detail. Additionally, *in vitro* and *in planta* tests of *ca.* 700 isolates have been conducted and led to the selection of isolates belonging to few genera for further testing of biocontrol performance. Partial results of such tests have now been published for the newly described mycoparasitic species *Calonectria hemileiae.* Excellent levels of control, comparable to those obtained with fungicide applications, were obtained.

CONCLUSIONS & PERSPECTIVES

The high level of reduction of Hv urediniospore germination *in vitro* and of CLR severity *in planta*, obtained for *C. hemileiae*, but also for a novel mycoparasitic species of *Fusarium* and endophytic species of *Trichoderma* and *Clonostachys* will be discussed. They justify our optimism towards the potential of antagonistic fungi as anti-CLR tools. Although only preliminary, and resulting from a limited survey for fungal antagonists in South America and Africa, this study has revealed a large hidden diversity of antagonistic fungi protecting the coffee plants and challenging its worst enemy Hv. This paves the way for a novel approach for CLR management.

S1-O-04

Comparative analyses between *Coffea canephora* and *C. humblotiana*, a caffeine-free species, provide insight to determine the origin of the absence of caffeine synthesis

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RATIONALE

Coffea humblotiana is an endemic wild coffee species found in the Comoro islands where it is locally consumed. Like the majority of coffee species from Madagascar, the beans derived from this species do not contain caffeine. To study the mechanism governing the absence of caffeine in *C. humblotiana* we focused on genes encoding enzymes in the caffeine biosynthesis genes and used a gene comparison approach against orthologs from the caffeine producing species *C. canephora*.

METHODS

The *C. humblotiana* genome was sequenced and the transcriptome profiling from leaves was performed. DNA sequence data for genes encoding the N-methyltransferase (NMT) enzymes involved in the last steps of caffeine biosynthesis was compared to DNA sequence from the caffeine producing species *C. canephora*. The caffeine content in young and mature leaves was determined by HPLC.

RESULTS

Here, we present the genome assemblies of *C. humblotiana* and *C. canephora* (initiative Arabica Coffee Genome Consortium). Despite the large difference of genome size, a detailed comparative genomics analysis performed between the *C. canephora* and the *C. humblotiana* genomes indicated an extensive and remarkable synteny. At the biochemical level, the absence of caffeine was confirmed by HPLC in young and mature leaves of *C. humblotiana*. In *C. canephora*, the genes encoding the N-methyltransferase (NMT) enzymes are located on chromosomes 9 and 1. The homologous loci have been identified in *C. humblotiana* and the DNA sequences were compared to that of *C. canephora*. On chromosome 1 the unique NMT gene is conserved whereas on chromosome 9, a complex collinearity with numerous different insertions of repeated sequences was observed close to the two other genes. Hence, we conclude that, the main three genes encoding NMTs are present in the *C. humblotiana* genome, and while one gene appears to be intact, the two others are either interrupted by a repeated sequence or a short deletion in the last exon thereby providing an explanation for the absence of caffeine.

CONCLUSIONS & PERSPECTIVES

Our study highlights the structural genome evolution between *C. humblotiana* and a caffeine-rich African species such as *C. canephora*. Genes encoding NMTs are impacted by insertions, deletions and gene duplications. The impact of the structural variations of the NMT genes activities and functions will be discussed. The analysis of the *C. humblotiana* genome may represent a valuable resource for comparative genomics and the study of genes of interest for cultivated coffee.

S2-O-06

Is the incidence of fungal diseases on Arabica coffee in it's native range related to genetic variation in coffee?

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RATIONALE
Fungal diseases are the major challenges of coffee production worldwide. With the current global climate change, disease and pest outbreaks are forecasted to escalate even to areas where they are not a major problem. We studied the relationship between coffee population genetic composition and diversity and the incidence of the major fungal diseases of the crop in southwestern Ethiopia, an area considered as main centre of origin and diversity of Arabica coffee *Coffea arabica*.
METHODS
To assess genetic composition and diversity of coffee populations, we used genotyping-by sequencing (GBS) approach using libraries prepared from DNA extracted from pooled leaf samples of 16 individual coffee shrubs from each of 60 sites selected along a gradient from almost wild forest coffee to intensively managed plantations. The reads of all pools were mapped onto *Coffea canephora* reference genome for SNP calling and subsequently filtered in different filtering steps. We calculated mean expected heterozygosity as a robust parameter for genetic diversity and used variant allele frequency to compare the variation in genetic composition among the 60 populations. We used both multivariate and univariate approaches to relate to the incidence of fungal diseases quantified on the same coffee populations at each site.
RESULTS
We found that genetic composition, but not genetic diversity, varied along the gradient of coffee management. The incidence of the four major fungal diseases was related to variation in the genetic composition of the coffee stands, but in a different way for each disease. In contrast, genetic diversity had no statistically significant relationship with the incidence of any of the diseases. At coffee shrub level, the incidence of coffee berry disease was related to the genetic diversity of coffee while the incidence of coffee leaf rust did not show such relationship.
CONCLUSIONS & PERSPECTIVES
Given that fungal diseases are major challenges of Arabica coffee in its native range, our findings that genetic composition of coffee populations had a relationship with major fungal diseases may serve as a baseline information to study the molecular basis of disease resistance in coffee. This would be of great importance for smallholder farmers who heavily depend on the crop for their livelihood.

S2-O-07

The mycobiome of wild Rubiaceae to improve the health of coffee plants

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RATIONALE

Plants host a complex internal microbiome from which endophytic fungi represent an important component. There is increasing evidence that many of these endosymbiotic fungi provide benefits to the plant (Gazis & Chaverri 2015). This diversity could then be manipulated and introduced into agriculturally important plants to improve their health and productivity (Pujade-Renaud et al. 2019). With this premise, we aimed at characterizing the endophytic fungi community of wild Rubiaceae species in natural forests of Costa Rica.

METHODS

We assessed fungal community composition using culture-dependent and -independent (metabarcoding) techniques. We then selected potential fungi to be used in antagonism tests against some important pathogens of coffee plants (i.e., *Colletotrichum* spp., and *Mycena citricolor*). In the antagonism tests, we included six fungicides to determine if the antagonistic fungi were able to tolerate the agrochemicals. In addition, we inoculated coffee seedlings with the endophytes to establish if they colonized the internal tissues of the plant and if they improved the plant's tolerance to drought. We collected samples from ca. 30 wild Rubiaceae genera and ca. 50 species, obtaining more than 1000 cultures and 200 tissue samples for metabarcoding.

RESULTS

From the fungi selected for the antagonism tests, we found that species of endophytic *Trichoderma* were the most aggressive against coffee pathogens and tolerant to the most commonly used fungicides in coffee production. In addition, we found that ca. 50% of the coffee seedlings inoculated with the endophytic *Trichoderma* were able to tolerate the drought effects.

CONCLUSIONS & PERSPECTIVES

The preliminary results from our studies indicate that the inclusion of the natural beneficial endo-phyto-mycobiome in Integrated Pest Management (IPM) strategies is an important alternative to the use of fungicides and for the amelioration of the effects of global climate change (i.e., drought).

References:

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ORAL PARALLEL SESSIONS

Session 7: Roasted coffee technology & processing

Session 8: Coffee chemistry & sensory sciences

S7-O-01

Coffee bean particle motion: implications for heat transfer during roasting

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RATIONALE

Understanding physicochemical coffee bean development during roasting is integral to unlocking key flavour and aroma characteristics, as well as improving process efficiencies and translating products from one roaster to another. Our predictive capabilities of roasting time-temperature profiles depend on semi-empirical heat and mass transfer models. By identifying coffee bean particle motion within a roaster, real particle dynamics can be coupled with heat and mass transfer models to improve both the accuracy and robustness of time-temperature profile simulations.

METHODS

Positron Emission Particle Tracking (PEPT) is a non-invasive technique that can characterise flow behaviour within granular systems and has been used here to capture the particle dynamics of labelled coffee beans within a spouted bed roaster. Beans of varying densities were studied - to emulate the effects of roasting - whilst the batch size and air mass flow rate were varied to acquire the impact of air-to-bean ratio on particle dynamics. Recorded tracer positions were used to identify and track the location and subsequent trajectories of a single bean with time.

RESULTS

Determination of occupancy profiles revealed two distinct regions: (i) a *dense* bean bed of high occupancy (ii) a *dilute* freeboard of lower occupancy. Results also showed the effects of coffee density and air-to-bean ratio on particle dynamics within the roaster. By examining the boundaries of the delineated bean bed and freeboard, and how they transform due to changes in coffee density and applied air-to-bean ratio, heat and mass transfer properties of the system can be inferred. Established empirical properties can thus be integrated with heat and mass transfer models to indicate regional heat transfer contributions.

CONCLUSIONS & PERSPECTIVES

The significance of air-to-bean ratio has been shown using the observed particle dynamics and inferred heat transfer properties. This work presents a foundational dataset to enable combination of particle dynamics with predictive heat and mass transfer models to specify temperature distributions within the roasting chamber and track temperature variations on the bean surface during roasting. Commercial implementation of these models has the potential to overcome the need for lengthy trial and error approaches to product and process development.

S7-O-02

Numerical Simulation of Coffee Extraction Based on Mass Transfer

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RATIONALE

Coffee extraction is the complex mass transfer process between hot water and ground coffee beans when the water passes through a bed of coffee grounds [1]. If the mass transfer during extraction process can be accurately estimated and a device is developed to brew a cup of coffee according to this estimate, coffee can be customized to personal preferences. As a first step toward establishing a complete prediction tool for coffee extraction, a general set of macroscopic governing equations for coffee extraction were derived using the volume averaging theory. For this purpose, the coffee bed was treated as a porous medium. The validity of these equations was elucidated by comparing the results of lumped parameter analyses to experimental data for the extractions of drip coffee, espresso coffee, and immersion-brewed coffee [2]. As a second step, a more versatile numerical simulation has been developed, that is applicable for processes ranging from the large-scale extraction of coffee in factories to small-scale extraction in coffee bars.

METHODS

In this study, the coffee extraction in a coffee factory was estimated by the proposed numerical simulation, and the development time for bulk coffee concentration, local coffee concentration obtained at the outlet of the extraction chamber, and even coffee concentration distribution inside the chamber were evaluated. Furthermore, we compared the caffeic acid concentration in the coffee solution over the whole extraction time as measured experimentally at a UCC factory, with that of the proposed numerical simulation.

RESULTS

The numerical simulation results showed that mass transfer occurred actively in the upper part of the chamber at the begining of coffee extraction, and the coffee concentration reached aconcentration equilibrium in the lower part of the chamber. In contrast, as time progressed, the place where mass transfer was active shifted to the lower part, and the coffee that did not reach a concentration equilibrium state was drained from the chamber. Furthermore, from the comparison of the simulated coffee concentrations with those measured in the extraction chamber, it was found that the present numerical result agrees well with experimental data. Thus, the proposed numerical simulation is validated.

CONCLUSIONS & PERSPECTIVES

A novel numerical simulation based on coffee extraction models was proposed for estimating coffee extraction. A comparison of the numerical result and the experimental data obtained using a large chamber at a coffee factory, validated the proposed numerical simulation. Therefore, we conclude that the proposed numerical simulation is useful for predicting coffee extraction.

References:

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S7-O-03

A New Method for Measuring Early Time Scale Coffee Extraction Kinetics in a Well-Stirred Batch Reactor

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RATIONALE

Development of a new method for measuring early time scale extraction kinetics is of interest for understanding how various treatments to roast and ground coffee affect effective diffusion of water soluble coffee compounds. Coffee extraction kinetics in well-stirred batch reactors (WSBR) have been reported for total dissolved solids on time scales greater than 30 seconds and up to several hours, although the majority of coffee extraction occurs within 60 seconds of wetting (Corrochano 2017, Moroney et al. 2015, Spiro & Selwood 1984). It is hypothesized that in order to measure the efficacy of various treatments on extraction kinetics of specific species in coffee, a new method for sampling coffee extract at early time scales is needed. Therefore, we developed a new method for brewing and sampling coffee extraction in a WSBR, with a focus on a time scale of less than 30 seconds.

METHODS

In this study we used a custom air over water pressure vessel to remove a continuous stream of coffee extract from the WSBR. This custom extraction rig was coupled with a rotary sample splitter, providing 16 samples of time resolved coffee extraction during a brewing event. Each brewing event was performed with 3600ml of 90 ºC water, and 100g of coffee. All samples were analyzed using LC-MS.

RESULTS

Brewed coffee samples of a dilute suspension within a WSBR were successfully obtained on a two second interval with the first sample occurring within three to five seconds of coffee-water contact. A total of 16 samples were taken within 35 seconds, with an additional four samples taken at approximately 60, 180, 300, and 600 seconds of brewing. Total dissolved solids and concentrations of citric acid, malic acid, quinic acid, caffeine and 3-CGA were measured for each sample. The results of the coffee extraction events proved to be repeatable, and treatments to the coffee demonstrated marked differences in extraction kinetics versus control.

CONCLUSIONS & PERSPECTIVES

This new method demonstrates the capability of sampling and measuring early time scale coffee extraction kinetics in a WSBR. The method permits the close examination of time resolved extraction kinetics of various soluble species found in coffee.

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S8-O-07

Towards smart on-line industrial coffee roasting process control: A new photoionization mass spectrometry (PIMS) for real-time monitoring of roasting process parameters

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RATIONALE

The coffee roasting process is the decisive step for development of the unique coffee flavor and value. Previous laboratory studies have shown that photoionization mass spectrometry (PIMS) allows a real-time monitoring of the roasting process and prediction of coffee product properties, such as roasting degree or antioxidant capacity (Czech 2016). In the framework of a project co-funded by the Federal German Ministry of Economics, a rugged industrial process monitor based on the PIMS-technology was developed and tested at the site of a roasting equipment manufacturer (Probat GmbH, Germany) as well at an industrial production site.

METHODS

A new Photo-Ionization Mass Spectrometry system (PIMS) for industrial coffee roasting process monitoring applications was developed. The system combines different soft photoionization processes. In detail, Resonance Enhanced Multi Photon Ionization (REMPI), using a new 248 nm Excimer Laser light source and Single Photon Ionisation (SPI), using a VUV lamp are applied in parallel. The ions are detected in a time of flight mass analyzer (TOFMS). Furthermore, a rugged sampling and calibration technique as well a software tool for on-line prediction of roast product properties (PLS model) were realized.

RESULTS

The developed system was tested in the laboratory as well at an industrial large scale roaster. We demonstrated that the roasting degree (measured by color value, Colorette scale) and the antioxidant capacity (measured by the Folin-Ciocalteu assay) of the product could be predicted from the roasting gas measurements. The antioxidant capacity is an important parameter related to health benefits of the coffee beverage (Czech 2020). Also correlations of the on-line recorded PIMS-signals to flavor descriptors (cup testing) are possible. After the evaluation of the system in the laboratory the system was used during large scale industrial production at the site of a Germany producer.

CONCLUSIONS & PERSPECTIVES

TA novel PIMS coffee roasting monitor was successfully developed and tested in the laboratory and at industrial production sites. The system is currently commercialized by Photonion GmbH and Probat GmbH.

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S8-O-08

Sensory Profiles of Cold, Ambient, and Hot Full Immersion Coffee Brews

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RATIONALE

Cold brew coffee has been rising in popularity over recent years among specialty coffees, with many marketing claims of being "sweeter" or "less acidic". However, there have been few studies to date that systematically characterize the differences in sensory properties of different brewing temperatures where strength (TDS) and consumption temperature are controlled. We sought to investigate three origins (Ethiopia, El Salvador, and Sumatra), three roast levels (light, medium, and dark), at three temperatures (4°C, 21°C, and 93°C).

METHODS

A modified descriptive analysis methodology was used under COVID-19 social distancing guidelines. Seventeen panelists underwent 8 tasting sessions via Zoom, with coffee delivered to their homes. During these tasting sessions, panelists discussed attribute definitions, created a tasting ballot, and agreed on reference standards. Ultimately, 26 attributes were chosen for sensory evaluation, with 3 basic tastes, 2 mouthfeels, and 21 aromas encompassing a wide range of attributes the Coffee Taster's Flavor Wheel. Panelists were delivered reference standards to be trained with as well. For data collection, the panelists were delivered samples and refreshed reference standards and asked to complete scorecards at home in a controlled environment the day of coffee delivery. Data was collected using RedJade sensory software.

RESULTS

Preliminary results have shown much success with the modified socially-distant descriptive analysis methodology. The data indicates a strong dependence on origin for how coffees will change with brewing, with more differences in Ethiopia coffees between 4° C and 94° C brewing temperatures compared to El Salvador and Sumatra. In particular, whether or not cold brew was less sour than hot brew also depended on origin – not all origins showed a difference in sourness, but it was notable for the El Salvador coffee.

CONCLUSIONS & PERSPECTIVES

Our results confirm that brewing temperature in this range makes a difference in the sensory properties of coffee brew, but it is highly dependent on coffee origin and roast. This is one of the first controlled, systematic investigations of cold versus hot brew sensory quality, and our results serve as a guide for coffee professionals to select the beans that will be best suited to brewing cold versus brewing hot.

S8-O-09

NMR metabolomics as a tool for Arabica green coffee traceability

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RATIONALE

All over the world, consumers demonstrated a preference for *Coffea arabica* L. because of its fine taste and flavor. Apart from coffee quality, consumers are now also interested in traceability, whether to ensure product authenticity or to support some agricultural practices. In the meantime, producers have to adapt to a fast-changing environment, and assure a sustainable production. Within BreedCafs 2020 project, Arabica coffee beans from several genotypes were grown in 4 locations in Nicaragua and green beans were investigated by non-targeted 1H-NMR to enhance traceability, which is usually limited to Arabica vs Robusta authentication.

METHODS

Green beans from 4 Arabica genotypes (H1, H16, Starmaya, Caturra) were harvested in 2018 in 4 farms in Nicaragua (Las Marias, Aurora, Albania, Boaco). 3 samples were collected for each genotype in each farm, resulting in 48 samples. Prior to NMR analysis, apolar fraction was extracted according to Portaluri *et al* (2020) while polar metabolites were extracted with methanol. Both fractions were analyzed separately. Spectrum were processed with TopSpin (Bruker) and chemometrics analysis were performed with Matlab R2013b (MathWorks).

RESULTS

Data obtain from both solvent extraction (methanol and chloroform) yielded into informative spectrum. Supervised analysis (PCA/LDA) of methanol-extracted spectrum allowed a correct modelisation of samples according to either their production farm or the plant genotype, although loadings interpretation was complex and did not directly allow the identification of discriminant signals.

Variable selection made possible a finer statistical analysis through the modelisation of more parsimonious models. Signals involved in genotype and environmental (farm location and practices) discrimination were then identified, giving information on potential metabolic markers, whose structure is yet to be elucidated.

CONCLUSIONS & PERSPECTIVES

Even though coffee apolar fraction helps Arabica/Robusta authentication, polar fraction turned out to be more relevant for environmental and genotype traceability of green coffee beans. Non-targeted NMR demonstrated great performances for our application and generated relevant information for a future identification of markers. Nevertheless, further work should include the production and analysis of more samples in order to consider all sources of variability including harvest period (year).

References:

- Portaluri et al. 2020 Food Chem. DOI: 10.1016/j.foodchem.2020.127129.

ABSTRACTS WEDNESDAY 30 JUNE

PLENARY SESSION - KEYNOTE LECTURERS

Session 4: Green coffee processing

Session 5: Sustainability, climate change & labels

S4-KN

Coffee postharvest - a crucial processing step for maintaining green coffee quality - or even more?

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Postharvest of coffee (*Coffea arabica* L. or *C. canephora* Pierre) obviously starts with harvesting the fruit, to which then different kinds of treatment follow (e.g. pulping, fermentation, drying), until an intermediate agricultural product is obtained, generally referred to as green coffee. However, also subsequent storing and transporting are relevant to the final green coffee quality, and consequently, it is in fact not until the beginning of *industrial* processing (e.g. decaffeination, roasting) that coffee postharvest treatment ends.

Any way the various postharvest sections are actually executed: The coffee bean that is finally being put under roast is by far not the same that it had been at the brink of its harvest. After postharvest, the coffee bean has undergone a number of changes, among which mere physical losses (i.e. removal of the pericarp, and of surplus water content) can be considered as the least quality affecting ones. Of much greater influence, and under (sub-) tropical conditions practically unavoidable, is the co-existent and treatment-specific microbiota. While infestation of the coffee bean itself usually is avoided, an interference with diverse metabolites or agents of microbial origin (e.g. organic acids, aldehydes, enzymes), and in case of traditional wet processing, a transient switch from aerobic to anaerobic conditions, seem inevitable. As final player, the coffee bean itself must be taken into account: being classified as intermediate between orthodox and recalcitrant seeds, during early postharvest, it exhibits a treatment-specific, in part germination-related, and in part stress-related metabolism, with impact on the coffee bean's composition and (micro-) structure. In later stages, in particular after storage has exceeded three months or more, the coffee seeds tend to lose their viability, and post-mortem reactions (e.g. lipid oxidations) take over, affecting quality-relevant coffee bean constituents as well.

Facing the diversity and complexity of these activities, events and changes in relation to their potential for quality interference, the question arises, how specific control can be maintained. Which of the above-mentioned issues are undesirable and should be diminished, and which may offer positive potential and should be improved? And with respect to the complex interconnectivity of many issues: could one case ever be managed without failing at another?

This presentation is giving an overview on the processes and events of coffee postharvest treatment. It discusses the coffee bean's physiological capacity to respond to its changing biotic and abiotic environment, and the potential for green coffee quality evolution.

S5-KN

Coffee and Climate Change: the wealthy will win, the poor will lose, and coffee will survive

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Arabica coffee traditionally grows in a narrow range of environmental conditions restricted to tropical humid highlands. Apocalypse messages that coffee will lose half of its production area over the next decades (e.g. Bunn et al, 2015) received much attention. Yet over the past decade global supplies have steadily increased and prices declined. Nonetheless, the 2020 Brazil drought and its 20th century frost events illustrate coffee's high sensitivity to climate variability and climate change. Latin America's leaf rust outbreak between 2008 and 2013 was partially attributed to increasing night temperatures (up to +0.9°C), aggravated by declining input use by smallholders who were demotivated by low prices (Avelino et al, 2015). Numerous other studies related increasing pest pressure to higher temperatures (e.g. Jaramillo et al, 2011; Groenen, 2018).

Warming night temperatures (Tmin) also correlated to Tanzania's decline in coffee export over the past half century (Craparo et al, 2015) as it shortens coffee ripening and reduces bean weight. Night temperatures during the bean filling above 16-17°C seem to particularly impact coffee yield. In Bahia (Brazil) Tmax (i.e. 34-39°C) is high during flowering, but 2018 yields averaged up to 5.4 t/ha in 100ha irrigation pivots nonetheless. However, when the 2020 season's Tmin increased from the past 14-16°C to 17-18°C, the bean weights dropped by 10-18%. It confirms common knowledge that smaller beans and lower cup quality are more commonin 'low altitude' Arabicas.

Da Matta et al (2018) reported a strong positive response of coffee plants to atmospheric CO_2 increase. In addition, Tmax up to 37°C hardly impacted growth. Coffee plants demonstrate more 'plasticity' than previously suggested. Modern hybrid varieties provide additional stress resilience. With the right irrigation, fertilization and pest control, high coffee yields can be achieved under future climates in many of today's coffee regions. Coffee growing in California (USA, 34° N latitude) further demonstrates there is still a lot of unexplored territory. However, adapting to climate change will require investment in new varieties, irrigation, fertilization and pest control. Resource-constrained smallholders might be unable to keep up. Regions with marginal climate and marginalised farmers are likely to lose out. The loss of coffee diversity and quality is a bleak picture for some connoisseurs. Larger premiums for smallholder origins, combined with technical and institutional assistance, might help mitigate this anticipated change partially. Else, the wealthy will win, the poor will lose, but coffee will survive.

ORAL PARALLEL SESSIONS

Session 4: Green coffee processing

Session 6: Biochemistry & biotechnology & composition of green coffee

S4-O-01

Untargeted metabolomics of green coffee and correlation to sensory attributes

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RATIONALE

Untargeted or comprehensive metabolomics is a powerful research tool whereby the relative abundance of hundreds to thousand compounds can be simultaneously evaluated across samples. In coffee, untargeted metabolomics has many applications including elucidating effects of genetic variation, environment and ripening, as well as studying in detail the impact of post-harvest processing on coffee quality.

METHODS

Here we used GCMS and LCMS based untargeted metabolomics platforms to determine the effects of whole cherry chilling, a novel post-harvest processing treatment, on green coffee chemistry and cup quality characteristics. Ripe C. arabica cherries from a single batch were stored at either +4°C or -18°C for up to 5 days, and the cold-treated as well as their control fruits were subsequently subjected to both dry and semi-dry processing methods. The resulting green beans were chemically profiled by both Triple Quad-GCMS profiling of polar extracts and Q Exactive Orbitrap-LCMS of semi-polar extracts, and raw data were processed in an unbiased manner using a dedicated workflow. The same beans were also assessed for cup sensory attributes after roasting, as described by Quantitative Descriptive Analysis using an expert tasting panel.

RESULTS

The untargeted GCMS and LCMS metabolomics approaches resulted in relative abundance values of 357 compounds mainly representing primary metabolism and 1593 compounds mainly representing secondary metabolism in coffee, respectively. Data mining including multivariate analyses indicated that the whole cherry treatments that were applied, and especially freezing before processing, exerted significant effects on the chemical composition of both dry and semi-dry processed green coffees, and enabled identification of those compounds and biochemical pathways mainly affected by each treatment. Correlating the metabolite profiles to sensory characteristics of roasted beans resulted in the identification of green bean compounds that were both associated to cup quality and influenced by cherry chilling.

CONCLUSIONS & PERSPECTIVES

Untargeted metabolomics can provide detailed insights into the chemical changes in green coffee taking place before, during and after post-harvest processing, their impact on cup quality and how these changes can be better controlled or may be fine-tuned by special treatments such as whole cherry chilling.

S4-O-02

Impact of post-harvest processing on bean composition and in-cup flavor of Arabica and Robusta coffee

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RATIONALE

Post-harvest treatments significantly impact the aroma and taste of the final beverage[1]. As the coffee's intrinsic quality is predetermined in the green bean by its precursor composition, a detailed knowledge and understanding of the molecular changes during post-harvest processing may allow to monitor and optimize these processes as well as to modulate in-cup flavor. In contrast to the extensive knowledge on coffee roasting, only few systematic studies were performed so far, monitoring green coffee bean changes along post-harvest treatments and linking these physical and chemical changes with an in-cup flavor. Thus, the aim of the present study was to systematically analyze how selected post-harvest processes can modulate the chemical composition of coffee beans and to reveal how these changes are influencing sensory profile of the final beverage.

METHODS

In two separate studies, different post-harvest treatments, wet, dry and honey processing on one hand and monsooning on the other hand, were applied to coffee beans. To follow the physical and chemical changes, samples were taken during post-harvest processing and the green beans as well as the roasted coffees were analyzed. Physical parameters, e.g. moisture content, bean density and color were measured and a large number of metabolites, e.g., sugars, amino acids, organic acids as well as aroma and taste compounds were analyzed using state-of-the-art analytics. The data obtained were correlated with the sensorial changes.

RESULTS

The results obtained give a clear insight how post-harvest processing is influencing the physical and chemical composition of green coffee beans. A clear change in the volatile profiles was observed. A formation of marker compounds in combination with a shift of intrinsic green coffee volatiles could be correlated with the respective changes in sensory profiles.

CONCLUSIONS & PERSPECTIVES

In summary, this study brings valuable insights how different post-harvest processes are influencing the characteristic flavor profile and is linking these changes to a shift in coffee bean physics and chemistry. The markers identified can be used to monitor and to optimize effectiveness of post harvest processes.

References:

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S6-O-01

In vitro inhibition of *Aspergillus carbonarius* growth and reduction of Ochratoxin A (OTA) contents using *Lactobacillus plantarum* strains isolated from coffee cherries

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RATIONALE

Coffee is one of the main agricultural crops in the world. Raw coffee cherries can contain the mycotoxin ochratoxin A (OTA). OTA is produced by several species of *Aspergillus* and *Penicillium*. International coffee organizations pay more and more an attention on statutory limits for OTA in Coffee products (**FAO, 2006**). OTA contents need to be reduced in coffee cherries to as low as technologically control of mycotoxin by avoiding fungal growth and OTA production.

METHODS

Identification of lactic bacteria (LAB) and *Aspergillus carbonarius* strains isolated from Ivorian coffee cherries were carried out using microbiological and molecular methods. Study of effect of cells and supernatant of liquid culture of *L. plantarum* strains were investigated on the growth of ochratoxinogenic *A. carbonarius* strain using various confrontation tests *in vitro*. Then, the best potential fungal growth inhibitor and OTA content reducing strains were inoculated with coffee cherries during post-harvest processing on farm. Fungal growth was measured by weighing the biomass during the incubation and at the end of coffee cherries drying. Samples of OTA contents reduction experiments were analysed by HPLC-FLD method (**Sueck *et al*., 2019)**.

RESULTS

Five OTA-producing *A. carbonarius* strains were detected. Sixteen isolates of LAB including 12 *Lactobacillus plantarum* strains, 2 *Weissella confusa* strains, 1 *L. pentosus* strain and 1 *W. paramesenteroides* strain. Ten strains of LAB showed potential activity with rates (17.3-79.1 %) against fungal growth and capacities to reduce OTA contents (6-96 %). Among these LAB, 7 strains of *L. plantarum* showed highest antifungal activities. Cells of LAB strains had highest inhibitory effect on fungal growth and reduction of OTA contents. On the farm, only the inoculation of *L. plantarum* D12 with coffee cherries result in reduction of OTA content without inhibition of fungal growth.

CONCLUSION & PERSPECTIVES

The inoculation of specific *L. plantarum* with the coffee cherries on the farm results in decrease of OTA level without inhibition of fungal growth. Using *L. plantarum* D12 strain could allow to control and reduce to as low as biotechnologically promising and excellent way in coffee cherries and probably in other foodstuff. The further study may be performed on the comprehension of OTA reduction mechanism.

References:

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- Sueck et al., 2019. Toxins 2019, 11, 329; DOI:10.3390/toxins11060329.

S6-O-02

Coffee (*Coffea arabica* L.) bean transcriptome and volatiles under stress

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RATIONALE

Stress is one of the major problems induced by coffee leaf rust (CLR), which is caused by Hemileia vastatrix Berk. et Br. This study evaluated the effect of CLR control and fruit thinning treatments on the gene expression of immature and mature coffee beans in two CLR susceptible cultivars of *Coffea arabica*.

METHODS

Eight treatments considered the interaction of two CLR susceptible coffee cultivars (*Coffea arabica* L.), two fruit thinning treatments (0% or 50% removal after pollination), and two rust control treatments (with or without cyproconazole and epoxiconazole spray application). Fruit samples were collected in the experimental plot once during the highest infection phase of CLR disease. Mature (red color) and immature (yellowish) fruits were manually collected from each plant. The profiles of volatile compounds from the green beans were identified using the Solid Phase Micro Extraction (SPME), Gas Chromatography-Mass Spectrometry (GC-MS). The total ion count – area under the curve of each peak (relative abundance) for each volatile was reported and used for later statistical analysis. The relative abundance of the volatile precursors obtained was statistically compared between treatments using an analysis of variance (ANOVA) and t test using a cutoff value of $p \leq 0.05$. RNA was isolated using the PureLink® RNA Mini Kit (LifeTechnologies Inc.) according to the manufacturer's protocol.

RESULTS

All differentially expressed genes (DEGs) were grouped into gene ontology (GO). The enriched metabolic pathways related to the DEGs revealed differences between the management practices and the physiology of the plant by genotype. A higher number of DEGs were found in the immature stage where synthesis of fatty acids and carbohydrates were most active.

CONCLUSIONS

The overall interaction of rust control and fruit thinning management showed that stress influences the bean's defense response and the chemical composition in a cultivar dependent manner.

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S6-O-03

XRF- and ICP-based multi-element and stable isotope profiling can be used to differentiate the geographical origin of Ethiopian coffee

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RATIONALE

Coffees with high quality and geographical indication of their origin are important for the world market and buying decisions of consumers. Ethiopia produces distinct coffee types (e.g., *Harar*, *Yirgacheffe*, *Sidamo*, *Limmu, Lekemt coffee*), which are recognized by the world market, in three major coffee regions varying in agroecology. To differentiate the geographic origin of coffee at different spatial scales, determinations of biochemicals, multi-elements, stable isotope ratios and a combination of these approaches, with several statistical techniques, have been proposed so far. We tested the potential of either X-ray fluorescence spectrometry (XRF)- or inductively coupled plasma (ICP)-based multi-element profiling with and without isotope ratio mass spectrometry (IRMS)-derived δ13C, δ18O and δ15N profiling for discriminating the geographic origin of Ethiopian coffee.

METHODS

One hundred three green arabica coffee samples from four Ethiopian coffee regions were subjected to multi-elements and δ13C, δ15N and δ18O determinations. Multi-elements were determined by using ICP- and XRF-based techniques, and δ13C, δ15N and δ18O were determined by using elemental analyzer-IRMS. Linear discriminant analysis was carried out for four datasets: (1) ICP-based multi-elements, (2) ICP-based multi-elements and δ13C, δ18O and δ15N, (3) XRF-based multi-elements, and (4) XRF-based multi-elements and δ13C.

RESULTS

XRF-based multi-elements with and without δ13C appeared to be most effective in discriminating the geographical origin of coffee, giving higher classification accuracy (89 and 86%, respectively) than ICP-based multi-elements with and without stable isotopes (80%, each). Combining XRF- and ICP-based multi-elements with stable isotope ratio data slightly improved the classification accuracy of coffee samples. This may suggest that the coffee geographical origin differentiating power of XRF and ICP multi-element determination can slightly be improved by including stable isotope compositions.

CONCLUSION

Our results demonstrate the potential of XRF-based multi-element data to discriminate samples of green arabica coffee beans according to their growing regions and provided the proof of concept for tracing Ethiopian coffee based on growing region via multi-element and stable isotope profiling. Considering a more rapid, an easier and a cheaper multi-element analysis via XRF with no sample material chemical digest constraints (i.e., ICP), the results indicate that the XRF-based multi-element approach can be a preferred method of choice to determine the geographic origin of Ethiopian coffee and be applied to help combating fraudulent activities in the coffee market.

S6-O-04

Metabolomic profiling of *Coffea mauritiana* leaf extracts from the Mascarene islands, and comparison with other coffee species for identification of novel biomarkers

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RATIONALE

Alkaloids, phenolics and isoprenoids are key compounds that contribute to the nutritional and aromatic properties of coffee beverages. Nowadays more and more studies focus on wild coffee species, primarily originating from Africa, and investigate not only green versus roasted beans, but also coffee leaves as a rich source of phytochemicals. In our study, *Coffea mauritiana*, an endemic tree from the Mascarene Islands, is registered since 2013 in French pharmacopoeia for its long term medicinal use. This species widely distributed in Reunion forest represents also an interesting model for highlighting patterns of within-island differentiation at small spatial scale. A metabolomics-based study was undertaken to evaluate the biochemical diversity from *Mascarocoffea* species originating from Indian Ocean islands in comparison with cultivated and wild African species.

METHODS

Mature leaves from 80 accessions were collected at the *Coffea* Biological Resources Center in Reunion, therefore grown in the same environmental conditions. Using proton nuclear magnetic resonance (NMR) and liquid chromatography, we profiled hydroalcoholic leaf extracts obtained from 42 genotypes of *C. mauritiana*, in comparison with four other *Mascarocoffea* and seven African species. We evaluated their radical scavenging capacity, as well as total phenolics, chlorogenic acids, trigonelline and caffeine contents. Unknown components from *C. mauritiana* extracts were isolated and characterized by high resolution mass spectrometry and multidimensional NMR data.

RESULTS

We quantified some of the major secondary metabolites extracted from coffee leaves. Our results show that *C. mauritiana* has a specific phytochemical profile with at least two glycosylated monoterpenoid components representing up to 4% of the leaf (dmb). We unambiguously identified these molecules as they have already been described in other plants and shown to display various potent biological properties. The occurrence of at least one of these derivatives which we showed to be present in at least three Mascarene coffee species, including C. mauritiana, may be considered as a new biomarker for *Mascarocoffea* species.

CONCLUSIONS & PERSPECTIVES

Our analytical approach aims at identifying chemotaxonomic markers for *C. mauritiana*, and more largely for other wild coffee species from the Mascarene Islands. Newly identified compounds in *C. mauritiana* support the traditional use of this plant. It will be interesting to screen more *Coffea* accessions, as well as to correlate biochemical and genetic data, in order to highlight the biosynthetic pathway leading to monoterpenoids in the *Rubiaceae* family and more precisely in *Coffea* genus.

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ORAL PARALLEL SESSIONS

Session 5: Sustainability, climate change & labels

S5-O-01

Sustainability projects – sustaining old governance structures?

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RATIONALE

Since the 1970s, a range of certification schemes have emerged as a reaction to the returning coffee crises, over time leading to a global sustainability and standards market. Originally shaped by social or environmental NGOs, more recently, we have seen an increase of trading companies, processors and retailers that promote similar sustainability agendas through projects, programs and service delivery models. While climatic changes are expected to add to the vulnerability of smallscale farmers, increased awareness among industry and consumers of vulnerable farming communities is fuelling a drastic increase in *sustainability* projects, facilitated by fast advances in ICT, digitalization and data capture. This study looks into the positive developments that may come of this, while also highlighting the uneven playing field in terms of data ownership and access to resources and information that may result in maintenance of the traditional governance structures in coffee and business-as-usual for the farmers.

METHODS

I draw on many years of coffee research projects, involving interactions with large numbers of farmers across the coffee, large traders and processors, service delivery companies, sustainability managers, NGOs and public agricultural offices. Here, I condense this knowledge, using a value chain lens in an investigation of recent initiatives by large coffee trading and processing companies that work with 1000s of farmers, and identify positive outcomes as well as aspects that may leave farmers disadvantaged.

RESULTS

Recently, sustainability initiatives by trading and manufacturing companies, often led by their sustainability branches, have reduced the role of 3rd party verified and basically mission-driven certification schemes. Companies collaborate with existing label organisations for legitimacy, while creating spaces with fuzzy accountability. Digital agriculture, incl. advisory apps, field sensors and input/output monitoring, play an increasing role for improved coffee farm and value chain management, but data ownership remains a contested matter and informs suppliers and buyers more so than farmers. Partly data-driven narratives empower consumers, but the narratives are increasingly formed by industry actors and not mission-driven NGOs.

CONCLUSIONS & PERSPECTIVES

Climate change, SDGs and market differentiation have opened up for new farmer facing sustainability initiatives, facilitated by new ICT tools and digitalization. This has created new opportunities for farmers, but also recreated asymmetries in the value chain. How this is handled by private and public actors will determine if the new era is indeed sustainable for farmers.

S1-O-05

Enhancing the adoption worldwide of Arabica hybrids through implementation of on-farm trials, transfer of propagation techniques and stakeholder dialog platforms

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RATIONALE

New coffee hybrids are important for addressing issues of quality, pests & diseases and climate change, but new plant varieties often encounter slow adoption among farmers due to uncertainties and access. Even when coffee hybrids are shown to be more productive and resistant to (a) biotic stresses, less than 5 % of the orchard in Latin America is planted with hybrids each year. A new dissemination strategy is needed, based on access to technology, data and networks, to increase farmer uptake. This is the aim of the H2020 project BREEDCAFS, based on sharing of propagation techniques locally, local assessment of hybrids in agroforestry systems (AFS), and setup of national stakeholder dialog platforms and sustainable agroforestry clusters. Here, we give a first glimpse of the implementation of this new approach.

METHODS

For hybrid assessment, on-farm demo-plots with 4 hybrids and a local control were planted in Nicaragua, Costa Rica, Cameroon and Vietnam. Agronomic observations, quality testing, and farmer surveys are carried out to evaluate productivity, profitability and farmer acceptance. Technology for combined somatic embryogenesis (SE) and rooted mini cuttings (RMC) were transferred to partners in Vietnam, Cameroon and Nicaragua, where dialogue platforms have also been created for all stakeholders in the sector. Further, implementation of agroforestry clusters has been initiated with groups of farmers cultivating new hybrids in sustainable AFS, targeted specialty buyers.

RESULTS

The combination of SE and RMC is showing to be a lower-cost approach than solely focusing on the complex and expensive method of SE for vegetative propagation of hybrids. The setting-up of rooted mini cuttings nurseries to Vietnam and Cameroon has allowed mass propagation of hybrids locally at reduced costs, while a women's cooperative in Nicaragua now runs a business producing and selling mini-cuttings. Initial field observations are encouraging; in Costa Rica and Nicaragua hybrids are more productive and produce a better coffee quality in AFS, while observations in Vietnam and Cameroon confirmed higher vigour and yield. In Nicaragua, a 1,250 ha agroforestry cluster is already running successfully, delivering high quality coffee to a specialty buyer; a setup that is now being replicated in Vietnam and Cameroon.

CONCLUSIONS & PERSPECTIVES

The hybrids assessed in BREEDCAFS show very promising results. As these hybrids are among the first on the market, many innovations are needed to promote their dissemination and adoption. Our approach to disseminate the Arabica hybrids in the coffee belt form a coherent strategy that appears to be effective in addressing replanting new varieties adapted in the coffee sector.

S5-O-02

Learnings of 15 years of coffee Cup of Excellence competition in 15 countries: Specific insight on the importance of varieties

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RATIONALE

During the last two decades, the Alliance of Coffee Excellence (ACE) has been organizing the Cup of Excellence (COE) competition in numerous countries. Coffee samples running in the competition are assessed by a national and international jury following the Specialty Coffee Association (SCA) score. Top samples are sold during auctions and are often fetching record prices. COE is hence promoting high cup quality coffees and rewarding the best of the best. The description (region, altitude, process, varieties…) of the samples and their scores are kept in an anonymized database. RD2 Vision and ACE saw an opportunity to dig this database and identify what are the more important determinants of cup quality with a specific insight on varieties.

METHODS

We gathered and standardized data from 3,176 samples assessed over 15 years in 15 countries. We applied linear regression and multivariable analysis to dig into the data and looked at the specific effect of altitude and varieties on the overall scores and the attributes of cup quality.

RESULTS

Most significant predictor of cup quality across countries and years appeared to be Altitude and Variety. All things being equal otherwise, an increase of 500 m in altitude, the variety being Gesha or the variety being Pacamara led to an increase of respectively 1.0, 2.1 and 0.96 points on the SCA score. While this result was not a major surprise, it was quantified in our study in a statistically robust manner.

The range of varieties presented at COE evolved along years with the emergence of 'exotic' varieties following the "Gesha" effect. Overall, the genetic diversity of the samples steadily increased over the years as a way to reach higher and maybe diversified cup qualities.

Regarding the post harvest process, the lack of standardized definition and denomination across countries prevented to assess its effect.

CONCLUSIONS & PERSPECTIVES

COE database appeared to provide an adequate database to statistically confirm the effect of altitude and varieties on cup quality across time and countries/continents. This supports the strategy towards breeding for aromatic coffee quality. Indeed, while Genetic x Environment Interaction will always be present, the varietal effect seems to be strong enough to justify a breeding strategy for cup quality. It also highlights the importance of varietal authentication and of a professional coffee seed sector that will allow farmers to pick the exact variety they wish, namely for expected cup quality. The denomination of post-harvest processes needs to be standardized to allow robust comparisons across countries.

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S5-O-03

Gorongosa Coffee: Sustainable coffee production in the Gorongosa National Park in the context of deforestation, climate change and food security

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RATIONALE

Biodiversity loss and ecosystem fragmentation due to anthropogenic and climate pressure are among the major concerns across the globe, especially in multi-use landscapes. Located in the southernmost tip of the Great Rift Valley, the Gorongosa National Park (PNG) is considered one of the world's most ecologically diverse conservation areas, encompassing several biomes. As part of the restoration strategy, a coffee agroforestry system (native trees and *Coffea arabica L.*) is being implemented in the Gorongosa tropical rainy forest, aiming to reconcile biodiversity conservation and human development, and to implement a sustainable model to produce fair trade, organic, certified, highest quality coffee. The research strategy and first results of the trilateral GorongosaCoffee project (Mozambique, Brazil, and Portugal) will be presented.

METHODS

Nine experimental permanent parcels have been established in three altitudes (650, 825 and 935 m) and three irradiance levels (deep shade, moderate shade, and full sun exposure). Plant performance will be monitored at phenological, eco-physiological, and agronomic (including bean characterization) levels. Distribution, taxonomic analysis, and plant genetic diversity analysis were also assessed, together with the microbiome of coffee and shadow trees.

RESULTS

Preliminary results point for a positive effect of altitude in the overall crop performance (growth, photosynthetic related parameters, yield and bean quality), and some contribution of moderate shade. Native *Coffea* species in Mozambique include five to six species, whose suitability for blend with Arabica coffee will be tested. Molecular analysis to assess genetic diversity is in progress.

CONCLUSIONS & PERSPECTIVES

The coffee agroforestry system is being successfully implemented in the Gorongosa Mountains and has a high potential for the recovery of tropical rainy forest and biodiversity, as well as to enhance socio-economic development.

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S5-O-04

The Tanzanian smallholder coffee growers investment decisions in coffee production

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RATIONALE

Majority of smallholder coffee producers in Tanzania complain about high cost of coffee production and low market price. This study assesses the coffee growers' investment decisions using real options theory that compare the variation in costs and its implication to prices. The study applies a model suggested by Luong and Tauer (2006) that uses the framework developed by Dixit and Pindyck (1994).

METHODS

In the simple entry–exit model, a firm must invest a sum K to build a project to produce a unit flow of output at a variable cost C. This investment project is assumed to last forever and be non-depreciating. The firm has to pay an exit cost per unit of output X if it decides to exit, and must incur the entry cost K again if it wishes to re-invest. K, X, and C are assumed to be constant and no stochastic. Empirical application of the entry–exit model was used to analyses the decision of smallholder farmers to invest in coffee production. This model uses data on coffee prices obtained from the Auction, coffee production, area planted, cost of capital, investment (fixed) cost, and variable production cost collected by using structured questionnaire from 250 respondents selected randomly from Mbinga, Mbozi, Hai, Karagwe and Muleba.

RESULTS

The study finds that smallholder coffee producers, with variable costs of 771 US$/ha of improved Arabica coffee varieties produce 1640 kg/ha and 913 US$/ha of traditional Arabica coffee varieties produce 687 kg/ha, would enter coffee production at a coffee price of not less than 2.5 US$/Kg. Producers with variable cost of 876 US$/ha of improved Robusta coffee varieties produce 2400 Kg/ha and 1083 US$/ha of traditional Robusta coffee varieties produce 1333 kg/ha, would enter coffee production at a coffee price of 1.5 US$/Kg. The result shows that, 15% of respondents have planted improved coffee varieties, 40% planted traditional varieties and 45% have planted both improved and traditional varieties. Regardless of relatively depressed price levels in the market, smallholder farmers continue to produce coffee.

CONCLUSIONS & PERSPECTIVES

The high cost of coffee production was due to high price of input, labor and low coffee productivity. The unstable market price affects smallholder farmers invest in coffee production. Therefore, government interventions to support farmer access to synthetic inputs, financial credits to support coffee production is vital for boosting nation coffee production and also increase smallholder farmer's profitability.

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POSTER PRESENTATIONS

Session 3: Farm management

S3-PO-01

k-SCAS: Framework for a Knowledge-Driven Specialty Coffee Agribusiness System

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RATIONALE

The agribusiness system for specialty coffees (SCAS) is complex and involves players from different sectors, depending on numerous areas of knowledge which tends to be field-specific (Goldberg, 2018; Megido et al., 2019), and rarely shared among different sectors. Knowledge representation contributes to the generation of value (Pacheco et al., 2011). As a way of obtaining the best quality of the final product, a knowledge-driven conceptual framework representing the SCAS, and a proposal for its application through the development of SCAS Agent Proto-Personas are presented.

METHODS

First, systematic literature searches were conducted. The next step was the development of an international survey with 369 agents and consumers of SCAS in 41 countries to identify essential knowledge necessary to obtain the maximum quality of the coffee. Subsequently, the SCAS conceptual framework knowledge-driven was developed, which was verified by eight domain experts from different areas of SCAS.

RESULTS

The k-SCAS Framework is structured on seven levels: 1) Knowledge Core; 2) Internal SCAS Agents and Stages; 3) Knowledge Sharing Events; 4) External Agents of SCAS; 5) Critical Success Factors; 6) Traceability and 7) Results, presented through Excellence in Coffee Quality, Integration with the Academy, Benefits for Consumers, Gains for Organizations and Wealth for Society.

CONCLUSIONS & PERSPECTIVES

The k-SCAS Framework contributes to science in a unique way, visually representing the relevance of the integration of previously field-specific knowledge in the agribusiness sector of specialty coffees, showing the importance of integrating agents and stages of this system.

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S3-PO-02

From Simulation Game to Early Warning System: an interactive Agent-Based Model to fight coffee rust in Central America

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RATIONALE

Coffee rust is an aggressive disease that infects all coffee plantations in the world. This fungus targets coffee leaves and spreads through release of spores. Its life cycle depends mainly on temperature and humidity. By analysing climatic conditions, coffee institutes issue warnings to producers. But due to lack of reliable data and knowledge, they tend to systematically recommend fungicide applications (chemical treatments), which are not affordable for small producers, let alone the environmental effects. Apart from biophysical factors, there is a clear correlation between rust outbreaks and socio-economic crises linked to coffee and input prices.

METHODS

To anticipate epidemics and implement prevention strategies, we designed an interactive multiplayer simulator. Based on the life cycle of rust and coffee trees, this game simulates coffee production according to climatic conditions and the treatments applied by producer agents. Intended for the coffee institutes, this game aims to generate recommendations for small producers with limited financial resources.

RESULTS

Sessions held in several countries in Central America showed wide disparities in results, even though the participants were experts in coffee and rust. By making players aware of the technical, economic and labour restrictions of small producers, participants realize that it is impossible to systematically apply fungicides. They are then obliged to rationalize their recommendations and adapt them to local conditions, which require accurate and continuous information on climate and local socio-economic context.

CONCLUSIONS& PERSPECTIVES

By highlighting the importance of communication between countries, this game aims to structure a regional network of meteorological and agricultural institutes. In order to find adaptive control strategies, it is also necessary for countries to exchange information on rust severity levels. The ABM underlying this game continues to be improved and will ultimately become a central tool of a regional early warning system. A Smartphone application will then allow customized recommendations to producers.

Keywords: Coffee rust; Agent-Based Modelling; Role-playing Games; Hybrid model; Early Warning System

S3-PO-03

Identification of constraints affecting the coffee productivity and quality in Burundi

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RATIONALE

The cultivation of coffee was introduced in Burundi in the early 1920s. It is currently cultivated in almost the whole country and represents 10% of the cultivated land. The favorable climate for coffee growing and the bourbon varieties which give high quality coffee are the great assets that the country has to develop this crop. However, the production and quality of coffee in Burundi is declining.
In order for the coffee sector to regain its competitiveness, a research project has been initiated on the agronomy of coffee in order to identify the agronomic and environmental constraints that affect the productivity and quality of coffee in Burundi.

METHODS

Data collection was carried out through a diagnostic survey to better understand the explanatory parameters of yield and quality. One hundred and ninety eight (198) coffee farms in the three main coffee production areas were targeted. The data collected were related to the description of the plot, plant vegetative parameters, yield parameters, soil fertility and the nutritional status of the coffee tree (soil and leaf sampling followed by chemical analyzes) as well as those in relationship with the sensory quality of the coffee.

RESULTS

Observed yields vary from 0.227 kg to 7.856 kg / coffee plant. Preliminary results revealed the effects of certain environmental factors on both yield and coffee quality. The exposure of the plots shows a highly significant difference ($P < 0.001$) on the yield, with plots oriented towards the North and North-East providing a higher yield compared to those found on slopes oriented towards the West and North-West. The altitude of around 1,700 m was the most favorable for yield. A planting density of around 2000 to 3000 plants per hectare and clay loam soils were associated with the best yields.
Regarding coffee quality, 74% of farms produced merchant coffee classified as «FW15 +» with 56% classified «FWAA». It was also noted that from 1700m up to 1900m altitude, the acidity of the liquor has a very good to excellent score compared to plots located at a lower altitude, with the presence of citric and malic acidity and the limited presence of quinic acidity. For this same altitude, the flavor and the body were better. This is also valid for the physical quality of the coffee beans because from 1700 m to 1900 m the green coffee beans become heavier and heavier.

CONCLUSIONS & PERSPECTIVES

Data related to the chemical quality of the soil are being analyzed in order to be able to draw further conclusions on their influences on the yield and quality of coffee in Burundi.

S3-PO-04

Inclusion of farmers in *Coffea canephora* selection process through surveys

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RATIONALE

Every breeding program is defined by two main pillars: the available germplasm and the breeding objectives. From farm to cup, objectives for *Coffea canephora* breeding are defined by the needs of farmers, factories and consumers. As farmers are the first clients, it is crucial to understand their requirements and how to include them in the variety's selection process. By collecting farmers feedback on Robusta varieties while they are under trials, Nestlé ensures that varieties fit the expectations of local farmers before plants distribution. As coffee culture differs from one country to the other, local preferences must be considered in each country. This ongoing study aims to investigate the expectations of Thai and Ivorian farmers regarding Robusta coffee varieties developed for each country.

METHODS

Nestlé agronomists have developed a survey based on important agronomic traits for farmers (plant growth, plant vigor, plot homogeneity, fruit setting, fruit size, yield and overall acceptability). Data is gathered on each participant to evaluate demographic effects on the vegetal material acceptability. So far, 430 farmers participated over 4 years in the South and the East of Thailand and 39 Ivorian farmers participated over 2 years in Divo and Yamoussoukro regions. Farmers were selected to be representative of each country's demographics regarding age, experience and cultivated surface of coffee. Surveys are conducted on a yearly basis over a full production cycle of 5 years to reflect the potential of the evaluated varieties.

RESULTS

There are significant differences between varieties regarding farmer acceptability in both countries. Preliminary results show that productivity related criteria, especially yield, are more important than vegetative traits for farmers in both countries. In Thailand, fruit size is essential for farmers as local Robusta population with big cherries have been shared between farmers in the past. Farmers of different age mostly agree on varieties acceptability even though older farmer tends to give higher scores. Farmers owning more surface of coffee are stricter regarding acceptability.

CONCLUSIONS & PERSPECTIVES

Farmers are satisfied to be involved in the selection process. Moreover, they are more confident to plant new material as they have already witnessed its potential. Two clonal varieties with more than 90% of farmer acceptability are registered and distributed in Thailand through the Nescafé Plan since 2018. Based on farmer feedback, breeding and selection objectives can be adapted to local preferences. Formats and overall organization of the surveys will also be improved based on this experiment and its results.

S3-PO-05

Influence of Balanced Nutrition on quality of *Coffee Arabica* L

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RATIONALE

The cup coffee quality is primarily driven by the physical and chemical characteristics of the green bean, which is determinate by the combinations of three main factors: Enviromental x Genetic x Agricultural practices (Lambot at al. 2017). Coffee is grown mostly on weathered, acid soils around the coffee belt. Nutritional practices of coffee growers are often poor, without any balance between nutrients, and too much emphasis on nitrogen. Such unbalanced fertilizer practices can reduce coffee yields and coffee quality. The objective of this research was to compare the impact of three different nutritional practices on coffee quality.

METHODS

Two trials on Arabica coffee varieties Caturra and Castillo® were carried out in El Pital-Huila region of Colombia. Three nutritional programs (NP) were tested: **NP1** represents farmer practices with the application of N, P ,K , S and Mg; **NP2** represents a balanced program providing all essential nutrients for coffee (N, P, K, S, Mg, Ca, Zn and B), but with a 50% reduction in the application of K, and **NP3** finally represents a fully balanced program providing all essential nutrients at optimum rates. The descriptive sensory analysis of coffee bean samples from the trials was made in the lab of Coffee Mind® in Denmark. Roasting was stopped at the #87 color on the Agtron Gourmet scale, and the brewing preparation followed the standards of the Specialty Coffee Association of Europe (SCAE).

RESULTS

Results reveal statistically significant impacts of the nutrition programs on coffee cup and physical bean quality. The principal component analysis shows that sweetness and balance were closely correlated with the fully balanced nutritional program (NP3), while rather negative aspects for quality such as astringency were correlated with the unbalanced program (NP1). Bean size was also affected by the nutrition: farmer fertilizer practices (NP1) and a 50% reduction in K supply (NP2) reduced green bean size, while application of all nutrients at the right rate and the right ratio (NP3) improved green bean size with 87% of all beans in mesh sizes 16+17+18.

CONCLUSIONS & PERSPECTIVES

Unbalanced nutritional practices without or reduced applications of essential nutrients such as Ca, K or micronutrients reduce green bean size and negatively affect coffee cup quality. Hence, coffee growers require more advise on improved coffee nutrition management, not only to improve productivity, but also to optimize coffee quality. Both, optimum yield and optimum quality are important pillars of farm profitability, especially in times of low coffee prices.

S3-PO-08

Grafting as a way to modulate expression of physiological and biochemical parameters linked to drought tolerance in *Coffea canephora*

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RATIONALE

Climate change is inducing longer and more intense drought periods in tropical regions. By 2050 a reduction (50%) of suitable cultivation area for coffee is expected. Consequently, it is imperative to develop varieties or to find grafting combination adapted to drought. Recently, Silva *et al*., (2018) demonstrated the benefit of reciprocal grafting (drought-sensitive Conilon onto tolerant rootstock). Our study evaluates the impact of reciprocal grafting of drought tolerant (dS) or drought sensitive (dT) clones on physiological or biochemical traits expressed during water stress.

METHODS

Coffea canephora cuttings (60-100 cm height) from two contrasting clones (dS FRT133=Group A x E and dT FRT140=Group A x D) cultivated in greenhouse (14 l sandy soil pot, control plants in 5 replicates and reciprocal grafted plants in 3 replicates) were submitted to 14 days of water stress. Leaf water potential (LWP) recorded with Schölander pressure chambers, proline and mannitol studied with HPLC.

RESULTS

Variations in LWP confirmed differences between dT & dS clones (Anova). Reciprocal grafting shows contrasting situations. A dS clone grafted onto dT rootstock presents higher drought tolerance and physiological/biochemical traits similar to dT clone. The opposite is observed for dT clones grafted onto dS clone. Proline and mannitol content show more contrasted results, aiming to be used as drought tolerance indicators (see Karunakaran and Ilango, 2019 on Tea). Our finding illustrates the impact of grafting on physiological and biochemical traits linked to drought tolerance. The use of dT rootstock leads to better regulation of water management and biochemical composition of the scion in dS clones as opportunities to improving drought tolerance of *Coffea canephora* genotypes limiting the impact of global warming.

CONCLUSIONS & PERSPECTIVES

Biochemical results of dS FRT133 and dT FRT140 clones agreed with phenotypes and physiological measurements. As grafted plants show 2-3-fold higher mannitol or proline content, reciprocal grafting highlights the impact of rootstocks use regarding water use and metabolites linked to drought stress mechanisms.

References:

- Silva et al., 2018: Plant Growth Regulation 85:221–229.
- Karunakaran & Ilango 2019: The Journal of Agricultural Science 157, 217–225.

S3-PO-09

First results on growth and yields of *Coffea arabica* L. var. Laurina plants grafted on 5 different rootstocks

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RATIONALE

Coffea arabica var. Laurina, also called Bourbon pointu, differs from the Arabica coffee plant by the shape of the shrub, leaf size, grain shape, lower caffeine content and its sensory characteristics. illycaffè's Laurina (internally called Bourbon Low Caffeine – BLC) is a result of a long selection process started in the nineties. The caffeine content of this selection is around 0,6-0,8 %. Despite the excellent quality on cupping, this selection over the years has not proved to be very productive nor very resistant to pests and diseases. In order to improve yields and performance, we grafted BLC on 5 different rootstocks. In this report the first vegetative and productive results will be described.

METHODS

The experimental trial was carried out in Finca Rabanales, located in Fraijanes, Guatemala. In March 2017, BLC scions were grafted onto 5 different rootstocks varieties (*Coffea arabica* var. Typica, var. Obata, var. Anacafe 14, var. Mundo Novo and *Coffea canephora* var. Nemaya) for a total of 50 plants per each grafted rootstock variety. In September 2017, the plants were planted in 5 different experimental plots, with 2 replicates of 25 plants for each plot. In the following years, vegetative measurements (including plant height, stem circumference, number of leaves and number of nodes per plagiotropic branch) were carried out monthly. In January 2020, measurements on the first cherry production (including number of fruits, their weight, number of empty fruits, medium average of fruit weight) were also performed.

RESULTS

Statistically significant differences emerged between the theses. The BLC plants grafted on Nemayas showed very small growths, scarce flowering and low productivity. In terms of yields, better results were obtained with grafted plants on the Mundo Novo and Typica rootstocks. The best yields have been obtained from the BLC plants grafted onto the Anacafé 14, a Guatemalan variety, which is supposed to have originated from a natural cross between a Catimor variety with Pacamara; this thesis has given much better results in terms of several parameters, including the growth rate and the average productivity per plant. The medium average weight of the coffee cherry was not influenced by the rootstock variety.

CONCLUSIONS & PERSPECTIVES

The vegetative growth results obtained 30 months after the planting are very promising. The data collected from the first harvest also show how BLC grafted on A14 could overcome the low productivity of this Bourbon pointu selection. Further investigation, especially around green coffee quality and yields, are absolutely required in the coming years.

S3-PO-10

Using local knowledge to identify shade tree species that best suit farmer's needs in coffee farms in Western highlands of Cameroon

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RATIONALE

Until the 80's, arabica coffee was the main commodity crop in Western highlands of Cameroon, but the culture has been almost entirely abandoned after forty years of low coffee prices (Uwizeyimana, 2009). The H2020 BREEDCAFS project (https://www.breedcafs.eu) aims at reviving the coffee sector via the introduction of new F1 hybrid coffee varieties well-suited to agroforestry systems, high-yielding and producing a high cup quality. Within this project, this study supports the design of coffee-agroforestry systems matching farmers' needs and adapted to local conditions.

METHODS

The study followed the Shade Tree Advice methodology, based on coffee farmers' local ecological knowledge of shade tree species (Van der Wolf et al 2016). Farmers' needs were identified through interviews. Associated shade tree species were listed through on-farm tree species inventories. Shade tree species performances were collected through interviews of farmers and rankings, of which around 100 were assessed in five geographical divisions (Noun, Bamboutous, Menoua, Nde and Grand Mifi) of the West region of Cameroon.

RESULTS

The 10 most important criteria for shade tree species selection were: 1) fruit production, 2) coffee-fertilizer saving, 3) timber production, 4) production of a third crop beneath shade trees and coffee, 5) impact on coffee yield, 6) reduction of coffee bi-annual production pattern 7) protection of coffee from anthracnose, 8) minimal need for pruning, 9) use in traditional medicine, 10) shading that improves working conditions. Meanwhile, 35 tree species were identified in coffee farms through the inventories, and the resulting agroforestry advices are available online on www.shadetreeadvice.org.

CONCLUSIONS & PERSPECTIVES

This study provides a user-friendly tool to support coffee farmers in their selection of shade tree species (Van der Wolf et al, 2016). On a local scale, this tool will contribute to promote the conservation of both economic and indigenous tree species as well as support efforts to revive the coffee sector in Western highlands of Cameroon through the development of sustainable agroforestry practices with the most performant Arabica varieties.

References:

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POSTER PRESENTATIONS

Session 4: Green coffee processing

Session 6: Biochemistry & biotechnology & composition of green coffee

S4-PO-01

The influence of microorganism succession at different coffee drying stages in beverage quality

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RATIONALE

Microbial population and the conditions that lead to one or other species to prevail, may influence the coffee beverage. The present research had the aim to analyze the fungal infection, total and lactic acid bacteria counts, the climatic conditions at different drying times and correlate these factors with the sensorial characteristics of the beverage.

METHODS

Coffee samples were analyzed at different sun drying stages. Three types of coffee preparation were analyzed: washed cherry; mature cherry and natural coffee. Fungal infection, lactic acid and total bacteria counts were performed according to Pitt & Hocking (2009) and Silva et al. (2017). Coffee samples were evaluated in two different degustation tests: infusion and *espresso* as described in Iamanaka et al. (2014).

RESULTS

At the beginning of drying all types of preparation showed high yeasts, total and lactic bacteria counts, decreasing with time and reduction of water activity. Washed cherry showed the highest fungal diversity and low infection with positive beverage attributes. The natural coffee had the highest fungal infection (22%) mainly by *Fusarium* spp., some of them showed positive attributes and others negative, slightly fermented. The mature cherry had the lowest fungal infection (2.5%) and the worst beverage quality (strong fermentation), indicating that the mature fruit dried partly on the tree has low fungal infection. However, during the period of drying yard, the whole fruit with sugar pulp and high water content was exposed to the sun for a long period favoring bacteria and yeast actions, causing possible lactic, alcoholic or acetic acid fermentation and harmful metabolites to taste, such as acetic acid (vinegar) and ethyl ester derivatives.

CONCLUSIONS & PERSPECTIVES

The drying time is a critical factor, as in this period several microorganisms can grow, produce metabolites that interact with coffee, affecting its quality and sensory characteristics.

References:

- Pitt, J.I., Hocking, A.D. 2009. **Fungi and Food Spoilage**. 3rd ed. New York: Springer.
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S4-PO-03

La Cumplida Refinada: sustainable coffee fermentation

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RATIONALE

Spontaneous fermentation is naturally occurring in coffee post-harvest processes and contributes to the quality consistency and to the specific flavour profile of a terroir1. The limitless choice in microbial ecology allows, in combination with well-chosen post-harvest conditions, to trigger coffee fermentation in order to create natural, innovative in-cup flavour profiles. In the past years, coffee producers innovated in their production & fermentation processes, not only increasing cup score, but also creating surprising, new flavour bouquets by controlling microbial communities during post-harvest processing. The challenge however remains to establish a robust process, delivering a consistent sensory profile, harvest after harvest. This study shows how a thorough scientific approach enabled to guide the optimization of a post-harvest process to consistently deliver new flavour profiles.

METHODS

During the 2018/2019 harvest, over 220 field trials were conducted to select the most appropriate post-harvest process and fermentation conditions. Microbial community dynamics during fermentation were evaluated using metagenomic tools. Gas chromatography was performed both on green and roasted beans to measure the impact of the processes on the chemical fingerprint. Finally, the analytical work was linked with extensive sensory evaluation to identify optimal process conditions enabling best in-cup profile.

RESULTS

The field trials generated coffees exhibiting various sensorial directions, both in terms of profile and intensity. The sensory profile modulation could be linked to an altered flavour fingerprint at both green and roasted bean level. A causal link was demonstrated between microbial growth dynamics and the novel flavour profile. The latter observations enabled to itendify the most optimal fermentation conditions applied at larger scale during 2019/2020 harvest season, while at the same time reducing the environmental footprint of the coffee process by reducing water consumption.

CONCLUSIONS

A harmonious flavour bouquet in-cup, generated through controlled coffee fermentation, is the result of a subtle combination of optimized process parameters, such as terroir, coffee variety, cherry maturity, fermentation conditions and drying process. Mastering the full coffee post-harvest processing chain at industrial scale requires rigourous control of each step, in order to reach consistent in-cup quality, batch after batch, harvest after harvest. The infinite diversity in microbial flora and process conditions paves the way for future innovation.

References:

- Sanz-Uribe, J.R., Yusianto, Menon, S.N., Penuela, A., Oliveros, C., Husson, J., Brando, C., Rodriguez, A. (2017). Postharvest processing – Revealing the green bean. In: The Craft and Science of Coffee, Chapter 3, pp.51-79.

S6-PO-09

Deep-dive into the role of coffee microorganisms on flavor generation during post-harvest processing

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RATIONALE

Coffee post-harvest processing significantly contributes to the sensory quality of the final cup. Microbial community dynamics are strongly dependent on processing type, resulting in altered cup profile. During post-harvesting, the epiphytic microorganisms can make use of the nutrients present at the surface and within coffee cherries and produce metabolites generating flavor impact or acting as flavor precursors before roasting. While the relationship between processing treatments and microbial communities has been gradually elucidated in the recent years, the role of the microflora in flavor generation is still vague. Therefore, this study aimed to deep-dive into the coffee microbial ecology of various processes to define the capability of specific native strains to generate flavors during fermentation.

METHODS

Various post-harvest treatments were applied on Arabica and Robusta coffees. Microbial enumeration highlighted the prevalent microorganisms, which were isolated on-farm. Additionnally, metagenomic analysis of full 16S and ITS amplicons was performed. The isolated strains were characterized and grown in a coffee simulation medium, mimmicking real fermentation conditions. During incubation, flavor formation kinetics was followed by GC-MS to target flavor-potent molecules. Statistical tools were applied to cluster the microbial strains based on their flavor generation behavior and a flavor map of coffee microflora was constructed.

RESULTS

Post-harvest processing conditions significantly impacted microbial ecology and its dynamics. Each process was represented by different microbial communities, resulting in various formation kinetics of flavour molecules along fermentation. The flavor potential of the coffee microorganisms seemed to be dependent not only on their taxonomy, but also their origin. While all strains could produce flavor-potent molecules related to fruity, floral or acidic notes, they differed in cumulative intensities and relative profiles.

CONCLUSIONS & PERSPECTIVES

The results generated in this study elucidated the roles of a subset of coffee microorganisms in flavor generation during various coffee post-harvest processing. This confirmed the potential to impact flavor profiles of the cup according to processing method and terroir. The methodology applied in this study can be used as a standard pipeline for understanding the flavor generation during the coffee post-harvest processing.

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- Sanz-Uribe, J.R., Yusianto, Menon, S.N., Penuela, A., Oliveros, C., Husson, J., Brando, C., Rodriguez, A. (2017). Postharvest processing – Revealing the green bean. In: The Craft and Science of Coffee, Chapter 3, pp.51-79.
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S6-PO-10

Transcriptome and biochemical analyses of diterpene synthases from *Coffea arabica* L.

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RATIONALE

Biochemical coffee bean composition is directly related to coffee cup quality and its healthy and nutraceutical properties. *Ent*-kaurene is a compound used as substrate for gibberellin biosynthesis, but also serves as an intermediate in more specialized diterpenoid metabolism, as exemplified by the more than 1000 known derived natural products. Cafestol (CAF) and kahweol (KAH) are two diterpenes found exclusively in the *Coffea* genus produced mainly in the perisperm during fruit development. In order to depict the diterpenoid biosynthesis in coffee, here we identified and functionally characterized genes involved in the middle step of *ent*-kaurene production.

METHODS

We used bioinformatic tools to identify diTPS candidate genes in a transcriptome data (RNA-seq). Five diTPS were selected for functional validation using protein heterologous expression and GC-MS analyses. We measured diterpene content using HPLC and gene expression profile using RNA-seq and RT-qPCR.

RESULTS

We identified 5 diTPS-related genes using bioinformatics approaches. They were cloned, and had their protein functionally characterized by protein hetereologous expression and GC-MS analyses. Our results showed that two diTPS are the genes responsible for *ent*-kaurene production, a precursor of CAF/KAH biosynthesis.

CONCLUSIONS & PERSPECTIVES

Ent-kaurene is produced by two distinct diterpene synthases in coffee plants. Futher analysis is on going to unravel the genes involved in the final steps of CAF/KAH biosynthesis. In the future, our results will open the possibility to develop plants with desirable content of CAF/KAH and improve beverage quality focusing on human health.

References:

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S6-PO-11

Changes in the sensory and volatile characteristics of coffee quality during storage in modified atmospheres

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RATIONALE

Coffee is one of the world's most widely consumed beverages. The quality of coffee used for beverages is directly related to the chemical composition of the roasted beans, which in turn is mainly affected by the composition of the green beans and postharvest processing conditions (namely, drying, storage, roasting, and grinding). The objective of this work is to evaluate the sensory changes and volatile compounds during the storage of green coffee in modified atmospheres during one year in pack MAP (vacuum and nitrogen) and determine its effect on these quality markers.

METHODOLOGY

Green coffee (*C.arabica*) from Chiapas, Mexico was packedin modified atmospheres, vacuum and nitrogen gas and stored at 18°C (GCV18 and GCN18) and environmental conditions (GCV and GCN) and they were stored for a year. For the identification of volatile compounds was used headspace solid-phase, chromatography–mass spectroscopy (HS-SPME/GC–MS)and the beverage was sensory evaluated by a panel trained in discriminatory and descriptive techniques (2AFC).

RESULTS

For green coffee, 39 volatile compounds were identified, and 60 compounds present in roasted coffee in N2and vacuum atmospheres, it was obtained a specific profile of volatile compounds for green coffee and roasted coffee, where volatile markers were found such as 2-pentanol, 2-furamethanol and benzaldehyde present in the 12 months of the storage in all treatments, that normally in conventional storage as in jute bags disappear or transform into compounds known as Off-flavors. For the sensory analysis the initial sample had a balance between the attributes of aromatic quality, aromatic intensity, body, acidity bitterness and astringency, in month 11 for the treatments (GCN18) and (GCV18) the fermented descriptor was found,with a low note described by the sensory panel.The note known as old was determined by the 2 AFC test, the panelists could not detect the difference between the samples stored for up to 12 months with a reference sample of coffee not stored in the four treatments.

CONCLUSION & PERSPECTIVES

This study provides a framework for future research on coffee storage such as obtaining volatile markers for coffee stored in hermetic sealed systems, while preserving sensory attributes and offering coffee industry an alternative for product preservation.

References:

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S6-PO-12

Quality comparison of Arabusta and Robusta grown in French Guiana

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RATIONALE

Arabusta coffee was created by crossing *Coffea arabica* L. and *C. canephora* Pierre ex-Froehner after doubling the chromosome number of *C. canephora*. For breeding studies and coffee quality improvement, clones have been planted in French Guiana. As green bean metabolite content largely influences the coffee cup quality, biochemical and sensory analyses of Robusta and Arabusta were done on green beans and compared as part of Breedcafs H2020 project.

METHODS

Arabusta cherries were harvested on 6 clones in 4 farms in Apatou and in Combi research station, and those from Robusta in Combi research station. They were wet processed before grinding. Phenolic compounds and alkaloids were quantified by HPLC (Campa et al, 2017), sucrose by enzymatic analysis (Megazyme kit, Ireland), diterpenes by NMR (Portaluri et al, 2020) and VOC by headspace SPME-GC/MS (Marie et al, 2020). Lipids were extracted (Folch et al, 1957) and quantified by cgfb (Bordeaux). Sensory analyses followed the protocol guidelines of the Specialty Coffee Association (SCA) for *C. arabica* (Marie et al, 2020).We used Statistica software for analyses.

RESULTS

Compared to Robusta, Arabusta had a lower caffeine content and higher trigonelline, sucrose and fatty acid contents. Arabusta evidenced both 16-O-methylcafestol and kahweol, known markers for Robusta and Arabica species, respectively. 17 of the 25 non-volatile and 23 of the 47 volatile compounds showed significant differences ($p \leq 0.05$) between Arabusta and Robusta. Except 5-caffeoylquinate and coumaroylquinate, chlorogenic acids accumulated more in Robusta.

A PCA based on volatile compound content separated samples in three groups: Robusta samples, most of the Arabusta from Apatou (richer in pyrazine, toluene, 2-propanone) and those from Combi associated with 3 from Apatou (richer in D-limonene, DMSO, methyl-butanoic acid, and IBMP). A sensory analysis based on 9 sensory parameters indicated that 3 samples received a final score higher than 80. A PCA confirmed that 2 Arabusta samples were particularly different from all the others.

CONCLUSIONS & PERSPECTIVES

With higher contents in sucrose, trigonelline, diterpenes, fatty acids and D-limonene and a lower content in caffeine, the composition of the Arabusta green beans seems significantly different from Robusta. The sensory analysis supports the hypothesis that some Arabusta clones possess the potential to produce quality coffee. However, it is necessary to continue the research to confirm these results.

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References:

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S6-PO-13

Genomics, lipidomics and metabolomics profiling of *Coffea canephora* L. cultivated and conserved in South-western Nigeria

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RATIONALE

Coffee production is an untapped source of revenue for developing countries. The metabolite and lipid diversity among *Coffea canephora* can be harnessed for genomic improvement of high cup quality trait.

METHODS

We examined the genetic diversity among 48 *Coffea* genotypes conserved in the germplasm of Cocoa Research Institute of Nigeria (CRIN), and 30 farmer cultivated genotypes collected from South-Western Nigeria, by analyzing 433048 single nucleotide polymorphisms (SNPs) identified through genotyping-by-sequencing. Gas Chromatography–Mass Spectrophotometer and Ultra-performance liquid chromatography coupled with mass spectrometry (UPLC–MS) used to quantify and profile metabolites and lipid molecules same genotypes of *C. canephora*.

RESULTS

We found three distinct diversity structures within the *C. canephora* genepool that were dominated by a single genetic group determined from passport descriptors to most likely be of Congolese (Democratic Republic of Congo) origin. High uniformity was also found among the farmer-cultivated accessions with 99% of them representing C. canephora var. Niaouli as their ancestral background. Across genotypes, the sucrose-to-caffeine ratio was low, a characteristic indicative of low cup quality. The sucrose-to-caffeine ratio was also highly correlated, indicative of common mechanisms regulating the accumulation of these compounds. Nevertheless, this strong correlative link was broken within the 'Niaouli' group, as caffeine and sucrose content were highly variable among these genotypes. The most abundance lipid was found to be triacylglycerols followed by fatty acyls while the least abundance was sphingomyelins, lyso-phosphatidylcholine and cholesterol ester. The three analyses differentiated Niaouli from the other genotypes

CONCLUSION AND PERSPECTIVE

'Niaouli' genotypes could therefore serve as useful germplasm for starting a Nigerian *C. canephora* quality improvement breeding program. This study revealed the narrow genetic base of the coffee germplasm in Nigeria. There is need to initiate research collaboration between Nigeria and other international and national coffee research institutes in other to access available coffee germplasm for high quality improvement.

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POSTER PRESENTATIONS

Session 5: Sustainability, climate change & labels

S5-PO-01

Local agroforestry knowledge and development of an online decision-support tool (shadetreeadvice.org) for selection of trees to be associated to coffee in North Vietnam

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RATIONALE

In Agroforestry systems (AFS), trees provide multiple services and contribute to 1) improve soil fertility; 2) buffer climate extremes and help adapt to climate change, 3) provide refuge for biodiversity and a micro-climate favourable to biological antagonists to pests and diseases (P&D), 4) provide fodder to improve diet of livestock and 5) diversify on-farm revenues (fruits, timber, fuelwood, fodder, medicinal products, honey...) and reduce exposure to price volatility. Still, trees can also compete water, light and nutrients, or favour some P&D, hence providing ecosystem disservices when farmers use locally inadequate tree species and/or poor agroforestry practices. Therefore, this study was undertaken to document local knowledge on trees and develop a decision-support tool to help select the right tree species adapted to local context.

METHODS

The study was conducted in Son La & Dien Bien Provinces, through interviews of 124 farmers in 12 villages, according to the following steps:

1) Inventory of existing tree species at the farm and landscape levels;
2) Documentation of ecosystem services and disservices that farmers associate with the various tree species;
3) Development of the decision-support tool (shadetreeadvice.org).

RESULTS

47 tree species were inventoried in coffee plots and less than 25 in non-coffee plots, including orchards, annual crops or forest plantations. Thai farmers conserve more tree species in coffee AFS compared to Kinh and H'mong ethnic groups.

Most farmers are aware of obvious ecosystem services such as reducing soil erosion, improving soil fertility, enhancing biodiversity, preventing damages from wind and frost, and providing shade to coffee plants. However, farmers have limited experience or knowledge on impact of trees on P&D, coffee yield and quality.

Farmers select tree species mainly based on economic benefits and market access. Farmers near main roads tend to plant more commercial fruit trees, while farmers far away from roads plant more timber trees.

CONCLUSIONS & PERSPECTIVES

The decision-support tool is available online (shadetreeadvice.org) for the NW coffee producing regions of Vietnam, to help agricultural services and cooperatives selecting the right tree species adapted to local ecological conditions and households' needs and constraints. On top of recommended tree species, the tool needs to be improved to give practical advices on tree planting density and management.

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S5-PO-02

Shaping climate-smart coffee landscapes to unite farm-based climate-smart practices with landscape scale benefits

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RATIONALE
The livelihoods and incomes of coffee farmers around the world are threatened by climate change. Adopting climate-smart practices (CSP) in coffee farming may help to secure incomes and taps synergies between climate adaptation and mitigation while allowing for sustaining farmers' livelihoods. Here, we apply the concept of climate-smart landscapes to coffee regions to explore climate-smart coffee landscapes.
METHODS
Our research aims to characterise climate-smart coffee landscapes, discuss potential benefits across multiple scales and develop a comprehensive framework for implementation. We conducted a review of scientific literature dealing with the topics of climate-smart landscapes, integrated landscape management and climate adaptation and mitigation in coffee farming. Based on our findings we created a framework how climate-smart coffee landscapes can be shaped.
RESULTS
We show that core elements of climate-smart landscapes can be adapted to coffee regions. This includes CSP on-farm, diversity of land uses within the landscape and active management of interactions. We identify various pathways how on-farm CSP can contribute to climate-smartness and can deliver additional benefits at landscape and global scale, including positive impacts on water flows, buffering climate extremes and conserving biodiversity. However, farmers need to see effectiveness of CSP and possess sufficient capacity and knowledge to adopt CSP. We propose a combined framework to combine on-farm climate-risk assessment with analysis of spatial patterns and embed this analysis within processes of integrated landscape management. Furthermore, governance, finance and markets are described as additional catalysts for change.
CONCLUSION AND PERSPECTIVES
Climate-smart coffee landscapes can be one important pathway to adapt coffee farming system to climate change while sustaining or even improving landscape sustainability. Our findings are of relevance to coffee practitioners all along the value chain, scientists, agriculture extension agents, NGOs and policy makers. The framework proposed allows to not only focus on farmers' benefits, but harmonizes adaptation processes with regional trends, advancing towards landscape wide sustainability and sustainable coffee value chains while assuring for farmers livelihoods.

S5-PO-03

A new approach to detecting deforestation in coffee growing regions

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RATIONALE

Many governments, corporations, and non-profit organizations have a strong motivation to protect rainforests and take action against deforestation, particularly if this deforestation occurs as a result of exportable commercial commodities such as coffee or cocoa. However, there have traditionally been significant limitations to measure and detect coffee region deforestation at scale. The main model utilized by major environmental organizations is generated by Global Forest Watch (GFW). This approach suffers from several constraints; inability to differentiate commercial plantations from old growth forest in protected areas, use of low resolution imagery that has difficulty detecting deforestation, and inability to penetrate cloud cover which can obscure 80% of optical satellite images.

METHODS

Recent developments in machine learning and satellite imagery can overcome these limitations. The first step involves verifying the GFW data set in select coffee origins. GFW uses the Hansen Dataset (University of Maryland, UMD dataset). The second step involves building a machine learning model using recent advances in satellite image resolution and also radar satellites for a more accurate deforestation detection model. The third step involves ground truthing the model by going on the ground to assess the degree of accuracy of the computer model predictions.

RESULTS

Such a rigorous assessment of deforestation in the coffee sector has not been carried out before. We will present a robust, methodologically-consistent approach to answer the question “how accurate is the Hansen data set for deforestation detection», as well as presenting the results of the machine learning model to determine if this approach is a more effective tool for the coffee industry. These answers will underpin future research and policy work.

CONCLUSIONS & PERSPECTIVES

We believe that this innovation has the potential to become an important new tool for the coffee sector in its efforts to combat deforestation and mitigate climate change. It can not only underpin future research but also has important policy implications for organizations on the ground. At the same time, we hope that it will open a broader discussion regarding the potential for machine learning to apply new innovations to systemic problems the plague the coffee sector.

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S5-PO-04

Statistical analysis of the weather impact on Robusta coffee yield in Vietnam

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RATIONALE

Weather and climate strongly impact coffee yield (Bunn et al., 2015). A data-driven approach is used here to identify how sensitive Robusta coffee is to weather, during which key moments weather is most influential for yield, and how long before harvest yield could potentially be forecasted. We focused on 19-year coffee yield of the leading Robusta coffee-producing provinces of Vietnam, where 40% of global Robusta is produced.

METHODS

To evaluate the long-term evolution related to weather/climate, we first determined the yield anomalies with respect to a long-term trend describing the management-related long-term changes. A simple linear regression method was then used to model the relationship between the observations of coffee yield anomalies and the weather input anomalies (i.e., derived from ERA-5 Land data (Hersbach et al.,2018)). Due to the scarcity of the available coffee yield data, we not only considered a simple regression model but also used several regularization techniques including principal component analysis and leave-one-out to avoid over-fitting the regression models.

RESULTS

The sensitivity of yield anomalies to weather varied substantially between provinces and even districts. On average, the weather could explain from 16 to 25% of the variation in Robusta coffee yield anomalies in the Central Highlands. The data suggest that Robusta coffee in Vietnam is most sensitive to two key moments: a prolonged rainy season of the previous year favoring vegetative growth, thereby increasing the potential yield, while low rainfall during bean formation decreases yield. These moments could be used to forecast the yield anomaly with3-6 months anticipation, depending on location. By applying our model at different spatial scales, while each scale has its advantages and disadvantages, our model for Robusta coffee in Vietnam shows more interesting results at the district level.

CONCLUSIONS & PERSPECTIVES

Identifying an appropriate statistical model for a relatively complex crop like coffee is not easy, particularly as there are limited observations. This explains why using regularization strategies such as favoring simple models over complex ones and applying good quality assessment diagnostics are required. In particular, we used the leave-one-out method to avoid potential over-training when a limited number of samples is available. In perspectives, we can also test more sophisticated models (e.g., mixed-effects models) that stay simple, combine data from multiple locations but still keep some specificities for each coffee region. This test would require additional coffee yield data to enable applying the model at a global scale more easily.

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S5-PO-05

Coffee cultivation at Mt. Elgon: perspectives and challenges in a changing climate

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RATIONALE

Coffee is crucial for the livelihood of millions of people and plays a central role in the economy of several developing and developed countries. There are warnings about the potential negative effects climate change with increased variability and extreme events have and will have on coffee production. However, adaptation strategies must be tailored to local conditions and resources. Here we present the major findings of three years (2014-2016) of detailed monitoring of coffee trees under smallholder conditions on Mt. Elgon.

METHODS

We worked on 27 smallholder coffee farms along an altitudinal gradient (1100 – 2100 m.a.s.l.) on the western slopes of Mt. Elgon, Uganda, collecting information at several levels:

- Coffee plant: water use, reproductive and vegetative growth;
- Cropping system: vegetation structure (i.e. shade cover, densitiy of plants, plot area) soil characteristics (pH, organic matter, macro nutriens), microclimate and soil water content;
- Farmers interviews and discussions.

RESULTS

Three types of cropping systems were identified: coffee-open, coffee-banana and coffee-shade tree. Coffee under 30 % shade and intercropped with bananas provided the highest yields. Contrary to expectations, altitude had no effect on yield during our study period, although temperature exceeded established thresholds. Poor management practices and nutrient depletion turned out to be main challenges in this area.

CONCLUSIONS & PERSPECTIVES

Coffee intercropped with bananas demonstrated to be the “winner system”. It provides moderate shade and an extra source of income and food. Coffee yields appeared to be more resilient to high temperatures than previously expected, given sufficiently available water. When aiming to increase coffee yields, efforts should be put into improving coffee tree management and tackling soil nutrients issues.

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S5-PO-07

Cost Structure of Specialty Coffee Production in Honduras and El Salvador

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RATIONALE

Specialty coffee has become very important for many coffee-producing countries. In Honduras, specialty coffee exports represent 30% of its exports, and in El Salvador, 50%. Several recent studies have analyzed the cost structure of coffee production (Gomez et al., 2017) but do not evaluate differences across production systems (e.g., specialty versus non-specialty). This study's main objective was to determine the cost structure of specialty coffee production in Honduras and El Salvador.

METHODS

Data was collected using a multi-stage process. First, coffee experts in each country were interviewed to outline typical management plans for coffee production. In the second stage, 20 farmers were interviewed: 14 in Honduras and 6 in El Salvador. The interview instrument focused on coffee production practices, input amounts, costs, yields, and prices received. Separate cost-profitability models were developed for specialty coffees produced in conventional (Honduras and El Salvador) and organic (Honduras) production systems.

RESULTS

In Honduras, the cost of producing conventional specialty coffee (CSC) and organic specialty coffee (OSC) was estimated to be $1,567/ha and $2,360/ha, respectively. In El Salvador, the average cost of producing CSC was $3,027/ha. Most of the expenses in Honduras corresponded to four cost categories: harvesting (53-60%), financing (12-14%), pest management (12-17%), and fertilization (7-135). Most of the costs of producing CSC in El Salvador corresponded to harvesting (27%), fertilization (26%), pest management (22%), and financing (9%). On a per kilogram basis, the cost of producing green CSC and OSC in Honduras was estimated to be $1.04 and $1.81, respectively. In El Salvador, the cost of producing 1-kilogram of green CSC was $3.17. The profitability analysis in Honduras revealed that 9 of the 14 farms obtained positive net income. In El Salvador, 4 of 6 farms had positive net income. Net income calculations did not include establishment, land, and owner management costs. Including these costs would help to evaluate producers' ability to cover all the expenses in the long term.

CONCLUSIONS & PERSPECTIVES

We find large differences in costs and cost structure of specialty coffee across two countries located in the same region. Differences are not only due to inputs costs (e.g., labor costs) but also due to differences in coffee management practices. The cost of specialty coffee production in El Salvador is higher than in Honduras, mainly due to differences in fertilization practices. We also found that conventional specialty coffee is more profitable than organic specialty coffee.

References:

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S5-PO-08

Impact of high atmospheric CO_2 concentration on the seasonality of gas exchange and carbohydrate metabolism in coffee trees under field conditions

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RATIONALE

The effects of climate change on coffee growth and production are particularly concerning given the importance of this commodity. In order to elucidate the mechanisms involved in coffee responses to enriched CO_2 atmosphere, the seasonality of gas exchange and carbohydrate metabolism of coffee were investigated under field conditions, at a Free Air CO_2 Enrichment (FACE) facility for coffee, in Brazil.

METHODS

C. arabica L. cv. Catuaí IAC 144 were grown in a FACE System at Embrapa Environment, under ambient (≅400ppm – CO_{2amb}) and high (≅550ppm – CO_{2high}) atmospheric CO_2 concentration. Seasonal leaf gas exchange and soluble carbohydrates were measured using a portable Infra-Red Gas Analyser and a GC/MS system, respectively.

RESULTS

Coffea arabica trees grown under CO_2 high conditions exhibited increased photosynthetic rates (averaging 121% higher in summer and 45% higher in winter) in both seasons, without displaying any significant changes in the seasonal photosynthesis pattern. Additionally, there was a tendency for the coffee trees grown under CO_2 high to exhibit increased levels of soluble carbohydrates, organic acids and amino acids in the leaves.

CONCLUSIONS & PERSPECTIVES

Our findings suggest that coffee trees adapt to CO_{2high} through increased photosynthetic rates, enhanced stomatal conductance regulation and augmented carbohydrate and organic acid synthesis. It is plausible that these responses could help mitigate the negative effects caused by climate change on coffee growth. These results should be considered when preparing impact assessments and when developing cultivation strategies for the anticipated increase in [CO_2].

References:

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S5-PO-10

Ecophysiological performance of *Coffea arabica* L. under agroforestry system on Gorongosa mountain, Mozambique

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RATIONALE

Management under Agro-Forestry System (AFS) is a growing important tool to fight the impacts of climate changes and global warming, improving microclimate environmental conditions to the plants and, thus, crop sustainability. Additionally, higher altitudes can reduce heat impacts and increase coffee bean quality, associated to slower fruit maturation (1-3). In this context, the *GorongosaCafé* project aims at assessing ecophysiological acclimation of *Coffea arabica* L. plants to different shade densities and altitudes, while addresses environmental issues related with the recovery of natural forest in the Gorongosa mountain, increases small farmers income, and promotes advanced training for local students.

METHODS

The experiments were carried out in the Gorongosa mountain, Mozambique, mostly in the area of Gorongosa National Park, at three shade densities (deep shade, moderate shade and full sun exposure), and three altitudes (650 m, 825 m, 935 m), with *Coffea arabica* L. plants. Ecophysiological evaluations were performed along the year, and included leaf gas exchanges, chlorophyll *a* fluorescence, and photosynthetic pigment content.

RESULTS

Deep shade reduced water loss through transpiration reduction, but reduced as well net photosynthesis (P_n) and some chlorophyll *a* fluorescence parameters. P_n values were higher under moderate shade and full sun expose during (both in dry and rainy periods), which showed as well the highest bean yields irrespective of altitude. The highest leaf photosynthetic pigment content was observed on the deep shade treatment.

Regarding altitude treatments, it was observed that the daily photosynthesis trends were similar among the different altitudes. However the beans yield among them varied significantly, with the highest yield obtained at 825 m and 935 m. Beans quality evaluation is ongoing.

CONCLUSIONS & PERSPECTIVES

The moderate shade condition and full sun expose condition have similar and greater values for leaf gas exchanges (photosynthesis and stomatal conductance) and higher bean yields. Furthermore, coffee bean´s productivity is substantially increased by altitude.

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ABSTRACTS THURSDAY 1 JULY

POSTER PRESENTATIONS

Session 1: Plant science

S1-PO-22

Root Microbiome of Arabica Coffee Plant Grown in Different Geographical Location

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RATIONALE

Coffee is a popular beverage and an important crop having many small producers in developing countries making their living by growing coffee. The microbial community associated with plants plays a crucial role in plant health and nutrient uptake and is also a significant ally for controlling biotic and abiotic stresses. In this study we determined the root microbiome of coffee plants grown in different geographical locations; results have shown the core (observed across all environments and plant genotypes) versus the variable microbiome (either location- or genotype- specific). This data will contribute to the understanding of microbial life associated with coffee roots providing possible avenues in management and to develop a more sustainable approach for the application of fertilizers.

METHODS

Four different arabica coffee growing areas were chosen: Burundi, Ethiopia, Colombia (open fields) and Italy (greenhouse). DNA was extracted from root samples using commercial kits and 16S amplicon libraries were prepared with the Illumina kit. Sequence reads obtained via the Illumina Miseq apparatus were processed and analysed using scripts and programs from DADA2 v1.1.5 and Phyloseq. Sequences were grouped into OTUs (Operational Taxonomic Units) with a 97% of identity and annotated using the RDP reference database.

RESULTS

Approximately 60 root samples were collected from coffee grown in open fields in Colombia, Ethiopia and Burundi, and 24 from greenhouse in Italy. Results show that greenhouse rice bacterial community is highly different from the others; this is not surprising considering the different management respect to the open field cultivation. Colombian and Burundi microbial communities are very similar while Ethiopian one partially overlaps with the others. The genus composition analysis of each sample was performed and the comparison between them shows a high level of uniformity among samples sharing field location or plant variety and/or age. Some microbial genera belonging to the variable microbiome are enriched in plants coming from a particular location while some genera are present in all the samples becoming candidates for the role of “keystone genera”.

CONCLUSIONS & PERSPECTIVES

Understanding the core and accessory microbiomes of the rhizosphere of coffee grown in different geographical areas represents the first step towards the development of microbial products, such as biofertilisers and/or biopesticides, for a more sustainable coffee production.

S1-PO-23

Seed purity of the first two commercial coffee hybrid varieties (*Coffea arabica*) Star2 and Star3 in Ecuador

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RATIONALE

Male sterility is a natural condition that could be used in coffee (*Coffea arabica* L.) to produce commercial planting material from hybrid varieties. In such strategy, male sterile plants are used as female parent in a dedicated seed garden where they are planted together with male fertile plants (pollinators). Throughout this approach it becomes possible to produce true hybrids seeds at low cost and quite high volumes, as compared to traditional vegetative methods. Genetic purity of hybrid seeds is a key aspect to be mastered before the commercialization process. In this poster, we report the results of a wide genetic analysis carried out to seedling issued to hybrid seeds, in order to verify the genetic purity of a first batch of hybrid seeds issued from two F1 varieties propagated in Ecuador.

METHODS

Seeds from two Arabica varieties named Star2 and Star3, developed by Nestlé PSRU (Plant Science Research Unit), were randomly sampled on male sterile parental trees, then they were planted and kept in the nursery until plants presented two to three leaves pairs. Leaves samples were used to extract genomic DNA from 188 plants for each variety. The genetic purity of hybrid seeds was assessed by studying the presence of parental discriminant alleles. Five microsatellite markers (SSR) developed by the PSRU lab, at Tours (France) were used to test the molecular polymorphism. The SSR markers were previously chosen for their specificity, genome distribution (four liaison groups) and variability regarding the parents. The allele segregation was investigated using GeneMapper software.

RESULTS

A total of 188 plants were analyzed using five SSR markers. Five loci were observed having a polymorphism ranging from one to three allele. Most of the hybrid seedlings, 93,6% for Star 2 and 95,2% for Star 3 appeared to be true hybrids with the expected allelic distribution. The frequencies of off-type plants ranged from 4.8% to 6.4%. Different patterns were observed for these off-types, some of them could be explained by the intrinsic heterozygosity of the male sterile parent.

CONCLUSIONS & PERSPECTIVES

This study is the first one to be conducted on coffee using a large number of samples. This huge sampling coupled with an efficient detection method by SSR markers, allows to obtain consistent results. It demonstrated the feasibility to produce commercial hybrid seeds in coffee having enough genetic uniformity to be deployed in the field.

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S1-PO-25

Exploring drought tolerance variation in Ugandan *Coffea canephora*

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RATIONALE

Climate in Uganda is expected to become more erratic, with frequent and severe drought periods. In order to underpin coping strategies, it is imperative to examine the degree of variation in drought tolerance in *Coffea canephora*. Such information also informs coffee breeding for drought tolerance.

METHODS

Plant material was collected from seven Ugandan natural forests and from two research collections. Two experiments were conducted. Expt1 was a screening experiment, run in a rainout shelter in Kawanda, evaluating 148 genotypes (90 wild, 11 feral and 47 domesticated). Rooted stem cuttings were grown for 20 months. Then, two treatments were imposed for four months, *i.e.* ample or restricted water supply. Expt2 investigated in more detail the drought responses of 15 genotypes, grown in a glasshouse in Wageningen, NL (drought treatment applied to 8 to 11 months old plants). Numbers and several properties of organs that were produced during the drought period were measured. The relative growth rate of leaf area (RGRA) was taken as a prime response variable, quantifying drought tolerance.

RESULTS

Restricted water supply reduced leaf variables like the number of leaves per plant, leaf area, leaf dry weight and specific leaf area from 12 to 38 % relative to ample water. Results showed a growth-tolerance trade-off whereby RGRA under ample-water was negatively correlated to tolerance in RGRA under restricted water. So, accessions with the best performance when amply supplied with water suffered most from drought. There was a weak tendency towards higher drought tolerance in accessions from drier climates than from wetter climates.

Expt2 yielded information on drought responses for a host of plant attributes. There was a negative relationship between drought tolerance and trait plasticity. Apparently, accessions with the largest trait responses to variation in water availability (high plasticity) are least able to maintain performance under drought. As in other C3 plant species, drought affected stomatal functioning, reflected in differences in 13C content and water use efficiency.

CONCLUSIONS & PERSPECTIVES

These results imply that breeding climate-resilient material should attempt to weaken the trade-off between drought tolerance and performance under well-watered conditions. One should also consider that plasticity in drought related traits may not necessarily confer drought tolerance. Further progress can be made by linking the current phenotyping data to genetic data, *e.g.* through genome-wide association studies (GWAS).

S1-PO-26

Water-use efficiency of new *Coffea arabica* F1 hybrids undergoing different water availability in an agroforestry system

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RATIONALE

Previous studies have shown that new F1 hybrids of *Coffea arabica* produce higher yield than commercial varieties in both full-sun and agroforestry systems (AFS). However, the physiological and molecular mechanisms controlling their improved performances are still largely unknown. Different water use strategies, such as a trade-off between maintaining photosynthesis while reducing water loss, have been described in drought resistant varieties. We hypothesize that a capacity to better regulate photosynthesis and water loss allow F1 hybrids to recover quicker from the dry season. To test this hypothesis, a field trial submitting these hybrids to different water regimes was set-up in the NOMAFSI station (Son La).

METHODS

As part of the H2020 BREEDCAFS European project, Starmaya, Centroamericano (H1) and Mundo Maya (H16) F1 hybrids, along as Marsellesa and a local Catimor pure lines used as controls, were planted (2018) in AFS with *Leucaena leucocephala* and subjected to three water treatments: rain-fed, water-suppressed and irrigated. The experiment followed a 3x4 design with the three treatments repeated in 4 blocks. Each block included a row of 8 plants by accession. Probes were installed in trunks to constantly monitor sap-flow. Photosynthesis, stomatal conductance, water potential, leaf area and dry matter content were also measured regularly. Plant reproductive and vegetative growth (fruit number, height, trunk diameter, number of nodes…) were periodically monitored since May 2019. Yield and fruit/leaf area ratio were assessed in 2020. Climate conditions and soil moisture were also monitored.

RESULTS

Irrigated plants had significantly higher leaf water potential, conductance and photosynthesis compared to those rain-fed and water-suppressed. For all agronomic traits, the lowest values were observed for control Catimor. For all accessions, sap-flow, photosynthesis, and conductance dropped down during the dry season in a similar way. However, daily sap-flow was higher for Catimor during the dry season but lower during the rainy season compared to other accessions. On the other hand, Marsellesa and H1 hybrid had a lower sap-flow during the dry season and higher sap-flow during the rainy season.

CONCLUSIONS & PERSPECTIVES

Differences in water use efficiency were observed among the tested accessions. However, no significant differences were observed when comparing photosynthesis and carbon allocation data. Larger differences among treatments and accessions are expected in the coming years. This should give us more insights about coffee F1-hybrids responses to water-stress and prolonged dry season.

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S1-PO-27

Genomic characterization of 10 Vietnamese elite clones of Robusta (*Coffea canephora*)

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RATIONALE

As a consequence of climate change, Vietnam, the world's largest Robusta producer, is facing the risk of losing 50% suitable area for growing Robusta by 2050 (Bunn et al., 2015). To deal with the ongoing challenge, it is therefore important to understand the genetic makeup and diversity of *Coffea canephora* clones cultivated in Vietnam. As a preliminary work, the genetic diversity of 10 clones considered as elites (with high productivity and pest resistance over the years) was assessed using two sets of genetic markers, SSRs and SNPs.

METHODS

Leaves of the 10 clones were collected in Robusta germplasm bank of WASI (Buon Ma Thuot, Dak Lak province) and used to extract DNA for further population genetics analyses. A collection of 233 African wild accessions of *C. canephora* covering the eight genetic diversity groups previously identified (Mérot-L'Anthoene et al., 2019) were included in the analysis as genetic references. We performed Principal Component analysis (PCA), sparse nonnegative matrix factorization (sNMF) (Frichot et al., 2014), neighbor-joining (NJ) tree construction, and population genetics statistics on genotypic datasets of 19 microsatellites (SSRs) markers (moccadb.ird.fr) and 1.3M biallelic single nucleotide polymorphism (SNPs) detected on resequencing data.

RESULTS

The PCA results of both SSR and SNP data presented a close genetic relationship between all the 10 Vietnamese Robusta clones with the accessions originating from the Democratic Republic of the Congo (DRC), corresponding to groups E and R (Mérot-L'Anthoene et al., 2019). Indeed, sNMF results showed high membership probability of the Vietnamese clones with groups E and R (higher than 90%), except for one variety representing approximately 25% introgression of group A and G (Cameroon-Gabon and Angola groups, respectively). The results were also confirmed by the NJ trees as well as the low differentiation coefficient between the Vietnamese clones and the group of accessions from DRC (-0.0048 and 0.0028 in SSR data and SNP data, respectively).

CONCLUSIONS & PERSPECTIVES

The genomic characterization of the 10 elite clones in Vietnam showed the presence of clones belonging to the E and R groups of Robusta diversity, while one presented a different percentage of introgression with A and G groups. These results now open the way to perform an in depth characterization of the genetic diversity of Robusta plants presented in Vietnam by checking the whole collection available in the germplasm bank of WASI. Such an approach should contribute to the selection of elite parental genotypes necessary to further launch new Robusta breeding programs.

References:

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S1-PO-28

WCSdb: A database of wild *Coffea* species

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RATIONALE

Two coffee species are mainly cultivated: Arabica and Robusta. Beside these species, the 139 wild coffee species/taxa belong to the *Coffea* genus are largely unknown to coffee scientists although these species may be crucial for future coffee crop development to face climate changes. These wild coffee species conserved in living collection revealed large morphological variations, but also growth habitats and adaptation. In addition to morphology, large variations were observed in terms of seed biochemical compounds involved in the quality of coffee such as caffeine, trigonelline, sucrose and mangiferin contents into others. However, this diversity was reported so far in any publicly available database.

METHODS

A database has been built using Pl@ntNet Publish. It is an IT platform dedicated to the dissemination of botanical data focused on taxa or specimen levels. It is based on Symfony (PHP) and MongoDB and allows users to manage data publication spaces.

RESULTS

In this study, we developed the Wild Coffee Species (WCS) database: http://publish.plantnet-project.org/project/wildcofdb_en. It presents: (i) each species held in collection on the sites of La Reunion island and Kianjavato (Madagascar) with a photo gallery (597 images); (ii) different detailed information such as synonymy, natural distributions, habitats, architectural, morphological, phenological, biochemistry, genetic/genomic data (chloroplast genomes, whole genome sequencing and GBS), trait of interest retrieved from the literature and personal observations on living collection; (iii) a general geographical map of the species distribution.

CONCLUSIONS & PERSPECTIVES

The WCS database represents the first comprehensive information about wild coffees species, to help researchers working in the preservation of coffee species, geneticists and breeders working with trait or genes of interest and improvement of cultivated species or breeders motivated to re-cultivate forgotten species adapted to climate changes or adapted to specific habitats.

References:

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S1-PO-29

Genetic variability and genetic structure of Thai Arabica coffee hybrids (*Coffea arabica* L.) based on SSR markers and a model-based genetic clustering method

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RATIONALE

Arabica coffee (*Coffea arabica* L.) plays an increasingly significant contribution to coffee industry in Thailand. Currently, the main important issues are the breakdown of rust resistance in improved coffee varieties due to the appearance of more virulent rust races and, the increase in population sizes in the country. This study aimed to investigate the genetic variability and genetic structure of Thai Arabica coffee hybrids (*C. arabica*) based on SSR markers and a Model-based genetic clustering method [1,2].

METHODS

The 70 coffee hybrid accessions together with 24 derived-spontaneous accessions maintained at the Royal Agricultural Research Center, Chiangmai, Thailand were assessed genetic variability and structure using 21 simple sequence repeat (SSR) markers and explored with the distance-based clustering and the structure-based methods.

RESULTS

All selected markers showed polymorphism with totally 100 alleles. The average number of alleles per locus was 4.7 and the average polymorphism information content (PIC) was 0.73, showing the same range with those previously reports in the literature. The average genetic similarity based on Jaccard's similarity coefficients was 0.60 and ranged from 0.20 -1.0. The analysis of PCoA plot and UPGMA tree showed three main clusters. The result revealed that the genetic clustering of the varieties or accessions was not correlated with their original varietal classifications. Therefore, the model-based clustering was applied to confirm the distance-based clustering and to more deeply understand the genetic variability and populations sub-structure within populations. The model-based analysis inferred three main genetic structures groups (K = 3) as the most suitable cluster and six sub-populations (K = 6) which provides a strong evidence of population substructure in *C. arabica* hybrids in Thailand.

CONCLUSIONS & PERSPECTIVES

Based on these findings, it is possible to minimize duplication and assist in the establishment of core collections that are representative of the full range of genetic variability. This information will provide coffee breeders with more efficient strategies for exploiting available germplasm resources in Thailand as well as informative data for varietal registration and identification.

References:

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S1-PO-30

Tailoring the creation of next generation of coffee varieties in Rwanda

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RATIONALE

Significant evidences report current coffee varieties will not tolerate the environmental threats of the 21st century, changing climate, disease and insects. This creates a disastrous decline in supply in the future. The best hope for sustaining the supply of high-quality coffee in the years to come is to focus on making the coffee plant more resilient. The creation of new varieties, supported by a vibrant seed sector, will result in major global productivity and quality gains.

METHODS

National performance trials involving 40 f1 hybrids and 28 new fixed varieties were evaluated for 3. The hybrids were developed by WCR and RAB. Materials were evaluated for growth and quality characteristics in three different locations. Statistical analysis consisted of AMMI, AMMI stability value (ASV) path analysis, and combining abilities (GCA and SCA).

RESULTS

From the AMMI analysis and ASV 10 best performers recorded a cherry yield per tree higher than 5 kg/tree and the overall quality scores above 85%. Varieties are Paraneima, IPR103, IPR 107, Geisha, RABC 15, H1, Batian, Oro Azteca, S4808, and EC 15. Tall hybrids included Geisha La Luisa X16691, Geisha HERBAZU X16691, Jackson X6A, Harrar X5A, Geisha La Luisa x 4877, BM139 X5A, and dwarf hybrids, Harrar x Ruiru 11, Obata x Geisha HERBAZU, Iapar 59 x 4550, Iapar 59 x4863, Iapar 59 x 4873[1].

24 months old varieties and hybrids already recorded cherry yield per tree higher than the national average (Ngango et al. 2019).

The path analysis revealed a correlation between stem diameter, number of nodes per branch, bean size, the weight of 100 beans, and overall cherry yield for both hybrids and varietal trials, and, hence the direct and indirect effects of cause variables on effect variables (Bondari 1990).

The GCA effects of parents and SCA effects of crosses were significant ($P < 0.01$) for stem diameter, number of nodes per branch, number of cherries per tree, bean size, the weight of 100 beans, cherry yield per tree, rust, and coffee berry disease scores. This indicates improvement programs should be directed towards the selection of superior parents (Fasahat et al. 2016). Crosses exhibiting high SCA effects would produce desirable transgressive segregants in advanced generations (Reyes 2019).

CONCLUSIONS & PERSPECTIVES

Performance trials exhibited good candidates for direct release for both F1 hybrids and fixed varieties. However, for hybrids, it will be necessary to put in place a sustainable strategy for mass multiplication of planting materials either through clonal propagation or exploitation of male sterility. Hybrids with positive SCA are valuable genetic resources for further breeding investigations.

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POSTER PRESENTATIONS

Session 8: Coffee chemistry & sensory sciences + others

S8-PO-09

Optimization of espresso coffee extraction to lower the amount of coffee

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RATIONALE

The espresso coffee (EC) quality is driven by several variables related to water, roasting profile, particle sizes and barista skills. Previous research has demonstrated that the sizes of coffee particles greatly affect on extraction kinetics. In fact, some studies highlighted that bigger particles could ease the percolation during the brewing process (Kuhn et al. 2017). However, the fine particles generate intensity of taste and can clog the filter baskets (Khamitova et al. 2020a). In this context, researchers have not studied yet in depth how different tools can be adjusted complementarily in coffee extraction, as how different filter baskets and perforated disc heights could be chosen according to the different particle sizes of ground coffee.

METHODS

The present project was based on the study and comparison of ECs prepared by decreasing the amount (from 14 to 12 g for double EC extraction) of specific particle sizes (from 200 to 1000 µm) of ground coffee in three variously designed filter baskets. The second part of the work was to investigate various heights of perforated disc under the shower (4-7 mm), and to prepare coffee with 14 and 12 g of ground coffee. The perforated disc is a perforated metal plate, assembled under the shower of each serving group of EC machine, which assures a homogeneous water diffusion over the coffee cake surface and adjusts the distance between the coffee cake and the shower. Thus, perforated disc can influence EC extraction, even if no studies have been reported yet; in fact, this parameter has been investigated for the first time by our research group (Khamitova et al. 2020b). ECs were analysed for the content of TDS, bioactive compounds, and organic acids with HPLC-VWD, while volatiles with HS-SPME-GC-MS.

RESULTS

Extracting with smaller particles escalates the quantity of bioactive compounds. The amount of caffeine per cup increased moving from 500–1000 µm to 200–300 µm particle size, both in Arabica and Robusta for all filter baskets. Using lower amount of ground coffee permitted to obtain the same extraction yield increasing the height of perforated disc. Keeping constant the volume of EC at various heights of perforated disc, the amount of bioactive compounds at 12 g were only around 9% lower than at 14 g.

CONCLUSIONS & PERSPECTIVES

The right implementation on EC machine of these tools, simple and feasible as they are, could lead to a more sustainable consumption of the beverage by reducing the amount of used R&G coffee and by producing lower spent coffee ground, while maintaining the same cup quality.

References:

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S8-PO-10

A metabolomics approach to discriminate which compounds contribute to the sensory characters of coffee brew

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RATIONALE Coffee is one of the most commonly consumed beverages in the world and its pleasant flavors appeal to many people. However, it is still unclear which compounds are the main contributors to the unique sensory characteristics of coffee. A gas chromatography-mass spectrometry (GC-MS)-based metabolomics approach, combined with sensory evaluation can be applied to identify markers for food quality1. In this study, we employed GC-MS metabolomics to discriminate which compounds contribute to the sensory characters of coffee brew.

MATERIALS & METHODS

A total of 28 samples, including five Arabica and two Robusta commodity coffees, were roasted to differing degrees. Each sample was then ground and brewed using a French press. Derivatization of the coffee brew and GC-MS analysis were conducted as previously reported2. The coffee brews were also evaluated by expert 'cuppers' for 12 sensory attributes. Each attribute was evaluated using a 10-point scale. A one-way analysis of variance (ANOVA) was applied to the sensory scores of the 28 coffee samples. The sensory evaluation and GC-MS datasets were subjected to multivariate analysis, principal component analysis (PCA) and partial least square regression (PLS-R).

RESULT

The ANOVA results revealed that the sensory scores of the 28 coffee samples were significantly different between samples and between all sensory attributes, except for "roasted flavor". The GC-MS analysis performed on the hydrophilic compounds in coffee brew tentatively identified 92 peaks by comparison with our in-house library and the NIST library. The PCA score plot derived from the coffee samples differentiated groups mainly on the basis of roasting degree and species (Arabica or Robusta) in both the GC-MS and the sensory evaluation results. Sensory predictive models of PLS-R were developed from the relationships between the sensory scores and the hydrophilic compound profiles. This model allowed us to predict the sensory scores of coffee brews. As a result, some compounds were identified as contributors to sensory attributes such as sweet flavor and acidic taste, which are required for high-quality Arabica coffees.

CONCLUSIONS & PERSPECTIVES

We used GC-MS analysis and sensory evaluation to differentiate the compounds that contribute to sensory attributes. Specifically, we found that organic acids were correlated with some of the desired sensory attributes of Arabica coffee brew. In the future, we will estimate the effects of these potential marker compounds on the production processes of coffee (e.g., post-harvest processing, roasting and brewing) to produce a high-quality coffee.

References:

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S8-PO-11

A simple predictive model for the espresso coffee Extraction Yield

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RATIONALE

The flavour of coffee is determined by the physico-chemical characteristics of the coffee powder and the settings of the extraction process. This is a very complex phenomenon whose formulation generally gives complicated models. The simplification of these computational tools for the evaluation of simple features, like the Extraction Yield (EY), is an important step for the coffee industry, providing a concrete possibility to predict the result of the preparation phase. We consider a simple simulation tool for the prediction of the espresso coffee EY.

METHODS

The computation of EY is based on a simplified mathematical model that describes the espresso extraction. This model considers the main features of the water percolation in coffee powder, i.e. the transport and diffusion of chemical substances by the water flow, and the diffusion process of the substances through the grains in the solid phase, see (Moroney, K. M. et al., *PLoS One*, 2019; Cameron, M. I. et al., *Matter*, 2020) for details. A simulation tool based on a finite difference approximation of such model, allows a fast computation of EY from the knowledge of the extraction parameters and the physical-chemical characteristics of the coffee pod. The accuracy of the model is tested by a comparison with EY calculation resulting from Total Dissolved Solids measurements, taken by laboratory experiments.

RESULTS

Several extractions and the relative EY values have been collected under different conditions of water pressure and temperature, as well as different granulometries and coffee types. The complete scenario of such experimental results will be shown. Also, the comparison between the experimental and numerical EY values will be discussed. The model is able to reproduce the EY variation given by different granulometries.

CONCLUSIONS & PERSPECTIVES

These results have a significant impact in relevant issues of coffee industry. In fact, the possibility of having these simple models allows the creation of tools for the EY prediction and control. In the future, such tools could be integrated into professional machines in order to increase the control of the extraction process. Moreover, having traced the EY, it is possible to study how to optimise the extraction while reducing the coffee powder used, in order to satisfy the sustainability goal of coffee industry.

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S8-PO-13

The role of chemometrics in the characterization of coffee quality

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RATIONALE

Coffee analysis is a fundamental step to guarantee its quality, safety and traceability and to comply with legal/regulatory standards and consumers' demand. The ever-increasing requirement of analytical controls, in the "omics" era, has made chemometrics an indispensable tool to manage the huge amount of data and extract relevant information to meet the demand to characterize industrial products.

METHODS

Analytical platforms on-line combining high concentration capability sampling techniques with either separative GC-MS (HS-SPME-GC-MS) or non-separative (HS-SPME-MS) techniques together with suitable chemometrics (PCA, PLS, PLS-DA, SIMCA) are here used to correlate aroma chemical profile and/or fingerprints with sensory properties, for coffee traceability and as a tool to monitor roasting process.

RESULTS

Prediction models of the coffee sensory notes based on analytical measurements have been studied for quality control. The chemical signatures enable to define sensory quality of in-cup coffee, although with some compromises. The most reliable models are those obtained for bitter, acid, spicy and woody properties, whose volatile fingerprint is representative of the sensory note. Chemometrics also provide tools to monitor the coffee roasting through correlation of chemical fingerprints with beans color, and/or profiling of aroma indices such as the 5-methylfurfural/2-acetylfuran ratio. Classification of Arabica mono-origin from blend of different origins through SIMCA algorithms affords to identify several volatiles characteristic of their origins.

CONCLUSIONS & PERSPECTIVES

Chemometrics with its discriminative, informative and predictive role can be a bridge between the complexity of coffee aroma and industrial requirements giving answers to challenge topics, and confirming and reinforcing relevance and significance of studying the relationships between coffee volatiles, aroma and quality in view of coffee sustainability.

References:

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S8-PO-14

Developing a milk coffee flavor wheel for Japanese consumers

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RATIONALE

Coffee is consumed in various styles, such as black or with milk and/or sugar. The flavor wheels of black coffee, for example by Hayakawa et al. 2010 and Spencer et al. 2016, are useful to describe the flavor characteristics of black coffee, but not those of milk coffee. The purpose of this study is to develop a milk coffee flavor wheel for Japanese consumers.

METHODS

Sixty milk coffee samples were prepared with combinations of the following 5 parameters: coffee beans (Brazil No. 2, Colombia Supremo, Ethiopia Sidamo G4, Indonesia Mandheling G1, Vietnam Robusta G1), roasting degree (L value = 18 or 23), sugar (with or without), composition (milk rich type or coffee rich type), and milk fat (full fat or low fat). Samples were presented to 203 consumer panelists and terms were collected by freely describing the flavor characteristics. Collected terms were selected using the following 2 steps: 1. Quantitative screening: terms used less than 1% were excluded, and 2. Qualitative screening: 12 experts engaged in the development of milk coffee beverages excluded terms thought to be unnecessary in describing the flavor characteristics of milk coffee. Check-all-that-apply (CATA) questions using the selected terms were applied to the analysis of 6 milk coffee samples by 74 consumer panelists. Cochran's Q test was performed using XLSTAT 2019 (Mindware Inc., Japan).

RESULTS

A total of 456 terms were initially collected. Quantitative and qualitative screening resulted in the selection of 53 terms. As a result of the CATA questions, all 53 terms were used at least once. For 42 terms, there was a significant difference between samples in the frequency of use (Cochran's Q test).

CONCLUSIONS & PERSPECTIVES

A flavor wheel of milk coffee consisting of 53 terms was developed. The results of the CATA questions showed that the flavor wheel contains adequate and appropriate terms for consumers to describe the flavor characteristics of milk coffee. Further study of correlations between terms is required to optimize the position of terms on the flavor wheel.

References:

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PLENARY SESSION - KEYNOTE LECTURERS

Session 7: Roasted coffee technology & processing

Session 9: Health & safety, consumption, quality & trends

S7-KN

Mass spectrometry based profiling of coffee chlorogenic acids

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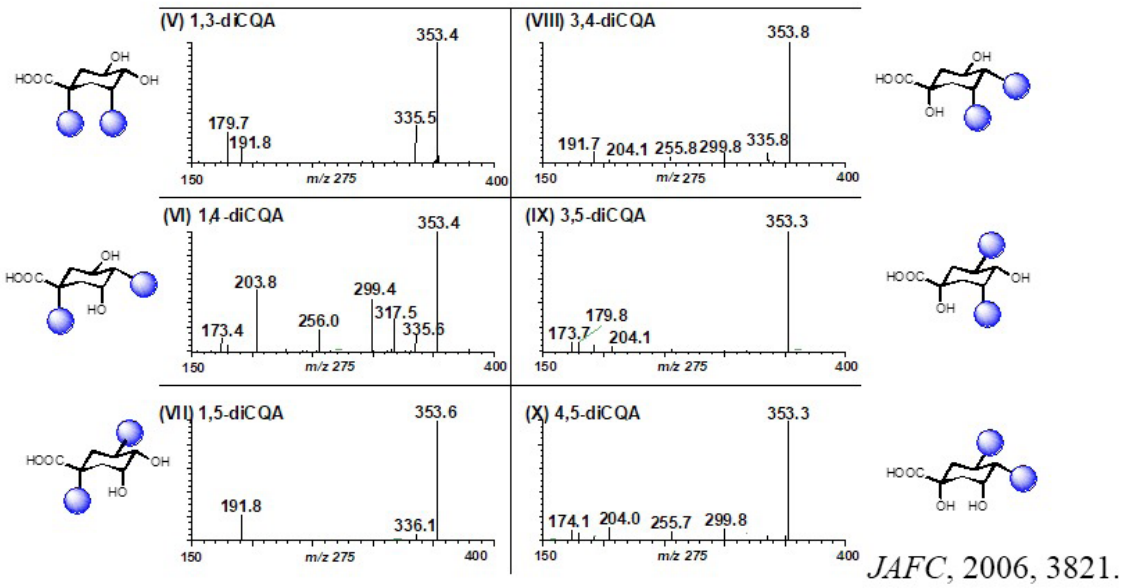
For the average consumer coffee is the most abundant source of chlorogenic acids (CGAs) providing an estimated 200 mg of CGAs per cup, possessing a multitude of beneficial effects for human health. Around 45 distinct CGA derivatives have been shown to be present in an Arabica green coffee bean and 80 in a Robusta green coffee bean. Upon roasting and brewing the number of distinct CGA derivatives increases to around 250 with green bean CGAs undergoing a series of chemical transformations including acyl migration, epimeriszation, trans-cis isomerization, dehydration and hydration.
We have developped over the last two decades a series of mass spectrometry (MS) based to techniques to unambigously assign CGA structures using high resolution MS, tandem-MS, energy resolved MS and ion mobility MS, which we review in this contribution. MS allows furthermore the possibility to quantify and profile complex CGA patterns allowing comparison between coffees of different botanical varieties, origins or produced by different agricultiral practices.
Finally we report on some selected biological activities of CGAs, in times of COVID-19, in particular on antiviral effe ts of coffee CGA derivatives.



Structures and tandem mass spectra of isomeric diacaffeoyl quinic acid isomers.

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S9-KN

A cup of coffee for a healthier aging

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Coffee is the most consumed beverage worldwide. Although rich in caffeine, it has over 1000 other constituents. Coffee is mostly consumed to counteract tiredness, increasing concentration and reaction time, which prompts the perception that coffee is as an excitatory beverage. Accordingly, health professionals often recommend patients to avoid coffee. Paradoxically, this perception is largely rebutted by scientific evidence, showing a global trend for an inverse association between coffee intake and the incidence of numerous diseases.
In particular, different studies following cohorts in different continents (Europe, Asia, America), converged to the conclusion that the regular intake of moderate doses of coffee is associated with increased longevity and particularly, increased healthspan on ageing. This was observed equally in men and women, in different ethnicities, in individuals with different polymorphic traits, and for the consumption of different types of coffee; the maximal beneficial effects of coffee on human health seem to occur with circa 2-4 cups of coffee a day and in individuals older than 50 years.
The healthier ageing associated with coffee consumption likely resulted from the ability of regular coffee consumption to attenuate the incidence of most age-associated chronic diseases; in fact, epidemiological studies show that the daily consumption of 2-4 cups of coffee attenuates the incidence of brain and cardiovascular diseases, chronic kidney diseases, diabetes, obesity, liver diseases and different types of solid cancers. However, the underlying mechanisms are not clear, since several coffee components, such as caffeine or chlorogenic acids to name a few, can target different pathways controlling physio-pathological features associated with chronic age-related diseases and it remains to be defined if coffee and some of its components may actually function as senolytics or senomorphics.
In conclusion, irrespective of some particular counter-indications and the current lack of knowledge on the mechanisms by which coffee affects human health, it is warranted that health professionals should evaluate the available scientific evidence before recommending the abstinence to coffee - a life-style habit that actually seems beneficial for patients, especially for the elderly.

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ORAL PARALLEL SESSIONS

Session 3: Farm management

Session 5: Sustainability, climate change & labels

S5-O-05

Patterns of Global Collaboration in Research and Innovation in Coffee Genetic Diversity and Breeding for Enhanced Sustainability

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RATIONALE

Research and innovation in coffee genetics plays a crucial role for the sustainability of the coffee global sector in a context of climate change. Effective management and improvement of coffee genetic resources (CGR) relies on the exchange of resources such as genetic material, data or knowledge between different countries and across continents. It often involves global collaboration among a range of diverse actors interested in CGR but with different capacities, aspirations and motivations. Considering that access and sharing of research inputs is central for well-functioning and sustained collaboration, this paper develops a framework that considers the interplay between regulations, organizations, collaborative relationships (projects) and resources as determinants of access and sharing of research inputs, which in turn affects the research processes, sustainability and science and innovation outcomes. Our expectation is that global collaborative networks actively address each of these characteristics in ways that help attain multiple goals.

METHODS

This paper draws from an empirical network analysis of an online survey conducted in December 2018 on a sample of 458 individuals involved worldwide in research activities related to coffee genetic resources and breeding as well as a case study analysis on how World Coffee Research sponsored projects govern the access, exchange and collaborative use of coffee genetic resources (CGR) for international coffee research in different research settings.

RESULTS

Social network analysis results are presented according the following three characteristics (Bodin & Crona, 2009): i) level of network cohesion; ii) groups connectedness; iii) network centralization. Findings reveal: i) a well-connected network around a giant component, which favors the pooling of resources and collective action strategies; ii) strong interdependences between producing and non-producing countries; iii) a limited number of organizations spread in the four continents have a central position in the network. A comparison of the profile of these various central organizations along with the WCR case study reveals different strategies undertaken to enhance global research collaborations.

CONCLUSIONS & PERSPECTIVES

Results from the survey and case study help characterizing collaboration patterns in coffee genetic diversity and breeding and identifying obstacles and opportunities for enhanced collaboration towards more global coffee sustainability.

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S5-O-07

Elevated [CO_2] buffers the effects of severe soil drought cycles on water status, photosynthesis and growth of *Coffea arabica*

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RATIONALE

Plant growth and development are frequently challenged by abiotic stresses and the increasing atmospheric CO_2 concentration ([CO_2]) contributes to climate change, including severe drought events. The elevated [CO_2] (e[CO_2]) has the potential to mitigate the negative effects of moderate water deficit (WD) in coffee plants, a worldwide important commodity. However, whether e[CO_2] reduce the effect of severe drought cycles in this species is not clear.

METHODS

C. arabica plants acclimated with ambient [CO_2] (a[CO_2] – 400 ppm) or e[CO_2] (800 ppm) for 60 days were exposed to the following water regimes: well-watered (WW – daily watered), water-deficit cycle 1 (WD1 – water withdrawal for 32 consecutive days) and water-deficit cycle 2 (WD2 – WD1 were irrigated during one week and subjected to a second cycle of water withdrawal for 20 consecutive days). Physiological measurements were performed at day 0 and in the last day of each water deficit cycle.

RESULTS

Before WD onset, a[CO_2] and e[CO_2] plants displayed similar physiological responses, except for the CO_2 assimilation which was higher in e[CO_2] plants. During the WD1, soil moisture drastically decreased, while the whole plant transpiration (WPT) reduced gradually from day 16 after WD1 onset reaching minimal values at day 32 compared to WW plants, regardless [CO_2]. In this sampling point, plant tissue humidity, relative water content (RWC) and photochemical efficiency decreased equally in both [CO_2] treatments under WD1 regarding WW plants. Additionally, shoot biomass was higher in e[CO_2] plants irrespective of water condition. After one week of rehydration, the photochemical parameters of WD2 plants recovered close to the WW plants. At the end of the WD2, soil moisture and WPT reached the same values found on day 32 of the WD1. In this second sampling point, leaf water potential and RWC strongly reduced in WD2 plants regardless [CO_2] in comparison to WW plants. The WD2 decreased the other physiological parameters to lower levels found in the WD1, but to lesser extent in e[CO_2] plants (especially photosynthesis, leaf humidity and shoot biomass), regarding a[CO_2] plants. Besides, WD2 increased leaf membrane damage mainly in a[CO_2] than e[CO_2] plants related to WW plants.

CONCLUSIONS & PERSPECTIVES

These results suggest that e[CO_2] is promising to buffer the negative effects of severe drought cycles and somehow induce plant stress tolerance in *C. arabica*. More studies involving biochemical and molecular biology approaches are needed to better understand the mechanisms underlying this process aiming to improve crop productivity in the future climate change conditions.

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S3-O-02

Effects of water stress on Arabica coffee production

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RATIONALE
Arabica coffee (*Coffea arabica*) is a highly valuable global commodity that is threatened by shifts in temperature and precipitation brought on by climate change. Coffee production is acutely sensitive to the quantity and timing of precipitation, so the increasingly volatile precipitation patterns that are predicted may become a major challenge. Therefore, the development and adoption of farm-level solutions for coffee producers is key. Our research uses an experimental approach to understand the role that cultivar selection could play as a tool for coffee farmers adapting to changing precipitation regimes.
METHODS
To evaluate how reduced rainfall affects the performance of multiple coffee cultivars in the field, we implemented a common garden experiment in Tarrazú, Costa Rica with transplants of five coffee cultivars (Milenio [H10], Centroamericano [H1], Catuai, Catuai 44, and Villa Sarchi). We used a randomized block design with ambient (control) and reduced rainfall (via rainout shelters causing an average of 14% reduction in soil moisture) treatments over the first two productive harvest seasons. At the first harvest, coffee fruit was harvested and weighed from each plant. Aboveground biomass was collected, dried, and weighed from a subset of the coffee plants. At the second harvest, the coffee fruit was again harvested and weighed.
RESULTS
Seedlings under the rainout treatment at the first harvest had over 200% greater total fruit weight and over 50% more biomass production than under control, potentially due to protection from the unusually high rainfall during this period of our experiment. This effect varied by cultivar where the F1 hybrids H10 and H1 had greater fruit production and biomass under both treatment and control conditions than the other cultivars. At the second harvest, following a year of more typical rainfall, the plants under rainout produced 66% more fruit by weight than under control. H10 produced the most fruit, consistent with our findings from year one.
CONCLUSIONS & PERSPECTIVES
While previous research has focused on increased drought stress under climate change, our results suggest that stress imposed by excess rainfall could similarly impair coffee production. However, cultivar selection could be a highly important tool for maintaining the viability of coffee production under such climate change effects. Specifically, the performance of the F1 hybrids in our experiment suggests that they may show more tolerance to projected variable precipitation conditions. Our results will be complemented by ongoing chemical and sensory analysis that will reveal the extent to which water stress impacts quality in addition to yield.

S5-O-08

Young shade trees buffer extreme climatic events and maintain high coffee yield and quality under their canopies in Yunnan, China

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RATIONALE

Local governments in southern Yunnan Province, China, started distributing free shade tree seedlings to coffee farmers in 2012. This prompted a large-scale conversion from intensive monoculture coffee systems towards coffee-agroforestry systems. In this study, we investigated the impacts of some commonly used shade tree species on microclimate and coffee yield and quality shortly after their introduction in coffee fields.

METHODS

We selected 3 commonly found shade tree species: *Jacaranda mimosifolia* (deciduous, light-moderate shade), *Bischofia javanica* (deciduous, moderate shade), and *Cinnamomum camphora* (evergreen, dense shade). We marked 90 coffee trees below (treatment) and around (control) the shade tree canopies, and recorded their fruit development throughout a whole coffee cycle, from flowering to harvest, all the way to cup quality testing. In parallel, we recorded the impact of shade trees on microclimate using temperature loggers.

RESULTS

The maximum potential coffee yield, indicated by flower set at the start of the growing season, decreased with shade intensity. However, fruit losses during the bean filling and maturation stages were higher in open conditions than under shade. Furthermore, shade trees buffered extreme temperatures and protected coffee trees from frost damages in December 2017 (+0.5 - 1˚C). Overall, coffee yield and quality under shade trees with moderate shade intensity (*J. mimosifolia* and *B. Javanica*, LAI<3) were similar to that of coffee trees in open conditions. Only *C. camphora* (dense shade, LAI=6) negatively impacted coffee yield.

CONCLUSIONS & PERSPECTIVES

If carefully selected and managed to enhance complementarity with coffee, young shade trees can rapidly provide benefits similar to those expected from older trees, here only 4 years within the transition towards agroforestry. In the case of southern Yunnan Province, the positive tradeoffs from young shade trees (positive externalities related to microclimate and coffee physiology and absence of negative impacts on coffee yield and quality) partly explained the success of local government programs to promote shade trees and the surge of agroforestry systems.

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S3-O-03

Application times and glyphosate residue on coffee beans

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RATIONALE

The coffee beans have a peak of nutrient absorption during the grain filling phase and the beginning of maturation, a period that varies from 153 to 175 days after flowering, being a phase of high capacity for foliar absorption, the herbicide absorption, with consequent transport and storage of this in coffee beans, may occur during this period, see Martinez et al. 2014, see Lima Filho and Malavolta 2003.

The objective of this work was to evaluate the residues of the herbicide glyphosate, in coffee samples, at different times of application.

METHODS

The work was carried out in a variety of Catucaí Amarelo 24/137, with a spacing of 3.2m x 0.7m, aged 4 years. The application of Glyphosate was made in the range of the coffee trail, with dosage of 1.200 gr ha-1 (active ingredient) , with a width of approximately 70 cm for each side, without protection for the coffee trees bottom part, as usually applied by producers, with a manual backpack sprayer, with a flow of 200 L ha-1 of syrup being carried out on the dates corresponding to the treatments.

5 applications were made on 5 different dates, with intervals of 15 days between them, with an experimental design with 4 blocks for each treatment, totaling 24 plots, with 10 plants each. The applications were made one before the absorption peak (140 days after flowering), two during the absorption peak (154 and 168 days after flowering) and two after the absorption peak (182 and 196 days after flowering).

The sample was prepared in type 3 standard, with a maximum of 12 defects according to the COB table, sieves 15 and above (maximum 10% sieve leakage 14), fine drink, good aspect, homogeneous dryness and maximum 11% moisture content.

Subsequently, the samples were sent to the Bioagri laboratory (Meuriex), which performed the specific analysis of glyphosate residues, within the European standard.

RESULTS

The study shows that there is a high risk of contamination by glyphosate residue mg kg-1 between 51 and 147.3 days before harvest, an average risk of contamination from 147.3 to 169 days before harvest and a low risk of greater contamination than 169 days before harvest.

CONCLUSIONS & PERSPECTIVES

It can be concluded that Glyphosate applied without protection for the coffee trees. The closer to harvest time, the more glyphosate residue will be in the grain. The ideal is to wait more than 169 days to harvest the coffee, a period that has a low risk of contamination by glyphosate residue in the coffee bean.

References:

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ORAL PARALLEL SESSIONS

Session 1: Plant science

S1-O-06

Stenophylla coffee (*Coffea stenophylla):* the forgotten coffee crop species of West Africa

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RATIONALE

Coffea arabica (Arabica) and *C. canephora* (robusta) almost entirely dominate global coffee production. Various challenges at the production (farm) level, including the increasing prevalence and severity of disease and pests and climate change, indicate that the coffee crop portfolio needs to be substantially diversified in order to ensure resilience and sustainability.

METHODS

We used a multidisciplinary approach to elucidate the identity, whereabouts, and potential attributes of a forgotten crop species: stenophylla coffee (*C. stenophylla)*.

RESULTS

We show that despite widespread (albeit small-scale) use as a coffee crop species across Upper West Africa and further afield more than 100 years ago, this species is now extremely rare in the wild and is not being farmed. Fieldwork enabled us to rediscover *C. stenophylla* in Sierra Leone, which previously had not been recorded in the wild there since 1954.

CONCLUSIONS & PERSPECTIVES

Stenophylla coffee may possess useful traits for coffee crop plant development, including taste differentiation, disease resistance, and climate resilience. These attributes would be best accessed via breeding programs, although the species may have niche-market potential via minimal domestication.

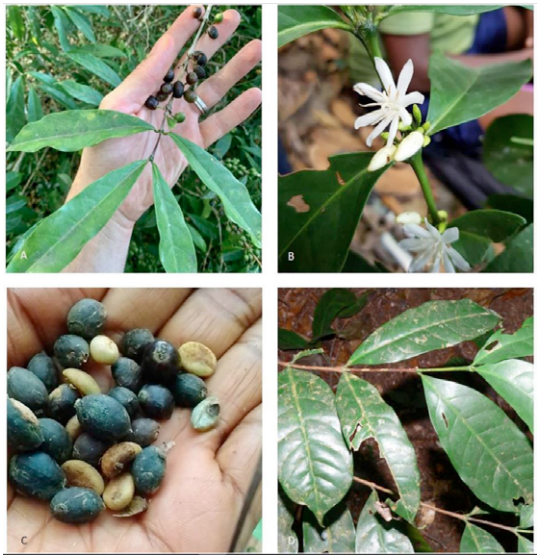

Figure 1. *Coffea stenophylla*. (A) in fruit, at Centre National de Recherche Agronomique (CNRA), Ivory Coast (image: Charles Denison); (B) flowers (image: Daniel Sarmu); (C) partially dried fruits and seeds (image: Daniel Sarmu); (D) leaves (image: Aaron Davis). Images B-D taken in eastern Sierra Leone.

References:

• Davis AP et al. 2020 Frontiers in Plant Science 11: DOI=10.3389/fpls.2020.00616.

S1-O-07

Evolution of *Coffea* leaf functional traits in relation to climate

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RATIONALE

The genus *Coffea* consists of over 100 species growing in (sub-)tropical regions of Africa and South-East Asia. Although most *Coffea* species are understory shrubs, considerable variation exists in niche occupation, ranging from species growing at high altitudes, lowland rainforest, periodically inundated riverbanks, seashores and savanna. As such, it can be expected that habitat adaptation is reflected in leaf functional traits that allow species to cope with prevailing climate conditions. We examined how leaf functional traits of about 38 *Coffea* and 2 *Psilanthus species* have evolved in relation to shifts in habitat and climate conditions.

METHODS

The aims are to (1) analyse intra- and interspecific variability of leaf functional traits (stomatal density, stomata size, specific leaf area) in the genus *Coffea*, (2) analyse macro-evolution of leaf functional traits by means of ancestral state reconstruction, (3) derive potential drivers of leaf functional trait evolution and adaptation of leaf traits to environmental conditions and (4) relate leaf traits to climate variables and other plant functional traits, including allometric relationships between plant traits. Stomatal density, specific leaf area and leaf size has been determined for 5 (herbarium)specimens (from different locations) per *Coffea* species investigated.

RESULTS

Present results show that *Coffea* species growing in dry areas have smaller and/or thicker leaves, as well as a lower stomatal density, suggesting that leaf traits in *Coffea* are adapted to water availability. We also found significant phylogenetic signal for certain leaf traits, indicating that leaf trait states are not only determined by the environment but also by descent.

CONCLUSIONS & PERSPECTIVES

Our study contributes to a more profound understanding of how *Coffea* species are adapted to their environment and how variable they are. Future analyses will be complemented with phylogenetic information, which will allow us to make inferences not only about the ecology, but also the evolution of leaf functional traits. Such information can contribute to provide tailor-made breeding practices and to determine more specific conservation priorities.

S1-O-08

Elevated air [CO_2] partly mitigates drought impact in *Coffea* spp. cultivars

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RATIONALE

Drought is one of the most critical abiotic factors affecting growth, C-assimilation and crop productivity. In the context of climate changes, it is important to study the interaction between the predicted increase in atmospheric [CO_2] and water shortage, regarding future coffee crop sustainability. We present results on the impact of elevated [CO_2] and drought in *Coffea* spp. at the photosynthetic machinery level.

METHODS

Seven-year-old plants from *Coffea canephora* cv. Conilon (Clone 153) and *C. arabica* cv. Icatu, grown under controlled conditions (RH: 70%; photoperiod: 12h; temperature: 25/20ºC (day/night); PPFD: *ca*. 700 µmol m^{-2} s^{-1}; two air [CO_2] (380 or 700 µL L^{-1}) and under well-watered (WW) conditions, were gradually submitted to mild (MWD - Ψ_{pd}, -1.5 to -2.5 MPa) and severe (SWD - Ψ_{pd} < -3.0 MPa) water deficit. The impact on photosynthetic machinery was assessed through leaf gas exchanges (net photosynthesis, Pn; stomatal conductance, g_s; photosynthetic capacity, A_{max}), chlorophyll *a* fluorescence, thylakoid electron transport rates, and RuBisCO activity (1-3).

RESULTS

Both MWD and SWD reduced P_n and g_s in both genotypes, with non-stomatal impacts (A_{max}) mainly in SWD plants, and mainly under 380 µL L^{-1}. In both genotypes, elevated [CO_2] increased P_n and A_{max} in WW plants, and mitigated (P_n, A_{max}, photochemical quenching, q_P) or cancelled (photochemical efficiency of photosystem II in light, F_v'/F_m') drought impacts only in MWD (despite some g_s reduction). In SWD plants, significant reductions were found in these parameters, usually without differences between [CO_2]. Notably, A_{max} kept relevant values even under SWD in both genotypes, denoting that the P_n values close to zero were chiefly related to stomatal closure. Among non-stomatal impacts, initial and total RuBisCO activities stands out, showing increasing impacts in MWD and SWD, irrespective of [CO_2] and genotype, contrasting with electron transport rates at both photosystems that remained mostly unaffected, even under SWD.

CONCLUSIONS & PERSPECTIVES

Elevated [CO_2] mitigated the negative impact of mild drought, maintaining higher C-assimilation performance (and, likely, lower photorespiration). This was linked to a high resilience of the photochemical machinery, but not RuBisCO. Thus, high [CO_2] contributed to this crop sustainability under future climate change scenarios that include lowered water availability.

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S1-O-09

Genome Sequence Assembly of *Coffea arabica* variety Geisha (UCDv1.0)

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RATIONALE

To develop a *Coffea arabica* reference genome, we sequenced a Geisha variety plant from Goodland Organics in Goleta, California. Geisha coffee is recognized for unique aromas and flavors, has achieved the highest prices in the specialty coffee markets, and therefore merits a deeper molecular understanding. The founding Geisha germplasm in California was provided to Goodland Organics by Price Peterson (Boquete, Panama) in 2006. In 2017 we released our first version of a *de-novo* reference genome sequence of *C. arabica* var Geisha (UCDv0.5, Phytozome). Here we report a revised, improved annotated version of the Geisha genome sequence (UCDv1.0). Currently genome sequences exist for *C. arabica* var Caturra and the two progenitors *C. canephora* (Denoud et al 2014) and *C. eugenioides* (de Kochiko et al 2018).

METHODS

DNA sequencing was performed using Pacific Biosciences (PacBio) SMRT sequencing (80x coverage) and Oxford Nanopore (ONT) (130x coverage) and assembled independently with Falcon-unzip and Canu, respectively. Dovetail Chicago and HiC libraries were used for scaffolding both assemblies. The original PacBio-Falcon-Dovetail contigs were then scaffolded to the ONT-Canu-Dovetail contigs using RaGOO and manual curation to create the final assembly and pseudomolecules. Ten different tissues were sampled and used for RNA sequencing using Illumina and PacBio IsoSeq to develop transcript assemblies for gene prediction, structural and functional annotation using AUGUSTUS and complementary software.

RESULTS

The total sequence assembled was 1.03 Gb in 236 scaffolds (N50=44Mb, L50=10) composed of 2,112 contigs (N50=1.1Mb, L50=234) and 1,876 gaps. Annotated gene models were 61,782 with 68,375 proteins. For comparison, our *de-novo* assembly was aligned to the available Caturra and progenitor assemblies. The progenitor comparison confirmed the accuracy of our assembled non-chimeric pseudomolecules. Most chromosomes were highly similar between Geisha and Caturra. One major inconsistency was observed in chromosome 6c in Caturra between 24-39 Mb in Geisha chromosome.

CONCLUSIONS & PERSPECTIVES

Critical challenges to coffee sustainability are impacts of climate change, disease and quality. The application of genomics technologies in breeding programs is the most efficient and reliable strategy to approach these problems and accelerate the development of new varieties for the sustainability of the coffee supply. The application of these technologies relies on the availability of high quality whole genome sequences of coffee. Our work is a contribution towards this effort.

Acknowledgement: Project funded by Suntory Global Innovation Center Limited, Tokyo, Japan.

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S1-O-10

Evaluation of F1 hybrid (*C. arabica* L.) performances and farmer acceptance on-farm conditions in three producing regions in Ecuador

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RATIONALE

Many Arabica coffee producing countries, particularly in Central and South America are facing to well-known threats like aged plantations, poor fertilization practices and increased prevalence of extreme climatic conditions. It is therefore urgent to implement new strategies to assure a durable sustainability for the coffee sector. Development of resilient varieties able to overcome climatic impact is part of this approach. Those new varieties should focus smallholder farmers with varied experience in coffee culture, who dependent of old traditional varieties and who are located either in marginal or productive environments. Here we report the results of a pilot study aiming to test a group of improved F1 Arabica hybrid varieties developed by Nestlé PSRU on real farm conditions along the three main producing regions in Ecuador.

METHODS

Thirteen hybrid varieties were deployed on thirty-one farmer trials in Ecuador. All varieties were cultivated following the current practices used by the local farmers. To ensure the success of this participatory research, a contract was signed with each producer. The agronomic performance, quality (bean and cup) attributes and farmer perception were assessed over four consecutive crops between 2014 and 2017.

RESULTS

Overall, data collected after a minimum of four crops show that most of the Nestlé hybrids over perform when compared to the traditional varieties cultivated by the farmers. The most performant hybrid, yielded up to 2.5 tons of green coffee/ha which represent three-fold more volumes compared to the traditional varieties Typica or Caturra. Seven out of 13 hybrids showed a high potential in cup quality, with cup scores fitting the 1.0 class in the Nestlé classification, which is equivalent to 90 to 100 points in the SCAA scale. Further, some Nestlé hybrids show a high sucrose/caffeine ratio, which is highly correlated to high-grade green coffees. Farmer survey assessment showed that most of the hybrid varieties (9 out of 13) were well accepted by the farmers reaching a 90% score of farmer preference. Among the different criteria considered by farmers, the plant vigor, architecture, overall development, plant healthiness and harvesting facility, were evaluated.

CONCLUSIONS & PERSPECTIVES

Based on these successful results and practical issues, two hybrids varieties (named Star2 and Star3) were selected for further propagation in the country. A local seed company was licensed by Nestlé Ecuador in order to produce commercial hybrid seeds using the male sterile approach. In 2019 a first batch of 900 Kg of hybrid seeds was produced, and rising volumes have been produced during last two years.

References:

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POSTERS

Session 1: Plant science

S1-P-01

Genotype-by-environment interaction in thirteen coffee cultivars (*Coffea arabica* L.) in five locations of Costa Rica

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RATIONALE

Multilocal trials for the evaluation of genotypes, is one of the most useful tools in agricultural research in plant breeding programs to determine the genotype-by-environment interaction (GEI) and to make recommendations for superior cultivars (Sa'diyah & Hadi, 2016). The aim of this research is to evaluate the adaptation of potential 13 coffee cultivars (*Coffea arabica* L.) introduced in five coffee growing regions through a genotype-by-environment interaction analysis for the identification of promising materials under different agro-environmental conditions of Costa Rica.

METHODS

The use of the multiplicative interaction (AMMI) model adjusts the additive main effects for genotypes and environments through an ANOVA procedure using BLUE (best unbiased linear prediction), and then applies principal component analysis (PCA) using DVS (singular value decomposition) to the remaining residuals, after setting the main effects. In addition, GGE Biplot model by Farshadfar et al. (2013) helps to interpret interaction of the environment with genotypes, since it visualizes similarities and differences between genotypes, localities and the differential response of genotypes. Genotype-by-environment interaction graphs were created to visualize cultivars' productivity in different harvests and their stability.

RESULTS

The AMMI graphs allowed us to understand the complex genotype-by-environment interaction existing in quantitative traits such as coffee production, understanding the interaction effects, improving the selection process and adding experimental efficiency by being able to form groups of, as well as of identify environments that contribute little to the interaction and discrimination of genotypes. The application of GGEBiplot Interaction Graphics are highly effective identifying genotypes in specific environments.

The results showed the applicability of these methods to define mega-environments, it is important to take into account the combination of subsets of environments in a larger group (mega-environments), since this allows to better represent targeted populations.

CONCLUSIONS & PERSPECTIVES

The application of the additive main effects and multiplicative interaction (AMMI) and site regression methods (SREG) were able to determine the genetic stability of a cultivar across different locations. The Genotype x Environment Biplot Interaction graphs could identify the cultivars' trends in possible mega-environments that may include factors like temperature, precipitation, relative humidity, wind speed, soil physical and chemical characteristics, among others.

References:

- Sa'diyah & Hadi 2016. Agriculture and Agricultural Science Procedia, 9, 163–169.
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S1-P-02

Identification of coffee cultivars (*Coffea arabica*) by quantitative and qualitative traits

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RATIONALE

The phenotypic identification are a common and cheap method to distinguish variation based on the observation of the morphological differences in different parts of the plant such as the size and shape of the leaf, the plant form, the color of the shoot tip, the characteristics of the fruit, the angle of branching and the length of the internodes (De Vienne et al., 2003). The identification of quantitative characteristics allows to determine the homogeneity for seed reproduction or in the standardization of a productive plantation.

METHODS

For the morphological characterization the descriptors established by the IBPGR were used. In the observations, the qualitative morphological descriptors such as: leaf shape, fruit shape, seed shape and seed color have the same events for the cultivars evaluated so they were discarded for data analysis. Measurements were made of plant height, stem diameter, leaf length and leaf width, plagiotropic branch length, internodes length, number of new and productive nodes, among others. 30 plants were measured in 35 *Coffea arabica* cultivars. A multivariate analysis was performed, the data were filtering and analysis performed with the aid of the language and environment for statistical computing R (R Core Team, 2016).

RESULTS

With the data collected, the number of variables that contribute more variance to have a greater differentiation between cultivars was determined. Out of 20 quantitative and qualitative variables analyzed, they contribute 37% of the variance between the main components 1 and 2. When analyzing the variables that contribute most to increase the variance, it was determined what characteristics contribute the most to the variance with 82% between the components main 1 and 2, among them the vigor of the plant (height / stem diameter), width x length of the leaf, width of the leaf, length of the leaf, diameter of the stem and the length of the plagiotropic branch. In addition, it was determined that the analyzed variables are directly influenced to the crop management, when comparing these variable in conditions in full sun, shade and pruning.

CONCLUSIONS & PERSPECTIVES

Determining what quantitative characteristics can be measured in coffee plants to determine the identification of the cultivar, is based mostly on the measurement of the leaves in a given and management. The adaptive capacity of coffee plants makes it difficult to identify cultivars that must be accompanied by a discard complemented with qualitative characteristics (color of the sprout and fruit, tolerance to diseases, bearing of the plant)

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S1-P-04

Assessment of the genetic diversity of Philippine coffee (*Coffea* spp.) using simple sequence repeats (SSR) markers

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RATIONALE

Due to the continuous increase in population, urbanization and promotion of registered and popular varieties in the Philippines, it is not far that the genetic diversity of coffee in the country is being threatened. Thus, collection of various accessions and varieties in the whole country was conducted and they are currently conserved *ex-situ* in a field genebank. The present study was conducted to assess the genetic diversity of Philippine coffee using simple sequence repeats (SSR) markers or microsatellites that serves as benchmark information in conservation and management of the collection, and for breeding and selection programs.

METHODS

DNA of 68 government - approved, and other cultivated varieties of *C. arabica*, *C. canephora* and *C. liberica* coffee were extracted using modified CTAB technique. The extracted DNA were amplified using 19 SSR primer-pairs. Polymorphic markers were analyzed using similarity index, cluster analysis, polymorphism information content (PIC) and principal component analysis (PCA).

RESULTS

All primers screened were polymorphic across all coffee species tested and were highly informative (PIC>0.5) except for primer M310 (0.30). A total of 128 alleles were amplified. The *C. arabica* of the country was found to have low genetic diversity while *C. canephora* and, *C. liberica* have relatively high genetic diversity. The low diversity in *C. arabica* can be attributed to its self - compatibility nature. The dendrogram generated from cluster analysis and PCA clearly resolved the collection into three distinct clusters/group. Cluster I was composed of all *C. arabica* accessions. Cluster II composed of *C. canephora* and Cluster III compromised of *C. liberica* var. liberica and *C. liberica* var. dewevrei. Another notable findings of this study is the detection of 3 SSR markers that are able to distinguish the 3 species. This is very helpful in the proper identification and authentication of coffee seedlings, green beans and even roasted ones.

CONCLUSIONS & PERSPECTIVES

The genetic diversity analysis done is important in managing the existing collection of coffee in the country. The information generated are also important in breeding and selection of coffee. Further, the use of SSR markers is essential in the identification and authentication of coffee at any growth stage.

References:

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S1-P-07

The yield performance and adaptability of coffee varieties across two coffee agro-ecological zones in Kenya

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RATIONALE

The focus in breeding over the years has remained on increased productivity per unit area and adaptation of genotypes to different environments. Testing of genotypes in different environments allows selection for the most productive and stable new varieties. The study aimed at assessing different coffee varieties sourced from different coffee growing countries for their yield performance and adaptation in Kenya.

METHOD

Ten (10) coffee varieties from four different countries were evaluated for their yield performance in Kenya across two different coffee agro ecological zones (Koru and Ruiru). The varieties included seven (7) tall and three (3) dwarf varieties. The trial was established in the year 2015 using the Randomized Complete Block design with three replications each plot having ten (10) trees. Data on yield was recorded in the year 2018 and 2019 and analyzed for the two environments and also over the two years' period.

RESULTS

There was significant difference on the yield performance among the coffee varieties, between the two environments and during the evaluation period (2018 and 2019). The interaction between the two environments was significant. In the year 2018, Mundo Maya and Batian produced significant ($P \leq 0.05$) high yield at Koru and Ruiru respectively. In the year 2019 K7 had significant ($P \leq 0.05$) high yield in Koru whereas S.795 produced high yield in Ruiru. Over the two years across the two environments, Ruiru 11 recorded significantly ($P \leq 0.05$) higher yields followed closely by Mundo Maya, Batian and K7. The varieties Sln. 5b and S6 recorded significantly low yield across the two environments over the two seasons. There was yield decline for the year 2019 when compared to the year 2018 for all the varieties. There was a positive interaction between the two environments over the two seasons.

CONCLUSION AND PERSPECTIVES

The study has led to the identification of coffee genotypes that are stable in the Kenyan environment. The Mundo Maya from Nicaragua is an already promising variety adapting well to the two environments and this can be used in the development of new coffee varieties in Kenya due to its high yield. The yield data need to be collected for another 2-3 years to have conclusive results on the performances status of all the coffee varieties being evaluated.

References:

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S1-P-08

Arabusta seed morphology: Arabica-like or Robusta-like? A preliminary comparison with parental species.

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RATIONALE

Arabusta coffee is an interspecific hybrid derived from *C. arabica x C. canephora*, often used in coffee breeding to study both the gene transfer from Robusta and its possible use in coffee production. The plants have very different morphological characteristics, as well as different levels of fruit production when compared with the parental species. Arabusta coffee was also studied from a sensory point of view, but in general the literature on this hybrid is rather scarce and the knowledge on it is still fragmentary. From a morphoanatomical point of view, as far as we know, no investigations have been devoted to study Arabusta seed morphology and to disclose possible Arabica-like or Robusta-like traits in its cell structure and this highly stimulated the present work.

METHODS

Seed samples of *C. arabica* L. (Costarica, 2019), *C. canephora Pierre ex-Froehner* (French Guyana, 2019) and F1 clones of *Arabusta* coffee (French Guyana, end of 2018) were harvested, properly processed and selected. Seeds were kept in a fixative solution for several days, then rinsed in tap water and cut at -20°C with a cryostat (Leica CM1520). Seed sections of 60 µ in thickness were observed by a Scanning Electron Microscope (Hitachi TM3030plus). Sections of 12 µ in thickness were staining in a Toluidine Blue O solution to highlight the main cell components. Measurements of cell wall area were performed on the electronic images by a Leica Software (Las X).

RESULTS

Arabusta is characterized by an elongated seed, slightly rounded, generally with a linear furrow. The endosperm cells are regular, characterized by a polygonal shape, with not frequent nodes present in their cell wall. Cell wall thickness is not significantly different from the parental species (5,7 ± 1,6 µ). However, the area occupied by the cell wall respect to the cell total area (28%) is more close to that measured in Arabica seeds (29%) than that in Robusta seeds (43%). Optical microscopy observations furtherly put in evidence this tissue aspect, showing similar cell size in Arabusta and Arabica seeds, and smaller cells for Robusta coffee. This feature is probably related to ploidy level (Arabica and Arabusta : 2n = 4x = 44, Robusta 2n = 2x = 22). No histochemical differences in the cell content were observed among the examined coffee species under the chosen experimental conditions.

CONCLUSIONS & PERSPECTIVES

The measurements performed on cell wall area and measured diameter as well as cell content area and endosperm cell size show that the morphoanatomical characteristics of Arabusta seeds corresponds more to those of Arabica than Robusta seeds. Further studies are necessary to confirm this preliminary view.

S1-P-09

Genes related to secondary metabolism and redox status are transcriptionally modulated in *Coffea arabica* leaves by applying hexanoic acid to roots

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RATIONALE

Hexanoic acid (Hx) is a short, naturally occurring monocarboxylic acid that is a potent natural priming agent against pathogens. The molecular mechanism that rely Hx induced resistance is not fully understood, since most studies were focused on reducing the symptoms of plant diseases. However, it was observed in some crops that the exogenous application of hexanoic acid can induce a long distance modulation in key genes of plant metabolism. We hypothesize that if Hx application can modulate genes related to defense responses, it would be a potential eliciting agent in Arabica coffee.

METHODS

Using RNA-seq, we analyzed the leaf transcriptome of two *Coffea arabica* cultivars that have a contrasting breeding history, cv. Catuaí Vermelho IAC 144 and Obatã IAC 1669-20, in response to the application of hexanoic acid in an eliciting concentration in nutrient solution. Total RNA was extracted with the RNeasy Plant Mini Kit (Qiagen) following manufacturer's instructions. Purity and quantity of the samples were checked with a Qubit device (Thermo Scientific). Library construction and RNA sequencing was performed by LC Sciences (Houston, TX, USA). cDNA libraries were generated using a poly-A selection method and paired-end reads (2 X 150 bp) obtained on the Illumina NovaSeq Platform. RNA-seq analysis used the same rational of Liu et al. (2020).

RESULTS

Using a publicly available *C. arabica* genome as a reference for RNA-seq data, we identified 121 differentially expressed genes (DEGs) in Catuaí leaves and 91 DEGs in Obatã leaves. A total of eight genes have significant similar transcriptional modulation in both cultivars, including genes related to redox balance, jasmonate signalling and the phenylpropanoid metabolism. Hx significantly repressed only an electron acceptor in chloroplasts. All other genes were upregulated. They include a glycosyltransferase associated to the salicilate-jasmonate signaling crosstalk, an ATPase, aldo keto reductases and genes related to the biosynthesis of hydroxycinnamic acids and terpenoids.

CONCLUSIONS & PERSPECTIVES

These results demonstrate that Hx application in roots can alter the gene expression patterns of leaves, activating genes involved in redox regulation and synthesis of secondary metabolites. This approach indicate that Hx have a priming effect, modulating genes involved in the establishment of systemic acquired resistance, becoming a promising eliciting substance in *C. arabica*. Funding: CAPES (Code 001) and FAPESP (#2016/10896-0).

References:

- Liu et al. 2020 Plant Cell Reports DOI:10.1007/s00299-019-02501-2.

S1-P-10

Morphological characterization and identification of commercially cultivated coffee (*Coffea* spp.)

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RATIONALE

Characterization of coffee is essential in crop improvement, genetic diversity analysis, conservation and in proper identification. Aside from Arabica and Robusta, Liberica and Excelsa coffee are also popularly cultivated in the Philippines and in some countries. However, difficulty in discriminating them is one major problem encountered not only by coffee farmers but also by plant material inspectors. This study was conducted to characterize the commercially cultivated coffee in the Philippines; and to develop a dichotomous key to quickly identify them.

METHODS

Five varieties/accessions of Arabica, Robusta, Liberica and Excelsa coffee were characterized using the descriptors for coffee of the International Plant Genetic Resources Institute and the Philippine National Seed Industry Council. Five qualitative and eighteen quantitative morphological descriptors were used to characterize and identify the samples and develop a key for the identification of cultivated coffee.

RESULTS

High genetic diversity (H'>0.70) were observed in all traits except for fruit disc shape, leaf margin and flower color. Dendogram generated four distinct clusters that was based on taxonomic classification (i.e. *Coffea arabica*, *C. canephora* for Robusta, *C. liberica* var. *dewevrei* for Excelsa and *C. liberica* var. *liberica* for Liberica). Further, it was found that leaf orientation and leaf margin were highly discriminating between Arabica and Robusta; and Excelsa and Liberica. Leaf area and texture clearly discriminated Arabica from Robusta. On the other hand, fruit size, leaf width and fruit clustering were found to be highly discriminating between Excelsa and Liberica. The highly discriminating traits were validated in Arabica by examining secondary data. On the other hand, 12 Liberica and 15 Excelsa accessions at the National Coffee Research, Development and Extension Center were used to validate the traits that highly discriminated Excelsa from Liberica.

CONCLUSIONS & PERSPECTIVES

The aforementioned results were used in developing an "identification key" for the effective and easy identification of Arabica, Robusta, Excelsa and Liberica. To the best of our knowledge, this is the first report on the quick identification of commercially cultivated coffee. The key to identification we developed also offers an unambiguous method to classify coffee based on morphological traits.

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S1-P-12

Study of genes related to the flowering process in coffee: toward genetic improvement in a climate change scenario

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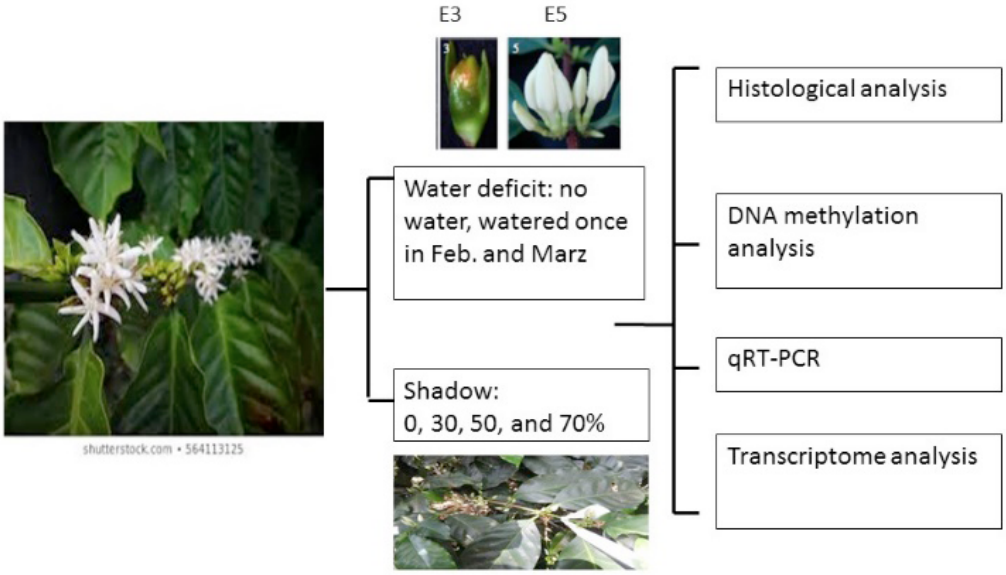
Coffee plants present the important problem of floral asynchrony that causes uneven ripening of fruits and, therefore, affects product quality. Although the molecular mechanisms involved in the flowering process are well characterized in herbaceous species, from the molecular point of view, the mechanism involved in the perception of these stimuli and the activation of reproductive development in *C. arabica* is little known. In coffee, the effect of climate change has been observed for several years in the different producing regions, damaging the crop. Therefore, the increase in temperature and changes in rainfall behavior have affected coffee phenology in important processes such as flowering and fruit development, causing in recent years numerous losses in many coffee regions. In this sense, analyzing the molecular process of flowering of coffee would provide useful information for agricultural practices, such as synchronization in flowering and, consequently, the ripening of the fruit with an improvement in the quality and production of the coffee drink.



Schematic representation of the performed investigation.

S1-P-13

Sensitivity of seeds to chemical mutagens, detection of DNA polymorphisms and agro-metrical traits in M1 generation of coffee (*Coffea arabica* L.)

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RATIONALE

Coffee (*Coffea arabica* L.) is threatened by biotic and abiotic stresses. Nevertheless, the breeding of Arabica coffee is restricted due to its low genetic diversity. Crop improvement via mutagenesis represents an alternative for increasing genetic variability and facilitating breeding.

METHODS

Coffee seeds cv. Catuaí were treated for 8 h with a solution of sodium azide (NaN3) (0, 50, 75, 100 and 125 mM) and ethyl methane sulfonate (EMS) (0, 80, 160, 240, 320, and 400 mM). The genetic variability induced in coffee plants after mutagenic treatment with sodium azide was determined by RAPD and AFLP analysis.

RESULTS

As the concentration of applied NaN3 and EMS increased, the germination, seedling height and root length decreased. The LD50 values for NaN3 and EMS were between 50-75 mM and 160-240 mM, respectively. The analysis revealed that both NaN3 and EMS induced variability within the DNA regions amplified with AFLP and RAPD markers. Finally, under field conditions, significant differences were noticed with respect to plant height, number of nodes in the orthotropic stem, and number of branches of the M1 mutant (NaN3 treated) plants compared to the non-mutant plants.

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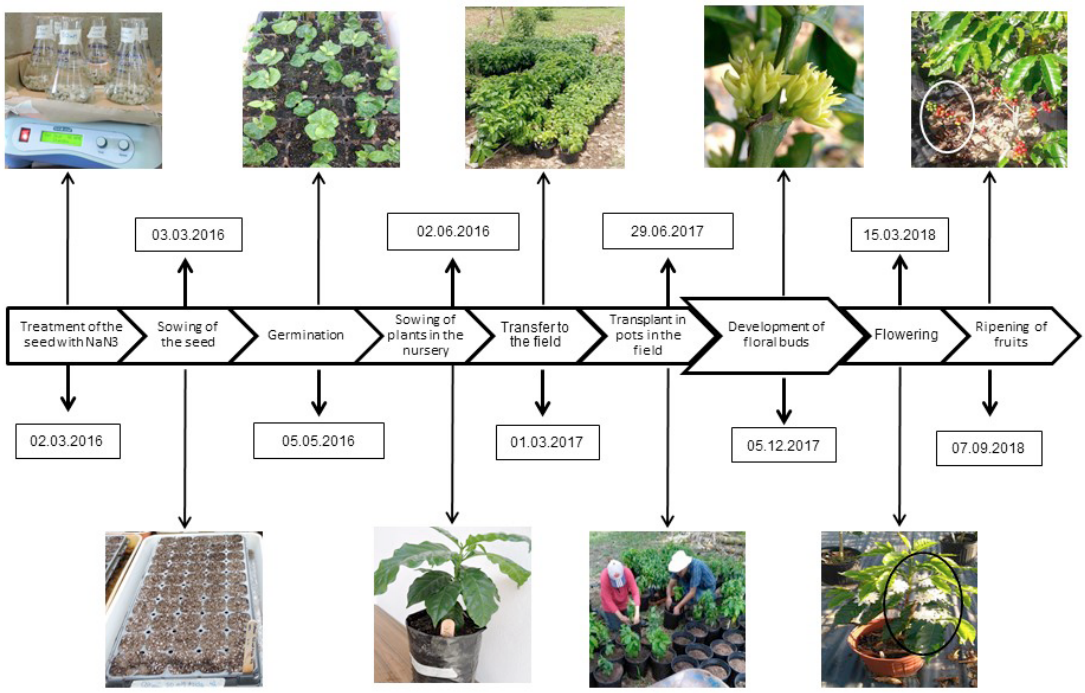

Steps and time required to produce a mutant M1 population from seeds of *C. arabica* L. cv. Catuaí using NaN3.

S1-P-14

Genetics of coffee wilt disease (*Gibberella xylarioides* Heim and Saccas) resistance in Arabica coffee (*Coffea arabica* L.)

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RATIONALE

Understanding the genetics control of coffee wilt disease (CWD) resistance and related traits in Arabica coffee is useful in planning breeding strategies in this economically important crop. The study was conducted to estimate combining ability, heterosis, heritability and identify the type of gene effects controlling the inheritance of CWD resistance, which are useful in designing appropriate breeding programs and CWD resistant variety development.

METHODS

The research was conducted on eight Arabica coffee parents possessing contrasting reaction to CWD, including its 28 F1 crosses through Griffing (1956) method 2 and model I, and one susceptible check in artificial inoculation test using Girma and Mengistu (2000) method at greenhouse at Jimma Agricultural Research Center, Ethiopia during 2015 to 2016. The reactions of inoculated seedlings were measured as wilted seedling percentage, incubation period, number of yellow leaves and defoliated leaves.

RESULTS

The mean performance of F1 crosses, parental lines and susceptible check for wilted seedling percentage ranged from resistant crosses P7 x P8 to susceptible parent P3. The crosses showed a relatively wide range of percentage death compared to the parents although only one cross (P7 x P8) recorded lower wilted percentage than the resistant parent (P2). Better parent heterosis (BPH) and mid parent heterosis (MPH) for wilted seedling and number of defoliated leaves showed inappreciable in desirable direction. However, considerable MPH noticed for the incubation period. Both additive and non-additive gene effects are found in controlling the inheritance of CWD resistance and incubation period; additive genetic effects being predominant. Parents P2 (971), P7 (974), P8 (370) and P5 (79233) exhibited highly significant negative gca effects and good general combiners for resistance. Moreover, sca effects of crosses P7 x P8 (974 x 370) and P4 x P8 (8136 x 370) revealed good specific combiners for resistance (low mean wilted percentage) and incubation period. Wilted seedling percentage showed high broad (88.27%) and narrow (75.41%) sense heritability coupled with 68.61% genetic advance.

CONCLUSIONS & PERSPECTIVES

Selection and hybridization could be an effective resistance breeding approach. Further research on F1, F2 and backcrossing (BC) generations, and quantitative trait locus (QTL) mapping is needed.

References:

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S1-P-16

The absence of the caffeine synthase gene is involved in the naturally decaffeinated status of *Coffea humblotiana,* a wild species from Comoro archipelago

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RATIONALE

Caffeine is the most consumed alkaloid stimulant in the world. It is synthesized through the activity of three known N-methyltransferase proteins. *Coffea humblotiana* is the sole *Coffea* species endemic to the Comoro archipelago. The feature of this wild and endangered species, is the absence of caffeine in seeds and leaves. So far no genomic characterization had been undertaken to discover the origin of this natural absence of caffeine.

METHODS

Using PacBio, Illumina and HiC, we assembled the *C.humblotiana* genome and the leaf transcriptome. Caffeine, theobromine and chlorogenic acids content were determined in leaves by HPLC.

RESULTS

Here we report on the 422-Mb chromosome-level assembly of the *C.humblotiana* genome. We predicted 32,874 genes andanchored 88.7% of the sequence onto 11 chromosomes. Comparative analyses with the *C. canephora* genome revealed an extensive genome conservation, despite an estimated 11 million years of divergence. The absence of caffeine is likely due to the loss of the *Caffeine Synthase* (*DXMT*) gene which converts theobromine into caffeine through an illegitimate recombination mechanism, corroborating the presence of theobromine in leaves.

CONCLUSIONS & PERSPECTIVES

C. humblotiana generated in the frame of the present study provides the first high-quality reference genome for the *Coffea* genus. It also provides valuable information for promoting the preservation of the diversity of this wild species in its environment and it represents a perfect resource for genomic and evolutionary studies on *Coffea* and *Rubiaceae*. It is also of interest in helping to develop new strategies for characterizing coffee cup-quality traits.

S1-P-18

Study of Arabica coffee bean characteristics (*Coffea arabica* L. var. Catimor) in five provinces of the upland of Thailand

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RATIONALE

In Thailand, Arabica coffee plantation (harvested area) increased from 8,600 ha in 2014 to 13,945 in 2020 [1], accounting for almost 62%. The study of Arabica Coffee Bean characteristics aims to develop the value and identity for use as a basis for registering geographic indications.

METHODS

We studied the geographical characteristics, physical characteristics and chemical composition of Catimor Arabica coffee in 29 plots in altitude of 839-1499 meter msl. in 5 provinces, such as Chiang Mai, Chiang Rai, Nan, Mae Hong Son and Phayao. All sample divide in 4 processing.

RESULTS

All plots had distinct geographical characteristics (intercropping, shade plants, planting systems, slopes of area, cultural practices, soil characteristics) and climatic conditions, corresponding to differences in physical characteristics (size of parchment and green bean, weight and number of green bean), aroma (macadamia, chocolate, dried flower, roasted coffee, cinnamon, nutty, peach, apricot, plum Hazelnut, ripe banana, ginger, caramel, butter, apple, jasmine, honey, lime, camphor, cocoa, spice, cereal and roasted fruit) and chemical composition. Arabica Coffee Bean characteristics of var. Catimor "Chiang Mai 80", which was released by Department of Agriculture, Thailand, did not differ in the width, length and thick of parchment and thick of green bean, but there was statistical differences in weight and number of green beans, percentage of grade A, percentage of pea berry. There were statistical differences in the sensory properties of the cup tasting, with an 79.72±0.97 scores, but no statistical difference in 4 process. And there are various aroma in each area and different process such as macadamia, cereal, bread, butter, caramel, honey, flower, fruit, chocolate, herbs and spices.

CONCLUSIONS & PERSPECTIVES

Arabica Coffee Bean characteristics var. Catimor in 5 provinces had differences in shape, size, chemical composition and cup taste, which depend on geographical characteristics and climatic conditions. The analysis of the nutrient content of coffee beans can be a good indicator for identifying the origin of coffee beans.

References:

- [1] Office of Economics, Ministry of Agricultural and Cooperative, Bangkok, Thailand

S1-P-19

Isolation, molecular characterization, expression and phylogenetic analysis of NAC 025 like transcription factor (TF) in coffee

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RATIONALE

NAC transcription factors play a pivotal role in regulating plant developmental processes and response to environmental stresses. Although the structural and functional characterisation of several NAC transcription factors has been reported in many plant species, there is a scanty report published on NAC transcription factors in coffee. In this study, we performed a comprehensive investigation on the molecular characterisations, phylogenetic and expression profiles of NAC 025 like genes in various coffee species including five indigenous coffee species from India.

METHODS

The full-length NAC 025 TF was cloned both from genomic and cDNA samples of seven coffee species and multiple sequences aligned using CLUSTAL W program. The SNPs and indels were calculated both in intronic and exonic regions, and the synonymous and non-synonymous SNPs, as well as frameshift and non-frameshift mutations, were determined. The sequence flexibility of the C-terminal region of the NAC TF was investigated in parents and hybrids. The expression profile of the NAC 025 TF was determined in the root, leaf, flower bud, flower and young fruit using QPCR analysis.

RESULTS

The size of the NAC 025 like genes in seven coffee species varied from 2471bp in *Coffea jenkinsii* to 2528bp in *C. wighitiana* both belongs to indigenous coffee species from India. The NAC gene has two introns interspersed between three exons. The frequency of occurrence of SNP in NAC gene, when compared with *C. canephora* varied considerably among the species being lowest (7) in *C. arabica* and highest (169) in *C. travancorensis*. In the exonic region, the frequency of non synonymous SNP is much higher compared with the synonymous SNP. Further, the occurrence of indels in the coding region of the NAC gene was computed which revealed the higher frequency of frame shift mutation caused by indels compared to non-frameshift mutation. This has resulted in lower protein sequence homology among the species. Interestingly, the sequence plasticity of the C terminal region of the NAC TF was confirmed both in the F1 hybrids and plants derived through *in vitro* somatic embryogenesis. Our study further asserted that NAC 25 like gene in tetraploid arabica was derived through the insertion of 89 bp sequence from *C. canephora*. The differential expression profile of the NAC gene in different plant tissue captured through real time PCR analysis was further elucidated.

CONCLUSIONS & PERSPECTIVES

The detailed molecular analysis of NAC 025 like transcription factor and its structural architecture will greatly facilitate our understanding of the functional perspectives of the NAC gene in coffee.

S1-P-21

Determination of the number of years in Arabica coffee progenies selection through repeatability

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RATIONALE

The repeatability coefficient is an indispensable information source for breeders, underlying the determination of the number of phenotypic data of annual yield required from each tree for an efficient selection of genotypes with less cost and labor. The selected genotype is expected to maintain the initial performance for the entire lifetime. This coefficient also represents the highest possible value of trait heritability in the broad sense. This study aimed to estimate the repeatability coefficient and the minimal number of evaluations required for an accurate prediction of the real value of the trees.

METHODS

The estimates of the coefficients of repeatability (r) were obtained by the methods of analysis of variance (ANOVA), Principal components based on the Matrices of correlations (CPcor) of covariances (CPcov) and the Structural analysis based on the Matrices of correlations (AEcor). The minimum number of measurements required to predict the real value of the plants, based on the pre-established coefficients of determination (R2), as proposed by Cruz and Regazzi (1997).

RESULTS

According to the method the repeatability values differed. The value of the repeatability coefficient estimated by ANOVA was lowest (0.26) while the highest was obtained by CPcov (0.63). The coefficients obtained by the methods of CPcor, and by AEcor, were 0.41 and 0.35, respectively. These results agree with Fonseca et al. (2004) and stated that the highest repeatability coefficient was estimated by principal components based on the covariance matrix (0.52), and the lowest by ANOVA (0.32). The mean values of the R2 were over 80% (92.74 to 83.00%), except by ANOVA (76%). Considering the method CPcov and a R2 of 80%, it can be concluded that three harvests would be necessary to infer the superiority of one progeny over another.

CONCLUSIONS & PERSPECTIVES

The differences in the values of the estimates of the repeatability coefficients indicate the importance of determining the most appropriate method. The estimate of the repeatability coefficient by principal components based on the covariance matrix was most appropriate, due to the minimization of the biennial effect on yield. The first three harvests were enough to select the best progenies of Arabica coffee.

References:

- Cruz and Regazzi 1997 Modelos biométricos aplicados ao melhoramento genético UFV, 390p.
- Fonseca et al. 2004 Crop Breeding and Applied Biotechnology 325-329, DOI: 10.12702/1984-7033

S1-P-23

Genetic diversity of Tanzanian advanced *Coffea arabica* germplasm and semi wild Ethiopian collection

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RATIONALE

Previous studies have revealed an extremely reduced genetic diversity in cultivated *Coffea arabica* L. The process of diffusion of *C. arabica* and the selection that followed have strongly reduced the genetic diversity present in its area of origin Ethiopia. The aim of the current study was to establish the genetic diversity of the Tanzania coffee research institute breeding pool of arabica coffee including the Ethiopian collection maintained at the institute.

METHODS

Ninety-one arabica coffee genotypes were selected from Ethiopian collection (45) and breeding fields at TaCRI Lyamungu (46). Leaf samples were collected from three trees per genotype for DNA extraction. DNA was extracted following the CTAB method at Sokoine University of Agriculture. Thirty primer sets were used in PCR amplification. The DNA reactions with the 30 SSR primer sets were performed using the touchdown PCR procedure. The reproducibility of the amplification products was checked twice for each primer. Fragments that were too difficult to score with certainty were excluded from the data analysis. Data analysis was performed using GenStat statistical software.

RESULTS

The observed heterozygozity was very high with a mean of 0.999 while the polymorphic information content generated ranged from 0.375 to 0.739 with a mean of 0.413. Three significantly diverse groups were produced from cluster analysis with the following percentages 56.75, 5.69 and 4.66.

CONCLUSIONS & PERSPECTIVES

High genetic diversity was revealed in this study hence the existing germplasm could continue to be used in improvement of the current superior coffee varieties and in development of new ones.

References:

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S1-P-24

Enhanced air [CO_2] and high temperature induced changes in membrane integrity and lipid composition in elite *Coffea arabica* L. genotypes

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RATIONALE

Coffee is among the crops threatened by climate change. High temperatures can have deleterious impacts in coffee plant physiology, particularly in membranes properties. In order to survive under extreme temperatures, plants must maintain adequate membrane fluidity and integrity, which requires dynamic changes in their profile. Membrane lipid metabolism plays a key role in plants tolerance to stress, and changes in fatty acid (FA) composition and saturation are amongst the first heat responses. In recent years important heat tolerance has been reported in coffee, and elevated air [CO_2] was found to strengthen plant vigour, and to mitigate heat impacts in leaf photosynthesis (1), membranes (2), and mineral content (3). Here we briefly describe changes induced by heat and/or elevated air [CO_2] in cellular membrane permeability and in chloroplast membranes lipid composition of elite *Coffea arabica* genotypes.

METHODS

Two-year-old plants from *C. arabica* (cvs. Geisha 3, Marsellesa and their hybrid), were grown under controlled conditions of relative humidity (70%), photoperiod (12h), temperature (25/20 ºC, day/night), irradiance (*ca.* 700 µmol m^{-2} s^{-1}), and two air [CO_2] (400 or 700 µL L^{-1}), without water restriction. Thereafter, temperature was increased from 25/20 ºC up to 42/34 ºC at a rate of 0.5 ºC day^{-1}, with 7 days of stabilization at 31/25, 37/30 and 42/34 ºC followed by a two week recovery period (Rec14). Quantitative and qualitative changes in chloroplast membrane lipids were assessed through the total fatty acid (TFA) content, individual fatty acid composition and global unsaturation degree. Cell membrane integrity was evaluated through electrolyte leakage and expressed as an injury index (I%) (for details see references 1-3).

RESULTS

In plants grown at 400 µL CO_2 L^{-1}, heat induced an electrolyte leakage rise in all genotypes. Under 700 µL L^{-1} CO_2, membrane permeability remained unaltered with temperature increase. Changes in chloroplast membrane lipids are under evaluation, and are expected to contribute to membrane stability and functionality.

CONCLUSIONS & PERSPECTIVES

Elevated [CO_2] can mitigate the negative impact of heat in membrane integrity, as inferred from injury index results. This can be related with greater membrane resilience associated to changes in lipid profile, which is being assessed in order to better understand coffee plant acclimation to heat.

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- 3- Martins et al. 2014. Clim. Change 126, 365-379. doi:10.1007/s10584-014-1236-7

S1-P-25

Genetic diversity of *Coffea canephora* assessed by using genetic parameters from the root system

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RATIONALE

The root system and the aerial part of coffee plants are interdependent (Partelli et al., 2014). Thus, the efficiency of nutrient and water uptake via the root system and the genetic variability of the crop is important to maintain coffee production in adverse conditions. A cluster analysis of 43 genotypes of *C. canephora* in the reproductive cycle was performed according to the root system distribution in different soil depths.

METHODS

The coffee plants assessed were composed of 43 different genotypes. Undisturbed soil samples were taken from a distance of 30 cm apart from the coffee stem in six soil depths (0-10, 10-20, 20-30, 30-40, 40-50 and 50-60 cm). Roots were washed, scanned and the resulting images were processed with the software Safira (Jorge and Silva, 2010) in order to quantify root surface area (mm2 cm-3), root length (mm cm-3) root volume (mm cm-3) and root diameter (mm). Dissimilarity between genotypes was calculated by the Mahalanobis distance. The cluster analysis was thereafter performed considering the Tocher's optimization method. The study was supported by Fapes, Capes and Cnpq.

RESULTS

The cluster analysis resulted in seven distinct groups, demonstrating a wide genetic variability within coffee genotypes, as the Tocher method favors the minimization of the intra-group distance and the maximization of the inter-group distance. Most (74.41%) genotypes were in the groups I and II, each of them comprising 16 genotypes. The group III was formed by four genotypes, followed by groups IV, V and VI, with two genotypes each, and group VII, with only one genotype. A similar cluster analysis for *C. canephora* was performed by Giles et al. (2018), enabling the identification of promising coffee genotypes with higher genetic variability.

CONCLUSIONS & PERSPECTIVES

There were genetic differences within the 43 genotypes of *C. canephora* assessed. There were genotypes with up to three times more roots than others, which can be promising alternatives to overcome water stresses and promote more nutrient uptake.

References:

- Jorge and Silva. 2010 Embrapa Instrumentação, 28p.
- Giles et al. 2018 Anais da Academia Brasileira de Ciências DOI 10.1590/0001-376520182017052
- Partelli et al. 2014 Pesquisa Agropecuária Brasileira DOI10.1590/S0100-204X2014000500004

S1-P-26

Assessment of Robusta coffee genotypes cultivated in the Brazilian Amazon

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RATIONALE

Robusta coffee (*Coffea canephora*) is widely grown worldwide. Several studies have investigated the crop variability (Giles et al., 2019; Partelli et al., 2019), mainly with genotypes of 'Conilon' aspects. However, the 'Robusta' coffee, which predominates in the Brazilian Amazon, is poorly studied in terms of crop breeding. This work aimed to assess different genotypes of *C. canephora* 'Robusta' cultivated in Rondônia State, within the Brazilian Amazon region.

METHODS

The work was performed in a farm located in Alta Floresta D'Oeste, Rondônia (12º08'51.86" S, 62º04'95.03" W, 440 masl and 28 ºC of mean annual temperature), which is within the Brazilian Amazon region. A total of 16 genotypes were studied (AS2, B80, AS7, AS1, V06, SV41, A106, J08, J25, B015, AS6, AS4, Z156, AS10, J03, L140), which were named by local farmers. The experiment was designed in randomized blocks with three replicates and 12 coffee plants in each replicate. Coffee plants were planted in May 2018 and the following characteristics were assessed in August 2019 (after 1.3 year): plant height, length of the plagiotropic branch, number of nodes, internodes distance and canopy diameter. The data were analyzed with the Skott-Knott test ($p<0.05$). The study was supported by Fapes, Capes and Cnpq.

RESULTS

All five assessed characteristics were significantly different for the studied genotypes. The number of nodes were the one with most variance, whereas the B015 genotype showed the lowest mean value (8.81) and the J25 genotype the highest mean value (14.68). In relation to plant height, it was observed two distinct groups: one ranging from 82.53 to 96.92 cm and other from 74.39 to 86.22 cm. Homogeneity in plant height favors some farming management, such as fertilization, pruning, semi-mechanized harvesting.

CONCLUSIONS & PERSPECTIVES

Initial assessments of the development of coffee plants within different genotypes are important to infer about the production capacity as well as the vegetative performance in future years. This pioneer study contributed to understand the morphological characteristics of the assessed coffee genotypes, which will be used as the basis for a future crop breeding program of Robusta coffee in the Brazilian Amazon region.

References:

- Giles et al. 2019 Scientia Horticulturae DOI: 10.1016/j.scienta.2018.09.038
- Partelli et al. 2019 Crop Breeding and Applied Biotechnology DOI: 10.1590/1984-70332019v19n4c68

S1-P-27

A possible underground exit: the histometric analysis of primary lateral roots aiming possible water deficit tolerance in coffee plants

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RATIONALE

Water deficit is challenging scenario that affect all culture crops. Plants have limited strategies to cope with stresses, which range from structural, morphological to anatomical and other features. The descriptive analysis and histometry of primary lateral roots from thirteen adult coffee varieties was performed as a base line for the seek for water deficit tolerance traits.

METHODS

Three plants from thirteen *Coffea arabica* accessions were selected based on the available information on their water deficit tolerance. Primary lateral roots were sampled and processed according to Pérez-Molina et al. (2021) for anatomical and histometric analysis.

RESULTS

There is a great variation of the histological traits of primary lateral roots of adult coffee plants conducted in only one environment. These traits variability is hypothesized to subsidize, at least in part, the differences in water deficit tolerance.

CONCLUSIONS & PERSPECTIVES

This variation is attributed to the coffee plants inherent phenotypic plasticity and possible interactions with microorganisms, developmental stage, soil properties among other features that may influence the differentiation of the tissues in roots.

Variable	Water stress		Cultivar																
(cross section areas - µm²)	Sensitive	Tolerant	P5B9P1	P7B13P14	Acauã	Bourbon	Catiguar.MG2	Catuaí.SH3	Geixa	IAC125RN	IPR100	Obatã	Sagarana.19	Sarchimor	Tupi	Water stress	Cultivar	F	R²	Pmodel
Total root area (	0.257 ± 0.022a	0.243 ± 0.019a	0.17 ± 0.005a	0.166 ± 0.021a	0.423 ± 0.044a	0.44 ± 0.045a	0.265 ± 0.039a	0.282 ± 0.042a	0.198 ± 0.032a	0.309 ± 0.076a	0.352 ± 0.112a	0.129 ± 0.001a	0.116 ± 0.011a	0.226 ± 0.033a	0.122 ± 0.032a	*	n.s.	5.9	0.2	***
Epiderme	0.034 ± 0.002a	0.036 ± 0.002a	0.031 ± 0.001a	0.027 ± 0.001a	0.052 ± 0.004a	0.047 ± 0.005a	0.036 ± 0.005a	0.035 ± 0.004a	0.031 ± 0.002a	0.036 ± 0.006a	0.041 ± 0.008a	0.025 ± 0.001a	0.024 ± 0.002a	0.032 ± 0.002a	0.019 ± 0.004a	*	n.s.	6.25	0.21	***
Cortex	0.206 ± 0.019a	0.208 ± 0.016a	0.126 ± 0.005a	0.129 ± 0.018a	0.343 ± 0.036a	0.365 ± 0.057a	0.209 ± 0.031a	0.223 ± 0.034a	0.156 ± 0.028a	0.255 ± 0.066a	0.284 ± 0.097a	0.097 ± 0.004a	0.085 ± 0.009a	0.175 ± 0.028a	0.094 ± 0.026a	*	n.s.	5.81	0.2	***
Estelo	0.017 ± 0.002b	0.019 ± 0.001a	0.013 ± 0e	0.01 ± 0.001d	0.029 ± 0.004d	0.028 ± 0.004c	0.019 ± 0.003d	0.025 ± 0.004bc	0.011 ± 0.002d	0.017 ± 0.004be	0.026 ± 0.008be	0.007 ± 0.001d	0.007 ± 0bd	0.019 ± 0.004a	0.009 ± 0.003be	*	n.s.	5.38	0.19	***
Xylem	0.006 ± 0.001a	0.007 ± 0.001a	0.004 ± 0a	0.003 ± 0a	0.011 ± 0.002a	0.01 ± 0.001a	0.007 ± 0.001a	0.011 ± 0.002a	0.004 ± 0.001a	0.005 ± 0.001a	0.009 ± 0.003a	0.002 ± 0a	0.003 ± 0a	0.008 ± 0.002a	0.003 ± 0.001a	*	n.s.	5.36	0.19	***
Phloem + Procambium	0.006 ± 0.001a	0.007 ± 0.001a	0.005 ± 0cde	0.004 ± 0cde	0.011 ± 0.001b	0.011 ± 0.002b	0.008 ± 0.001ade	0.008 ± 0.001ade	0.004 ± 0.001cde	0.007 ± 0.002abe	0.01 ± 0.003ab	0.002 ± 0c	0.002 ± 0c	0.007 ± 0.001acde	0.004 ± 0.001cd	*	n.s.	5.72	0.2	***
Number poles protoxylem	4.822 ± 0.137a	4.722 ± 0.113a	4.789 ± 0.122cdef	4.08 ± 0.294def	5.067 ± 0.179b	5.889 ± 0.281ab	4.778 ± 0.258cf	5.875 ± 0.307cef	4.5 ± 0.298cdef	4.923 ± 0.487acef	4.429 ± 0.388abc	3.455 ± 0.109de	4.158 ± 0.191de	4.941 ± 0.277cef	4.462 ± 0.312d	*	n.s.	5.58	0.19	***
Number cortex cells	8.358 ± 0.29a	8.19 ± 0.246a	7.192 ± 0.216cdef	6.854 ± 0.425cdef	10.1 ± 0.344b	10.188 ± 0.806b	8.646 ± 0.728adf	8.423 ± 0.702adef	7.518 ± 0.508cdef	9.077 ± 1.387abf	8.714 ± 1.24ab	6.227 ± 0.097ce	6.421 ± 0.314c	8.039 ± 0.465acdef	7.154 ± 0.541cde	*	n.s.	4.62	0.17	***
Pericycle	0.004 ± 0a	0.005 ± 0a	0.004 ± 0def	0.003 ± 0.001ef	0.006 ± 0.001c	0.007 ± 0.001bc	0.004 ± 0.001adf	0.005 ± 0.001abcd	0.003 ± 0ef	0.005 ± 0.001abdef	0.007 ± 0.003abc	0.002 ± 0e	0.002 ± 0e	0.004 ± 0.001adf	0.002 ± 0.001ef	*	n.s.	4.25	0.16	***

mean ± standard error; n.s.: P>0.05; *: P<0.05; ***: P<0.001; F: Fisher value: R2: coefficient of determination; Pmodel: model probability; Statistical significance among main effects was determined by two-way ANOVA; Means followed by different letters within rows (among tolerance or cultivars) indicate significant difference according to a Fisher's least significant difference (LSD, P<0.05).

Table 1. Lateral primary root histometry of 13 *C. arabica* seedlings cultivars categorized into two degrees of tolerance to water stress (sensitive or tolerant). Cultivars: P5B9P1, P7B13P14, Acauã, Bourbon, Catiguar.MG2, Catuaí.SH3, Geixa, IAC125RN, IPR100, Obatã, Sagarana.19, Sarchimor, and Tupi.

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S1-P-28

Resilience of C-assimilation to drought and/or heat conditions in *Coffea* spp.

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RATIONALE

Climate changes have been pointed to exacerbate water deficit and high temperature events, affecting crops sustainability, namely due to deleterious impacts in C-assimilation pathway that is the basis of crop productivity. Since limited data exists on drought and heat stress superimposition, a most common situation in nature, we unveil the sensitivity of key points of the photosynthetic machinery to these stresses in coffee plants.

METHODS

Seven-year-old plants from *Coffea canephora* cv. Conilon (Clone 153) and *C. arabica* cv. Icatu, grown under controlled conditions (RH: 70%; photoperiod: 12h; temperature: 25/20ºC (day/night); PPFD: ca. 700 µmol m^{-2} s^{-1}; air [CO2]: 400 µL L^{-1}), and under well-watered (WW) conditions, were gradually submitted to severe water deficit (SWD, Ψ_{pd} < -3.0 MPa). Temperature was then increased from 25/20 ºC up to 42/30ºC (0.5ºC day^{-1}), followed by a two week recovery period (Rec14). Photosynthetic impacts were assessed through gas exchanges (net photosynthesis, stomatal conductance; photosynthetic capacity, A_{max}), chlorophyll a fluorescence, thylakoid electron transport rates, and RuBisCO activity (see details in 1,2)

RESULTS

Single drought affected all gas exchanges and most fluorescence parameters in both genotypes. Yet, Icatu kept F_v/F_m and RuBisCO activity, and reinforced electron transport, denoting low non-stomatal limitations of photosynthesis, whereas photosystems (PSs) and RuBisCO activities declined in CL153. WW plants of both genotypes showed heat tolerance up to 37ºC (or 39ºC), but at 42ºC a limit was exceeded, with non-stomatal limitations on photosynthesis (A_{max}, F_o, F_v/F_m, and, especially, RuBisCO), although electron transport was mostly unaffected. Stresses interaction was found at the harshest conditions (SWD, 42ºC), with aggravated impacts in PSs and RuBisCO, but unregulated energy dissipation ($Y_{(NO)}$) was reduced by photoprotection increase ($Y_{(NPQ)}$). By Rec14 some aftereffects persisted in SWD plants of both genotypes.

CONCLUSIONS & PERSPECTIVES

Different tolerance was observed: Icatu was more tolerant to drought than CL153, whereas heat affected both genotypes mainly at 42ºC, stronger in SWD and Icatu. Photochemical components were highly tolerant to heat and stress interaction (42ºC), contrasting to RuBisCO that deserve special attention by breeders, to preserve coffee sustainability in climate change scenarios.

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S1-P-33

Enhanced air [CO_2] mitigates high temperature impact in elite *Coffea arabica* L. genotypes

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RATIONALE

Climate changes have been pointed to threat coffee crop sustainability. Yet, relevant coffee heat tolerance has been reported, and elevated air [CO_2] can mitigate heat impact on photosynthesis, mineral content, and increase productivity (1-3). We briefly assess the role elevated air [CO_2] in the photosynthetic heat resilience in elite *Coffea arabica* L. genotypes.

METHODS

Two-year-old plants from *C. arabica cvs*. Geisha 3 (G3), Marsellesa (Mar) and their Hybrid (Hy), grown under controlled conditions (RH: 70%; photoperiod: 12h; temperature: 25/20ºC, day/night; PPFD: *ca*. 700 µmol m^{-2} s^{-1}; air [CO_2]: 400 or 700 µL L^{-1}; without water restriction), were exposed to a temperature rise (25/20 ºC up to 42/30ºC, 0.5ºC day^{-1}), and a two week recovery (Rec14). Photosynthetic impacts were assessed through leaf gas exchanges (net and maximal photosynthesis, P_n and A_{max}; stomatal conductance, g_s), chlorophyll fluorescence, photosystems (PS) electron transport rates, and RuBisCO activity (2).

RESULTS

P_n was usually reduced at 39 ºC and, mostly, 42 ºC in all genotypes, but G3 showed minimal P_n and g_s values under both [CO_2]. The 700-plants showed increased P_n across genotypes and temperatures, and were less affected at high temperatures. Important P_n, g_s and A_{max} aftereffects were observed in all genotypes by Rec14. Yet, non-stomatal limitations (assessed by A_{max}) in P_n occurred only at 42 ºC and in the 400-plants of all genotypes (and G3 700-plants), in line with the photochemical efficiency of PS II (F_v/F_m, F_v'/F_m') and energy driven to linear electron transport ($Y_{(II)}$) values, which showed no reductions in 700-plants of Mar and Hy at 42 ºC. Only G3 (both [CO_2]) showed raised deregulated energy dissipation in PSII ($Y_{(NO)}$), suggesting photoinhibition.

Potential activity of PSII and I showed marginal impacts, irrespective of heat, genotype and [CO_2], whereas RuBisCO showed large activity reductions (only) at 42ºC, stronger in 400-plants (and worse recovery).

CONCLUSIONS & PERSPECTIVES

In normal air [CO_2] G3 was marginally more heat sensitive (P_n, g_s, A_{max}, PSII and RuBisCO), without clear differences between Hy and Mar.

High [CO_2] promoted photosynthetic performance (all temperatures and genotypes), and mitigated heat impact (less in G3), keeping relevant (P_n, RuBisCO) or unaltered (A_{max}, F_v/F_m, F_v'/F_m', $Y_{(II)}$) values at 42 ºC. RuBisCO thermal sensitivity deserves special breeders attention.

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S1-P-35

'Koffiestories' (Coffee stories): exploring and highlighting material and intangible heritage of coffee

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RATIONALE

Coffee is one of the most consumed beverages in the world. People come together through coffee at a wide range of occasions. Coffee connects people, literally and figuratively. Together with various partners, from the field of heritage and beyond, we investigate, register, document and value material and immaterial coffee heritage in Flanders and Brussels. Public-oriented activities highlight this coffee heritage from different perspectives, ranging from artifacts to history, from scientific knowledge to stories, traditions and rituals.

METHODS

The "Coffee Stories" project is a collaboration between Center for Agrarian History (CAG), Meise Botanic Garden, the Royal Association of Coffee Roasters in Belgium and Cera. Together with volunteers, 'the Coffee Noses', we are mapping the coffee heritage. The Coffee Noses are exploring public and private archives and collections, and they are looking for interesting stories on coffee. Public-oriented actions such as traveling exhibitions through Flanders and Brussels, workshops, dialogue tables, a publication and a Coffee Festival highlight the theme of coffee from different angles and for multiple audiences. The project will culminate in an exhibition informing the public on the coffee value chain, the cultural-historical heritage and past and current knowledge and research on coffee in Meise Botanic Garden.

RESULTS

The project brings together people with different background: botanists, historians, coffee professionals and volunteers experienced or not experienced on coffee. This resulted in an interesting cross-over of expertise and knowledge and resulted in a broad view on coffee which is accumulating in a website 'koffiestories.be', an exhibition, a book and other public oriented activities on coffee. Unfortunately most of the public events were cancelled due to COVID-19 but the travelling expo was reworked to an online expo.

CONCLUSIONS & PERSPECTIVES

Coffee is a topic which appeals a wide range of people with different cultural or ethnic background, or from different age and education level, because almost everybody has some kind of connection with coffee. On the other hand the knowledge on coffee of the average citizen is rather limited. Linking the general interest in coffee with different kind of activities, a broad public can be outreached and knowledge on coffee can be transferred. We are eager to share our experience and information with you. As the overview exhibition is in three languages (Dutch, French and English) the content can to be re-used in other countries and multiple settings.

References:

- Greet et al. 2020 Koffiestories een complete koffiegeschiedenis van brander tot Barista Draye G. & Paulissen P., Hannibal, 240 pp.

S1-P-36

CoffeeBridge: Bridging knowledge to the field. Evaluation of the agronomic and socio-economic potential of Robusta genetic resources as a cash crop in the Congo Basin

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RATIONALE

Coffee is an important cash crop in the Global South. Although Arabica coffee still counts for 60% of world production, the Robusta coffee share (ca. 40%) is expected to increase. Research on the diversity of Robusta genetic resources, its management in agroforestry systems, its nutrient requirements etc. is lagging behind. The CoffeeBridge project intends to fill this gap by working with a multidisciplinary team on Robusta in the DR Congo, an important center of origin of diversity for Robusta.

METHODS

The project focuses on the Yangambi area and has five objectives each with own methodology: 1) evaluating the local coffee chain, its sociological dimensions and economic relevance by socio-economic surveys; 2) characterizing and evaluating Robusta genetic resources, for cultivation and breeding by genetic, phenotypic, chemical and organoleptic assessments; 3) Evaluating and analyzing i.e. macro- and micro-nutrient deficiencies in the coffee leaves in different genetic lines under different agro-ecological management systems; 4) recuperate knowledge on Robusta kept in archives and grey literature, esp. on past agronomic research and on the origin of the Robusta coffee; 5) formulate recommendations and policy advice to improve agronomic practices and cropping systems in order to arrive at a sustainable and profitable coffee culture in Tshopo Province.

RESULTS

Although the project started only in 2020, we already notice some interesting developments: - the project raises new interest by different stakeholders in the Congolese coffee genetic resources like INERA and UNIKIS; - local scientists of different disciplines and different institutes are involved in research; - historians, economists, sociologists, biologists, agronomists, earth-scientists and an independent quality-grader are collaborating in a project with one common objective.

CONCLUSIONS & PERSPECTIVES

Although the project has hardly started we can already see some positive effects: it brings local scientists of different disciplines and institutes together and generates local and international awareness on local genetic resources, which were until recently deprived from research. Local involvement and awareness is essential in order to install an effective conservation of these genetic resources with huge international importance. The project contributes to the conservation and valorization of coffee genetic resources and strengthen local skills. Although the project focuses on Yangambi and the Tshopo Province, it will contribute to the global coffee challenge as Robusta coffee from the DR Congo is of global importance. Belgian Science Policy is financing the project.

References:

- Stoffelen et al., BGJournal 16(2), 2019, 5 pp. https://www.bgci.org/resources/bgci-tools-and-resources/bgjournal/

S1-P-37

BP 1001: The future variety for producing outstanding fine robusta coffee in Indonesia

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RATIONALE

Indonesia is the fourth largest producer of coffee whereas Robusta is the main crop. However, this crop is having low contribution for farmer income due to low price, and recent price crisis make sustainability of this crop is become more questioned. Fine Robusta is now a new trend for how Robusta to be consumed by Indonesian due to its better cup quality. Therefore, this situation is giving an opportunity for Robusta farmers to get a better price. In Indonesia, cup quality is one of our major criteria in the Robusta breeding. However, this is the first time we discovered a promising clone having distinct cup attributes compared to the generally Indonesian fine Robusta.

METHODS

The BP 1001 was resulted from the double crossing of two parents having different genetic group of (E x R) and (A x G) according to recent genetic differentiation by Merot-L'anthoene et al. (2019). The assessment of cup quality was done at medium altitude of 666 m asl in East Java. Cupping assessment followed the system developed by The Uganda Coffee Development Authority. Two years of cupping assessment has been suggested for this clone as it has a promising cup quality for the production of outstanding fine Robusta.

RESULTS

Indonesian fine Robusta is generally chocolaty, caramelized and spicy, while the BP 1001 has more floral and vanilla aroma, which has never been reported in Robusta, but is well known in Arabica. High sweetness score, mild, and acidity were making the cupping of this clone close to Arabica taste. Therefore, we define the outstanding fine Robusta when the cup attribute is reaching the taste similar to Arabica.

CONCLUSIONS & PERSPECTIVES

This result suggested the promising of BP 1001 as a clone for producing outstanding fine Robusta. Further selection to obtain similar clones to BP 1001 is needed regarding the naturally allogamous mating system of this species. We expect that the price for this kind of Robusta green bean will be more respected in the global market for better income of Robusta farmer. Therefore, sustainability for this crop will be more secure in the future, considering Arabica production is more vulnerable to the impact of climate change.

References:

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S1-P-38

Genetic diversity of *Coffea* spp. in Mozambique

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RATIONALE

The genus *Coffea* is native to Africa and comprises more than 125 species, of which only two dominate the world market: *C. arabica* L. (Arabica type of coffee) and *C. canephora* Pierre ex A. Froehner (Robusta type of coffee). In Mozambique, a community-based flagship project (GorongosaCoffee) is being developed in the Gorongosa Mountains, where an agroforestry management system with coffee and native trees was implemented, aiming to promote socio-economic development and revert deforestation. Integrated in this project, we are characterizing the introduced and native *Coffea* species based on distribution, taxonomic and genetic diversity studies of plants.

METHODS

Current distribution of the genus *Coffea* was assessed through herbarium data (IIAM, UEM, LMU and LISC), and validated through field surveys. Taxonomic analysis was based on morphological descriptors, and genetic barcodes (*mat*K, *rbc*L, and ITS). Moreover, Microsatelites (SSRs) were used to assess genetic diversity and gene flow between *Coffea* populations in Mozambique.

RESULTS

Genetic barcodes revealed the presence of cryptic species of *Coffea*, which is also supported by the presence of wide genetic variation values. A decrease in population genetic diversity was found between data obtained from herbarium vouchers and recently collected samples.

CONCLUSIONS & PERSPECTIVES

The studied populations of *Coffea* in Mozambique showed high levels of genetic diversity, quickly reacting to changes in landscape structure and anthropogenic pressure despite the loss of native germplasm.

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S1-P-40

Adapting temporary immersion tissue culture system to enhance mass production of *Coffea arabica L.* composite hybrid Ruiru 11 in Kenya

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RATIONALE

The use of Temporary Immersion System (TIS) of tissue culture to produce seedlings of Arabica coffee is well documented. The system offers great promise in mass production of coffee seedlings particularly those of hybrid varieties such as cultivar Ruiru 11 in Kenya. The system overcomes a number of constraints associated with hand emasculation and pollination and those inherent in the classical tissue culture techniques using solid media approaches.

METHODS

The current paper reports the results of a study aimed at adopting the use of RITA® bioreactors in the commercial production of hybrid Ruiru 11 in Kenya. The parameters studied included time efficiency, prolificacy of the TIS system as influenced by growth regulators combinations and acceptability of TIS generated seedlings.

RESULTS

The results indicated higher efficiency under TIS and high acceptability of the TIS-mediated tissue cultured seedlings. Both the genotype and growth regulator combinations had significant impact on the efficiency of the TIS system.

CONCLUSIONS & PERSPECTIVES

Making the TIS system work for composite hybrids such as *C. arabica* F1 hybrid, Ruiru 11 requires adjustment of the standard tissue culture protocols to accommodate variations in the responses of the constituent clones to tissue culture manipulation. The approach thereby resulted in optimum efficiency in the production of tissue culture seedlings was achieved.

References:

- Mwaniki et al. 2019 AJOB. DOI: 10.5897/AJB2019.16913.
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POSTERS

Session 2: Plant pathology & protection

S2-P-01

Induction of resistance in *Coffea arabica* against coffee berry disease using plant defense activator

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RATIONALE

Coffee berry disease (CBD) is a major cause of crop loss in Africa and a serious threat to Arabica coffee production. The use of fungicides for the control of CBD is usually effective. However, residual toxicity resulting from the wide spread use of fungicide shows the urgent need of other disease management alternatives. Moreover, chemical control is constrained by the present tendency towards organic coffee production. Breeding for resistance to CBD may provide a sustainable long-term management of the disease. However, it is highly influenced by the environmental conditions since the nature of resistance to CBD is believed to be quantitative (Van der Graaff 1981). Induced resistance may provide an alternative approach to plant protection especially for problems not satisfactorily controlled by conventional methods. Thus, we studied potential of exogenous application of plant defense activator in triggering systemic resistance in Arabica coffee against CBD.

METHODS

Effect of exogenous application of Monopotassium phosphate, Dipotassium phosphate, Jasmonic acid and Salicylic acid (SA) in triggering systemic resistance in Arabica coffee against coffee berry disease (CBD) was studied in vitro and in artificially inoculated cultivars with known resistance levels. The greenhouse experiment was designed in RCBD with factorial combination. Data on disease incidence, severity and AUDPC were collected and analyzed using SAS software

RESULTS

The results showed that the chemicals don't have direct antifungal effect at the concentrations tested except salicylic acid at its higher concentrations; however, all chemicals significantly ($p<0.05$) reduced severity of CBD in all coffee cultivars. There was a significant ($p<0.05$) interaction effect between PDIC and coffee cultivars on disease development. The highest disease reduction was observed with treatment of the hypocotyls with SA followed by Jasmonic acid. Application of SA at 10.0mM reduced the disease severity by >50% and 65% on standard susceptible 'cv 370' and moderately susceptible coffee cultivar 74110, respectively.

CONCLUSIONS & PERSPECTIVES

Exogenous application of SA can be used to trigger systemic resistance in Arabica coffee and could serve as a tool for the management of coffee berry disease. This clearly shows a potential avenue for the exploitation of plant defense inducing chemicals for the control of CBD as a safe alternative to synthetic fungicides. Further studies on application of PDIC with other management tools like biological control agents as part of integrated coffee disease management is crucial to reduce yield losses due to coffee berry disease.

References:

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S2-P-03

Anti sporulative potential of crude extracts of common medicinal plants against coffee leaf rust

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RATIONALE

Commonly used medicinal plants produce secondary metabolites that have been shown to have antimicrobial properties. The aim of this study was to determine the anti sporulative potential of crude extracts of *Allium sativum*, *Capsicum annuum*, *Piper nigrum*, *Lantana camara*, *Tagetes minuta*, *Zingiber officinale*, *Azadirachta indica*, *Salvia rosmarinus* and *Eucalyptus grandiis* against *Hemilleia vastatrix,* the causal agent of coffee leaf rust.

METHODS

The leaf rust infected coffee leaves with uniformly spaced lesions were detached up to the 4th node from the tip of the branch from the susceptible variety SL 28. The trial was laid out in an RCBD of 3 replications and was repeated twice. Plant extracts were prepared by mixing 10gms of either fresh or dried test medicinal plants in 100 mls of double distilled water, 70% methanol and 70% ethanol. The leaves were sprayed with the solvents containing the different extracts and incubated at room temperature for 21 days. The control was double distilled water, while standards were Copper Oxychloride at 0.38 gms/100mls and Cyproconazole at 0.05 mls/100mls.

RESULTS

The percentage of sporulation was scored on the 7th and 10th day, of which, sporulation was significantly higher on the 7th day (21.22%) than on the 10th day (11.93%). Sporulation was higher in freshly prepared plant extracts, in distilled water (23.23%), methanol (18.31%) and ethanol (15.53%), while using dry extracts, sporulation was 22.28%, 12.69% and 8.09% for distilled water, methanol and ethanol respectively. The average percentage of sporulation in distilled water was 42.10% while copper oxychloride and cyproconazole had 13.89% and 2.13% sporulation respectively. Among the extracts, *A. sativum* had the lowest sporulation of 2.66% as compared to *P. nigrum* 8.38%, *T. minuta* 9.95%, *Z. officinale* 11.92%, *S. rosmarinus* 13.77%, *C. annuum* 13.43%, *L. camara* 20.16%, *A. indica* 16.44% and *E. grandiis* 19.14%.

CONCLUSIONS & PERSPECTIVES

All the tested extracts have a potential to inhibit sporulation of *H. vastatrix,* especially *A. sativum* (garlic), however, preparing these extracts when dry is more effective. Furthermore, sporulation declines overtime as the extracts as applied. Further studies to determine the specific metabolites that inhibit sporulation as well as rates of application and performance under field conditions are on going.

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S2-P-05

Detection and counting of coffee berry borer (*Hypothenemus hampei*) using computer vision algorithm.

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RATIONALE

Counting coffee berry borer (CBB) from field traps is a tedious process due to the number of traps (BROCAP®) that may be operating, the important number of individuals that can be captured in each trap, and the litter remains (moss, insects, leaves, etc.) found within the traps. The sample processing time to identify and count CBB can be reduced with the use of photographs and computer vision algorithms.

METHODS

We trained Corigan, a pipeline developed for small object detection with high resolution images that uses yolo3 engineering as the object detection core. The photos used to train Corigan came from a study to assess the effects of adjacent land uses on CBB dispersion, which is currently in the field phase. We used photographs with a resolution of 4000 x 2672 pixels of 180 dpi and 24bit depth, taken with a Panasonic DMC-G2 camera with a light aperture of 3.5, exposure time of 1/125s and an ISO of 100 with a focal length of 14 mm. We used 318 photographs to train the model and 30 photographs to validate the efficiency of the model.

RESULTS

Out of a total of 897 CBB found in the 30 photographs, the model identified 851 CBB (true positives; recall = 95%), identifying 230 objects (false positive) that did not correspond to CBB (accuracy = 72%) and not recognizing 53 CBB samples (5% of false negatives). The model mean average precision (mAP) was 74%.

CONCLUSIONS & PERSPECTIVES

The use of the pipeline detection reduces processing time considerably. Litter remains difficulted detection of CBB by the pipeline. Therefore, we suggest the use of at least three different photographs of the same sample each one taken after stirring the sampling material, and the use of the average detected CBB from these three counts for subsequent analysis. We believe that the use of detection averages will reduce the error caused by false positive detection. We will continue to improve the accuracy of the algorithm to reduce false positives, and work to improve the pipeline to detect CBB different stages (egg, larva, pupa, adult).

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S2-P-06

First insights on the differential expression of adaptive candidate genes among contrasting pathotypes of *Hemileia vastatrix*

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RATIONALE

Hemileia vastatrix (Hv) causing coffee leaf rust, remains the major threat to Arabica coffee production worldwide. Under the constant risk of new Hv pathotypes emerging under a strong selective pressure, a better understanding of the adaptive genetic variation of Hv populations is needed. Since genes involved in coffee-rust interaction are expected to evolve under strong selection, this study aimed at the analysis of expression differences in putative candidate genes under positive selection that could provide insights on the pathogen virulence evolution.

METHODS

In this study, we selected seven candidate genes (CGs) with a signature of positive selection identified in a previous genome-wide scan integrated into a phylogenomics framework (Silva et al., 2015). To assess their potential association with Hv virulence profiles, expression analysis of CGs was initiated by qPCR for five isolates with contrasting pathotypes during compatible interactions at three key stages of the infection process [penetration stage (24hpi), >50% infection sites with haustoria (96hpi) and a stage preceding sporulation (8-11dpi)].

RESULTS

qPCR data analysis showed that most of the CGs studied were mainly activated during the penetration phase, suggesting their involvement in the early stages of the infection. Comparison between Hv isolates revealed significant differential expression of CGs involved in signaling for Hv1427 (lowest virulent pathotype) at the two first key stages of infection. However, in general, largest differences of expression were detected at different infection stages for each isolate, rather than among isolates at each time point.

CONCLUSIONS & PERSPECTIVES

First results revealed differences in expression among isolates, either regarding up or down-regulation at different infection stages, or the level of expression at each time point studied, suggesting that these candidate genes may be involved in Hv virulence. Putative causal relations and possible adaptive significance is being assessed. This study provides a first insight on the molecular variation underlying virulence divergence in coffee rust.

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S2-P-07

Significance of minor coffee diseases in Ethiopia

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Ethiopia has high genetic diversity and favourable ecological conditions for growing coffee in wide area coverages. However, the country has not fully exploited its vast natural endowment of genetic and unique natural coffee forest environments due to several factors. This is partly due to the limited use of improved technologies and best practices by most small-holder farmers, the widespread and prevalence of insect pests, diseases and coffee weeds. Coffee suffers from a range of major diseases including coffee berry disease (CBD), coffee wilt disease (CWD) and coffee leaf rust (CLR). Currently bacterial blight of coffee (BBC) and coffee thread blight which is caused by *Pseudomonas syringae pv garcae van Hall* and *Corticium koleroga* which were considered as minor coffee disease become an emerging constraint in major coffee growing areas of Ethiopia. Bacterial Blight of coffee attacks coffee leaves, branches and shoots with characteristic blight symptoms. The infected branches and shoots start die-back from the point of infection towards the tip while coffee berries on infected branches are also completely destroyed leading to total crop failure. Currently the spread of the disease was reported at Gedeo, Sidama, Wolita and Kembata-Tembaro Zone of SNNP regional state. Moreover, Coffee thread blight is becoming an important disease at most major coffee producing areas of Ethiopia. Southwest part of Ethiopia is one of the major coffee producing areas where the damage by coffee thread blight is frequently reported. The disease noticed mainly on the succulent twigs, berries, stems and leaves of the coffee trees. The existence of variation in terms of tolerance against BBC and thread blight among land races and released coffee cultivars was observed in Ethiopia. Integrated disease management of these diseases which includes cultural management (using wind break, manure/fertilizer application, pruning and sterilization of farm implements) and sensitizing and use of information systems, and dissemination of information are equally important. Further more in-depth research on the disease epidemiology, detailed characterization of the bacterial and fungal pathogens and management practices are required along with exploring and developing resistant varieties.

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S2-P-09

Metabolomics analysis of interaction of *Coffea arabica* resistant and susceptible to *Meloidogyne paranaensis*

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RATIONALE

The importance of nematodes as factors limiting coffee growing is increasing. The spreading of species *Meloidogyne paranaensis* is a threat to the coffee growing in Brazil and Latin America. Given the difficulty of crop management in infested areas, breeding programs have focused on the selection of cultivars resistant to this nematode. Besides the knowledge of resistance sources to *M. paranaensis* in *Coffea arabica*, little is known about resistance mechanisms involved. A histopathological study indicate impairment of giant cells development in resistant coffee plants derived from germplasm Amphillo at 14 days after inoculation (DAI) (Alves et al. (2019). The present work aimed to identify resistance-related metabolites of the *Coffea arabica* genotype 16-6-I, derived from the germplasm Amphillo to *Meloidogyne paranaensis*.

METHODS

The genotype 16-6-I resistant to *M. paranaensis* (R) and cultivar Catuaí Vermelho IAC 144, susceptible (S) were evaluated in this study. Plants were inoculated with 2500 J2 de *M. paranaensis*. Leaves samples were collected of three plants at 2, 4, 8, 14, 22, e 32 DAI (days after inoculation). Leaf extracts were obtained according to Kim et al. (2010) and were analysed by hydrogen nuclear magnetic resonance.

RESULTS

Among the substances identified by nuclear magnetic resonance analysis, the most significant change observed was the increase of trigonelline concentration at 14 DAI in susceptible plants, probably a late response of resistance. Higher sucrose concentrations were observed from 8 - 22 DAI in resistant plants, possibly blocking the action of cytokines, indispensable for the formation and maintenance of feeding sites.

At 14 DAI, slight differences were also observed in caffeine, chlorogenic acid, α-glucose, β-glucose concentration between resistant and susceptible plants, and generally maintained until 22 DAI.

CONCLUSIONS & PERSPECTIVES

These results complement the study of Alves et al. (2019) highlighting that resistance responses are full active at 8 DAI. Future works in gene expression and physiology must be conducted in order better understand this pathosystem.

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S2-P-10

Climate change; its impacts on coffee production of Ethiopia and mitigation

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RATIONALE

Climate change has become an internationally recognized problem. One of its impacts have been a decrease agricultural production in certain areas. The impacts of global warming are already being seen as temperature rising steadily in the World and likely to affect our coffee growing areas. In Ethiopia also Farmers observing a longer, more extreme dry season and more intense rain as a result yield reduced. It is also clear that climate change can significantly affect the genetic diversity of coffee gene pool. Finding out facts and generating information to mitigate climate change is critical.

METHODS

Results of different research disciplines reviewed and secondary data assessed.

RESULTS

As Ethiopia is the center of origin and diversity for arabica coffee a loss of gene pool is a great loss not only for the country it is also mess for the whole world. Following the climate change the disease and insect pest effects on Ethiopian coffee production is showing visible problem. As a current emerging problem Bacterial Blight of Coffee (BBC) and Thread Blight of Coffee (TBC) prevalence considered in this review. In addition insect pest problems like coffee Thrips and Blotch Leaf Miner; which were not serious previously now becoming visible constraint. Abiotic factor like occurrence of frost is also facing a problem intermittently.

CONCLUSIONS & PERSPECTIVES

In general to mitigate the prevailing climate change effects on coffee initiated conservation efforts need to be scaled to capture all variability from future risks of climate change. Supporting innovation and implementation of climate-resilient technologies is necessary. Enhancing the awareness and capabilities of coffee-farmers to deal with climate change is still critical. Ethiopia have not only variable genetic diversity it has also geographic diversity where previously areas which are not known for their coffee production are now growing coffee considerably. This adaptation allows coffee producers to both reduce the negative impacts of climate change and benefit from new opportunities that might arise from it. Appropriately and fairly valuing climate-smart solutions for coffee production and sustainable land use needed as coffee is one of the crop useful for conservation agriculture. Generally if appropriate measures are taken to use coffee genetic resources and the environment, Ethiopia has best resilience to produce coffee because majority of our coffee is grown under shade tree.

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S2-P-11

Comparative characterization of resistance proteins in *Coffea* species

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RATIONALE

Plants have evolved highly complex defense mechanisms to protect themselves from various pathogens. One of such mechanisms is activated when a given pathogen-derived molecule, called effector, is 'specifically recognized' by plant receptor proteins encoded by R genes. Understanding the role of R genes in plant defence is of utmost importance because the transference of particular R gene variants to susceptible individuals may confer resistance to specific pathogens. Dozens of thousands of R genes have been identified to date in hundreds of plant species and several tools are available to characterize R genes in a given sequence. The unprecedented power of next generation sequencing to produce genomic data has resulted in the release of entire genomes for dozens of plant species.

METHODS

We have identified R genes present in the available whole reference genome sequences of *Coffea canephora*, *C. eugenioides*, and *C. arabica*, as implemented in DRAGO2 (https://github.com/sequentiabiotech/DRAGO2-API). Then we performed reciprocal BLAST searches using these R genes and their flanking regions to identify homology and paralogy across coffee species.

RESULTS

The number of R genes found in the three coffee species ranged between 2345 for *C. canephora* and 5218 for *C. arabica*. Despite a basic analysis showed that the number of homologous proteins between species pairs ranged between 1350 (for the comparison *C. canephora-C. eugenioides*) and 1700 (for *C. eugenioides-C. arabica*), a deeper characterization of the genomic regions containing R genes provided additional details about the ancestry of these genes in the three species.

CONCLUSIONS & PERSPECTIVES

Our results shed light into the molecular structure, distribution, and ancestry of R genes in *C. arabica*, *C. canephora*, and *C. eugenioides*. Using this knowledge, we aim to understand the molecular mechanisms underlying disease resistance in *Coffea* species.

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S2-P-12

A first insight on the *Hemileia vastatrix* urediniospores proteome

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RATIONALE

Coffee leaf rust (CLR) is caused by *Hemileia vastatri*x, an obligate biotrophic basidiomycota. CLR is one of the most common and damaging diseases of Arabica coffee, leading to yield losses of more than 35%. The high pathogenic variability in CLR has been recognized for long as it is associated with the breakdown of coffee resistance. Distinct *H. vastatrix* races have been identified through the differentiation of isolates on a set of 27 coffee plants (differentials), but their molecular characterization has been limited. In order to shed light on *H. vastatrix* diversity, a high-throughput analysis of the proteins from the urediniospores of *H. vastatrix* races was undertaken.

METHODS

Urediniospores of three *H. vastatrix* races were used: race VI (v?-unknown) non-pathogenic to all known *C. arabica* genotypes and races II (v5) and XXIV (v2,4,5) pathogenic to the majority of *C. arabica* genotypes. The proteins from urediniospores were extracted with SDS buffer and purified using the 2D clean-up kit (GE Healthcare). Protein identification was achieved by nanoLC-MS/MS and searching in the NCBIprot Hv33_RustFungi_annot database (Porto *et al.* 2019). Protein functional annotation was based on Blast2GO/EggNOG analysis.

RESULTS

This approach allowed us to identify significatively 1874 proteins and obtain functional annotation for about 70% of them. Considering the biological processes identified, proteins were classified as being involved mainly in DNA integration, RNA-dependent DNA biosynthesis, proteolysis, translation, oxidation-reduction process, primary metabolism, nitrogen compound metabolism and macromolecule metabolic processes. A principal component analysis revealed a clear separation of the three rust races.

CONCLUSIONS & PERSPECTIVES

Our work provides the first tentative approach to establish the proteome of *H. vastatrix* urediniospores. In addition, a comparative analysis of the proteomes of the urediniospores of three *H. vastatrix* races is being undertaken in order to identify protein factors that clearly differentiate the three rust races and may contribute to pathogenicity.

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S2-P-13

Cercosporin quantification in *Cercospora coffeicola* isolates by spectrophotometry and high-performance liquid chromatography: a comparative analysis

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RATIONALE

Coffee brown eye spot (BES) is an important disease, caused by the agent *Cercospora coffeicola.* BES affects all coffee growing stages, from seedlings in nurseries to adult plants, leading to losses up to 30%. Cercosporin is activated by light toxin produced by *Cercospora* and has been detected in some *Pseudocercosporella* and *Colletotrichum* species. Cercosporin has been considered a possible component of aggressiveness in coffee plants. Sensitive and accessible methods for cercosporin detection and quantification are required for better understanding its role in plant pathogenesis. A comparative analysis of cercosporin quantification by spectrophotometry (SPEC) and high-performance liquid chromatography (HPLC) was performed in *Cercospora coffeicola* isolates

METHODS

Teen monoconidial isolates *C. coffeicola* were cultured in 9 cm diameter Petri dishes containing 9 mL PDA (potato dextrose agar) culture media and were maintained in BOD incubator (Bio-Oxygen Demand) under 12 h photoperiod at 25 0C for 12 days.

Four mycelial plugs (6 mm diameter) removed from each petri dish colony were immersed in 8 mL of 5N KOH and maintained in the dark for 4 hours. Cercosporin determination by SPEC was performed at 480 ηm (Jenns et al. 1989, Yamazaki and Ogawa 1972) and by HPLC was performed based on Gunasinghe et al. (2016).

RESULTS

The cercosporin production varied among the *C. coffeicola* isolates, ranging from 0.01 to 34.52 µM. When comparing cercosporin quantification obtained by SPEC and HPLC for each isolate, although SPEC quantification was always higher than HPLC no significantly differences were obtained (Tukey test, $p \leq 0.05$). Furthermore, the Spearman correlation showed a significant linear association between cercosporin quantification values obtained by SPEC and HPLC methods (r=0.94).

CONCLUSIONS & PERSPECTIVES

Both methods are equally valid for the cercosporin evaluation from *C. coffeicola* grown *in vitro.* The HPLC is an analytical procedure more sensitive, accurate and precise. On the other hand, SPEC is a fast and simple technique involving ordinary lab equipment; the choice of technique depending on lab facilities available.

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S2-P-15

Salicylic acid induces plant systemic resistance to against anthracnose in coffee

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RATIONALE

Anthracnose is a major disease which has severe impact on coffee yield and quality. The outbreak of Anthracnose usually started during coffee flowering and fruit bearing stage, so the early stage of coffee production is a crucial time point to prevent the disease. Salicylic acid (SA) is a signaling molecule in plants which can induce SAR pathway and against pathogens. The purpose of this study is to induce the systemic disease resistance of coffee trees at the early stages, so as to increase the effect of preventing and controlling coffee anthracnose in the field.

METHODS

The experiment was conducted on two coffee estate in Southern Taiwan. Tested varieties were *Coffea arabica L.* SL34 and Gesha, which are the two major varieties in Taiwan. Farmers often use Azoxystrobin before and after raining season to prevent the outbreak of anthracnose, we introduce SA into conventional fungicide practice and test the effect of SA to control anthracnose (*Colletotrichum* spp.) in the field. We evaluated the Infection Rate on leaf and fruit by calculated the infected leaves, branches and fruits. The treatment group used 100 mg/L SA, foliar spray, during the flowering stage and fruit developing stage; Trees without SA as the control group. Field Trial A was conducted at altitude 1450 m with 5 year-old SL34 planted under 48% of shade and 0% of shade respectively. Therefore we separated the field into two blocks according to the light condition. Field Trial B was conducted at altitude 1200 m with 3 year-old Gesha planted under 0% of shade. Both experiments were investigated in 2020.

RESULTS

In Field Trial A, SL34 planted under shade condition with SA treatment had 1.30% of Total Infection Rate on leaf, and 5.00% without SA; In open sun condition, SA treated group had 4.40 % of Total Infection Rate on leaf, and 10.83% without SA. In Field Trial B, Gesha trees with SA treatment had the Total Infection Rate of 16%, and 49.1% without SA. Both experiments in open sun condition had significant effect of reducing infection rate when treated with SA. We also investigated the severity of fruit on SL34. Under shade condition, trees treated with SA had 5.97% of Infection Rate on fruit, and 7.5% without SA; 4.66% with SA, and 11.53% without SA in open sun condition.

CONCLUSIONS & PERSPECTIVES

Use of SA with fungicide at the early stages of coffee production can reduce the severity of Anthracnose infection, especially in open sun condition. We think it is a promising way to improve plant disease resistance without affecting conventional agricultural practice.

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S2-P-16

A survey of *Hemileia vastatrix* physiological races emerged in the coffee germplasm resource nurseries located in the main coffee regions of China

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RATIONALE

The Yunnan Province, the main coffee region in China, has been responsible for more than 90% coffee annual production of China. The first report of the coffee leaf rust (CLR), a disease caused by the fungus *Hemileia vastatrix*, in China took place in 1998. In 1990's the traditional susceptible coffee cultivars were replaced by cultivars S. 288 and Catimor. Over the past few years the main coffee cultivar *Catimor* in China has become susceptible to CLR and the physiologic race XXXVII ($v_{2, 5, 6, 7, 9}$) was identified as predominant in the main coffee regions. Most of the important coffee germplasm resource nurseries, which involve some genotypes collected from worldwide and some newly breed resources are located in the main coffee cropping regions of China. In this way, these coffee germplasm nurseries can increase the adaptative evolution of *H. vastatrix* through a high selection pressure.

METHODS

Rust samples were collected on 4 representative coffee germplasm nurseries distributed in the southern of Yunnan Province during epidemics of CLR in 2018. Their spectra of virulence were evaluated on a set of coffee differentials at Centro de Investigação das Ferrugens do Cafeeiro (Oeiras, Portugal).

RESULTS

A total of 57 CLR samples were divided into 4 groups: Group1, WS-MLPCB (23°11′N 104°55′E, 550m); Group2, PE-YAUCB (N 22°47′45″, E 100°58′59″,1320m); Group3, PE-ACB (N 22°37′36″, E 100°59′50″, 1010m); Group4, from RL-MACB (24°01' N 97°51' E, 1260m). Twenty-seven new pure cultured isolates were derived from single rust pustules taken from the contrasting sub-groups. In this sampling, the races XXXVII ($v_{2, 5, 6, 7, 9}$) and XXXIV ($v_{2, 5, 7}$ *or* $v_{2, 5, 7, 9}$) predominated. Moreover, a new race with the virulence genes $v_{2, 5, 6, 7}$ was characterized.

CONCLUSIONS & PERSPECTIVES

The occurrence of the new race ($v_{2, 5, 6, 7}$) indicates the coffee germplasm nurseries can be a potential threat to development of new rust races to the current coffee cultivars. Monitoring the occurrence, dynamics, distribution and pathogenicity of *H. vastatrix* is essential to quickly detect and track new races as well as provide a necessary information for resistant variety breeding.

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S2-P-17

Preliminary evaluation of coffee germplasm collection for resistance to root-knot nematodes (*Meloidogyne* spp) in Tanzania

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RATIONALE

Root-knot nematodes (*Meloidogyne* spp) is one of the important pest devastating Arabica coffee in Tanzania. A survey conducted in 1984 by John Bridge identified root-knot nematodes causing yield losses estimated to be more than 20% especially in the Northern part of Tanzania. The survey was carried out in 2019 to identify the resistant coffee lines in the germplasm collection at TaCRI, Lyamungu.

METHODS

The root-knot rating index of 0-10 as described by Bridge and Page (1980) was used to identify the resistant coffee lines in the Variety Collection Extension (VCE) at TaCRI, Lyamungu, Kilimanjaro. Total of 108 coffee lines replicated into 16 trees each was assessed by scoring for nematodes infestation after observation of roots removed from standing coffee plants in the field.

RESULTS

Results indicated different levels of infestation by root-knot nematodes observed in different coffee lines ranging from "0" to "9" (root-knot nematode rating index). Most of the lines were found to be susceptible to the nematodes, including some Robusta coffee. No coffee lines which was observed to have all roots severely knotted with nematodes ("10" rating index). Among the 108-coffee lines, crosses and selections screened in the field, ten of them which includes; Conuga - 261/4-2-4/1030/3 Clone, Hybrid 262/6 387 (Arab x Dew) Clone (A), C. can. 1604/5 Bengelan - col. l. Clone, Arab. Hyb. F1 H.127/ 6-213/18 (H.39) x 87/1 (Geisha), Arab. Hyb. F1 H.50/4-93/6 (H.66) x 134/4-23 (S.12 Kaffa) clone, Arab. Hyb. F1 26/13-19/1 (Caturra) x 832/2-13 (Hybrid of Timor clone), 34/63 (C.R 1345/5 Racemosa), 32/63, 30/63 and 29/63 were found to have no galling with root-knot nematodes ("0" rating index) and one coffee line, *Dewevrei var. excelsa*-879 was found to have very few galls.

CONCLUSIONS & PERSPECTIVES

The eleven coffee lines which indicate to be resistant ("0" galling index) to *Meloidogyne* spp may be used in the breeding programme in the future if proved to be resistant. Evaluation to confirm the level of resistance of these coffee lines is underway by planting the seedlings in the soil infested with *Meloidogyne* spp and examining the infection.

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S2-P-20

Diversity in the regulatory region of genes in the S_H3 *locus*

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RATIONALE

The S_H3 *locus* is implicated in plant defense against the leaf rust disease caused by the fungi *Hemileia vastatrix*, which reduces coffee plant production. A cluster of genes coding for NB-LRR proteins is allocated to this *locus*. Investigating the regulatory region of these genes can be worthy in knowledge and economically.

METHODS

The cis-acting elements (cis-els, conserved motifs for the interaction with transcription machinery) in the promoters (2000 pb up-stream translation start) of 20 genes allocated to paralogous S_H3 *loci* in four *Coffea* spp. genotypes were identified and counted (www.dna.affrc.go.jp/place). Data were analyzed using Sigma Plot.

RESULTS

Cis-els more frequently identified are depicted in Fig. 1 (in average, 3.5 times/promoter for SEF4MOTIFGM7S up to 14.1 times for CAATBOX1). Genes diverged for the frequency of cis-els. At least one gene/genotype was found in the highest cis-el frequency class (11.46 < N3, A2, C1, B8 > 11.9 copies of each cis-el), probably displaying complex patterns of induction. Genotypes did not diverge.

CONCLUSIONS & PERSPECTIVES

Information presented here are going to be correlated with the transcription level of different genes.

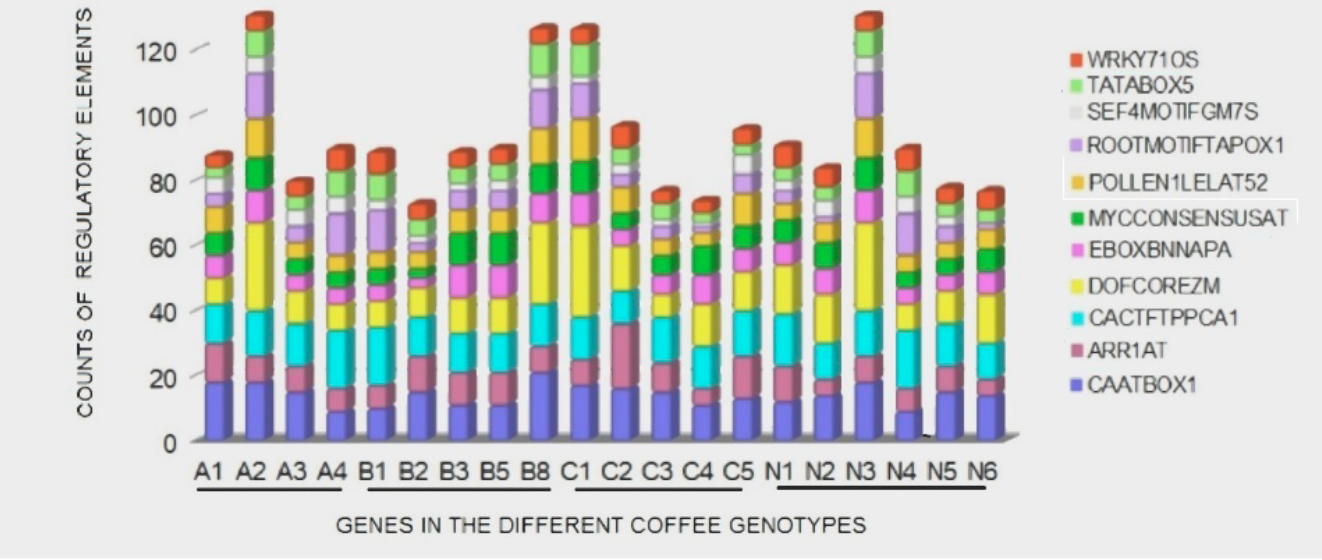

Fig. 1. Cis-acting elements in the regulatory region of genes clustered in SH3 paralogous loci of different Coffea genotypes. A1-4 and N1-6: C. arabica; B1-8: C. eugenioides; C1-5: C. canephora genes. Besides ROOTMOTIFTAPOX1 and CACTFTPPCA, which are possibly involved in tissue specific transcription in the roots and mesophyll cells, and WRKY71OS, which is involved in SA-triggered defense, most of the cis-elements identified are transcription enhancers.

S2-P-21

Mikania micrantha: its management in coffee plantations of North East India

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RATIONALE

The North East Region of India offers vast potentiality for production of premium coffee and it can help to wean the indigenous people from shifting cultivation, sustain the bio-diversity and to improve the socio-economic status of the people. Infestation of most of the coffee plantations in the region by the invasive perennial vine *Mikania micrantha* reduced productivity of coffee, induced black rot disease and fruit drop which made coffee cultivation economically unsustainable. However, the problem has not received due importance by the stakeholders and so far no recommended practice has been developed for management of the weed.

METHODS

The study was conducted in 2016-17 and 2017-18 at the Regional Coffee Research Station, Diphu, India to develop management strategies against the *M. micrantha*. Attempts were made to break dormancy of the weed seeds and hasten the germination by soil application of gibberellic acid. The germinating weeds were then killed by pre and post-emergent application of either Oxyfluorfen and Glyphosate or Oxyfluorfen and Glyphosate+ 2,4-Dichlorophenoxyacetic Acid.

RESULTS

Application of gibberellic acid (500 ppm) on soil significantly increased seed germination of *M. micrantha* over 250 ppm and control without effecting growth and yield of coffee. Improved vegetative and yield attributing characters, higher yield, net return and benefit-cost ratio of coffee was recorded in the treatments comprising Oxyfluorfen (0.29 kg ha-1) followed by Glyphosate (0.99 kg ha-1) and Oxyfluorfen (0.29 kg ha-1) followed by Glyphosate (0.99 kgha-1) + 2,4-Dichlorophenoxyacetic Acid (0.73kg ha-1) as compared to the weedy check.

CONCLUSION & PERSPECTIVES

It could be concluded that soil application of gibberellic acid did not have any effect on growth and yield of coffee. *M. micrantha* on coffee could be managed effectively by the application of Oxyfluorfen (0.29 kg ha-1) followed by Glyphosate (0.99 kg ha-1).

It was the first detailed study on effect of *M. micrantha* on coffee and its management. The findings will help to overcome the problem caused by *M. micrantha* on sustainable coffee production in the infested areas.

S2-P-22

Characterization of *Colletotrichum* species causing anthracnose in coffee (*Coffea arabica*) plantations in Costa Rica

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RATIONALE

Colletotrichum spp. infect coffee leaves, stems and berries. Several species of *Colletotrichum* have been identified in coffee crops worldwide, of which the specie of greatest concern is *C. kahawae* subsp. *Kahawae* that causes the anthracnose of green berries, which is currently restricted to the African continent. Recently, in Costa Rica a wide variability in symptoms and aggressiveness of anthracnose has been detected, affecting many of the coffee regions.

Study was performed to identify and describe *Colletotrichum* species complex present in coffee crops in Costa Rica.

METHODS

Colletotrichum spp. was isolated from coffee leaves, branches and berries with anthracnose lesions collected at five coffee regions in Costa Rica. Single spore cultures of each isolate were obtained and fifteen representative *Colletotrichum* strains were selected for molecular identification by partial sequencing of actin, β-tubulin , GAPDH, glutamine synthetase (GS) and ITS region. Multiple sequence alignment was performed using Clustal W in MEGA X (Kumar et al, 2018). Concatenated alignments of all data were used for phylogenetic analyses by maximum likelihood method with TN93+G model of nucleotide substitution and 500 bootstrap replications.

Biochemical test was done for four isolates according to the method of Bridge et al. (2008) to discard CBD agent *C .kahawae* subp. *kahawae.* Also, a morphological description for the identified *Colletotrichum* species were performed by description of colony growth, conidia and apressoria size and shape, perithecium and ascospores formation (Weir et al, 2012).

RESULTS

Morphological, biochemical and molecular analysis help us to identified six *Colletotrichum* species. We identified *C. fructicola, C. siamense, C. costarricense, C. karstii, C. theobromicola* and *C. kahawae* subsp. *ciggaro,* being *C. theobromicola and C. kahawae* subsp. *ciggaro* the most frequent species identified.

CONCLUSIONS & PERSPECTIVES

This is the first report of the *Colletotrichum* species complex causing anthracnose in coffee crops in Costa Rica. These data will provide the basis for the study of the *Colletotrichum* species complex and its relation with the variability in the aggressiveness and symptomatology of anthracnose symptoms observed in coffee crops in Costa Rica in recent years, but more studies about the biology and the epidemiology of *Colletotrichum* species should be carried out.

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S2-P-23

Relative water content in *Coffea arabica* leaves in response to Brazilian *Pseudomonas syringae* pv. *Garcae* infection

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RATIONALE

Studies on defense mechanisms against pathogens are hampered by numerous biological processes in plants. However, it is recognized that there are physiological mechanisms involved in the gene-to-gene response, in order to quickly limit the growth of the pathogen see Duniway (1973). In this sense, one of probably responses is the interruption of water flux at the infection site (Freeman and Beattie, 2009). A study was conducted to establish whether the response *of Coffea arabica* to bacterial halo blight induces a reduction in vascular flow at inoculation sites.

METHODS

Seedlings of two wild accessions of FAO collection from Ethiopia, selected according to resistance or susceptibility to bacterial halo blight, had young leaves infiltrated with bacterial suspension of *Pseudomonas syringae* pv. g*arcae* strain adjusted to contain 108 CFU.mL, two control groups were established, with plants infiltrated with distilled water and healthy plants. Six, 24 and 48 hours after the infiltrations, leaf discs were cut out and the relative water content (RWC) was estimated according to the Barrs (1968) methodology. Obtained data were compared by analysis of variance considering the factorial arrangement 3x2x3 (three treatments, two genotypes and three periods after inoculations).

RESULTS

It was observed that the treatment infiltrated with *P. syringae* pv. *garcae* suspension showed lower relative water content. This aspect indicates that, regardless of the reaction of the genotype to bacterial halo blight, infiltration with the pathogen results in a reduction in the RWC at the inoculations sites. If, the reduction of vascularization at the inoculation site may indicate the occurrence of hypersensitivity reaction (HR) and consequently the resistance response, as demonstrated by Freeman and Beattie (2009) in the *P. syringae* - *Arabidopsis thaliana* pathosystem, the fact of equality between treatments vs. reaction of the genotype, may be associated with the absence of this mechanism of resistance in the interaction *C. arabica – P. syringae* pv. *Garcae* Brazilian strain.

CONCLUSIONS & PERSPECTIVES

There was no induction of HR in a coffee genotype resistant to Brazilian *P. syringae* pv. *garcae* strain in the period between the moment of infiltration up to 48 hours after inoculation of the pathogen. Therefore, a more efficient mechanism in relation to the occurrence of HR must be associated with the defense mechanism of coffee trees. Knowledge of the defense mechanism of *C. arabica* against the causal agent of the bacterial halo blight will be use in breeding programs that seek to incorporate the durable resistance to pathogen.

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S2-P-24

Portraits of a mycoparasitic fungus: *Calonectria hemileiae* – a newly discovered coffee leaf rust antagonist, with particular reference to it antifungal metabolites

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RATIONALE

The fungus *Hemileia vastatrix* (Hv) is the etiological agent of the worst disease of coffee – coffee leaf rust (CLR) – infamous for having devastated the coffee industry of Ceylon (Sri Lanka) in the 19th century and, since the early 2010s, for the major epidemics in northern South America and Central America. Among other national and international organizations, the World Coffee Research reacted to this crisis on several fronts, including funding research aimed at revisiting the use of natural enemies as biocontrol agents of Hv. A multinational team of researchers was assembled and focused its studies on fungi growing as mycoparasites on Hv pustules and endophytes (growing inside healthy coffee plant tissues, and possibly providing protection against biotic and abiotic stress). A highly diverse mycobiota was found in Africa and in Brazil and is being progressively described (Colmán et al. 2021; Rodriguez et al. 2021). Among them was the new species *Calonectria hemileiae* (Ch) – collected in Brazil on Hv pustules. This was selected, during *in vitro* tests preliminary screenings, for the best candidates for CLR biocontrol. Very promising results were obtained, some of which have been recently published (Salcedo-Sarmiento et al. 2021). Those results will be discussed. A continuation of the studies, now involving the evaluation of metabolites produced by Ch, generated additional information, which will be presented here.

METHODS

Filtrates, from Ch-colonized liquid CZAPEK medium, were obtained and tested for their potential to inhibit Hv urediniospore germination at various dilutions. Similarly, hexane, dichloromethane and ethyl acetate extracts from Ch filtrate were obtained and similarly tested against Hv. These extracts were subjected to analysis by gas chromatography coupled to a mass spectrometer as part of a composition analysis.

RESULTS

The filtrate obtained was capable of completely inhibiting urediniospore germination at 100%, but the inhibition dropped to 50% when the filtrate was diluted at 75% in water. The dichloromethane extract showed the highest level of inhibition of urediniospore germination. At concentration values of 1.0 mg ml-1, the extract completely inhibited the germination of Hv. The chemical analysis of the extracts revealed a complex combination of substances, some of which may represent novel molecules. Their identity is under investigation.

CONCLUSIONS & PERSPECTIVES

Ch was found to have potential for direct use as a biocontrol agent against CLR but, in addition, it was also found to produce metabolites which may prove useful as natural fungicides for controlling Hv.

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S2-P-27

Identification of NLR proteins in the coffee genotype HDT 8232/2 challenged with *Hemileia vastatrix* (host resistance) and *Uromyces vignae* (nonhost resistance)

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RATIONALE

The natural hybrids between *Coffea arabica* and *Coffea canephora*, known as "Timor Hybrids - HDTs", have been used as the main source of resistance to leaf rust (*Hemileia vastatrix*) in coffee breeding programs. However, the molecular nature of the resistance depicted by the HDTs remains elusive. The identification of resistance proteins, specifically NRL (Nucleotide-binding and Leucine-Rich proteins) that are responsible for the immunity triggered by effectors is of major importance and NLR-coding resistance genes are widely used to protect crops against many diseases.

METHODS

We used three cDNA libraries generated by highthroughput sequencing of RNA from leaves of the Timor Hybrid HDT 832/2; non-inoculated (control), inoculated with *H. vastatrix* (incompatible interaction) and inoculated with *Uromyces vignae* (nonhost interaction) (Diniz et al. 2012). The sequences obtained were analyzed by a bioinformatic pipeline aiming to identifying NLR proteins. In recent years, the genomes of *C. canephora*, *C. arabica* (Denoeud et al. 2014) and *C. eugenioides* were sequenced, which allowed us to compare the NLR from the three different species with the sequences obtained from the HDT transcriptome.

RESULTS

We could identify almost one hundred putative NLRs proteins in the transcriptome libraries that showed the conserved domains of NLRs protein. The majority was present in all cDNAs libraries, a small group was only detected in inoculated leaves, and an equally restricted group was present in the control and in the nonhost interaction. Interestingly, the upregulated sequences in leaves inoculated with *H. vastatrix* (incompatible interaction) were equally upregulated in leaves inoculated with *U. vignae* (nonhost interaction).

CONCLUSIONS & PERSPECTIVES

NLRs are extremely abundant in plant genomes, for instance in *C. canephora* genome more than seven hundred different proteins sequences were predicted. This high number of NLRs is reflected in the transcriptome of the HDT 832/2, however most of the sequences seem to have an unaltered transcription level when compared to the control. These findings reveal the diversity of NLRs and the existence of specific groups for the different interactions. We intend to identify NLR in other coffee genotypes with high spectrum of resistance to *H. vastatrix*, such as HDT derivatives and the Kawisari hybrid (*C. arabica* x *C. liberica*), under our new project CoffeeRES, to unveil the role of these proteins in the coffee immune system.

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S2-P-28

Identification and reaction of XXIX race of *Hemileia vastatrix* in Timor Hybrid derived coffee plants

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RATIONALE

Coffee rust is a disease caused by the fungus *Hemileia vastatrix*, which has been causing many losses in the coffee production worldwide. In Brazil, *Coffea arabica* coffee trees are all susceptible to rust. After the discovery of the Timor Hybrid (HT), several research institutions have been using it in genetic improvement programs for the development of rust-resistant cultivars. In 1999, the IAC developed and registered the Obatã IAC 1669-20 (Sarchimor) cultivar which is rust resistance. In 2010, this cultivar showed a moderate reaction to rust in routine field evaluations. The objective of this work was to determine the virulence spectrum of one rust isolate and also to evaluate the resistance of HT plants and some of their derivatives to the new race.

METHODS

The studies were based on the leaf disc method (Eskes and Toma-Braghini, 1981). Four clones of rust coffee differentials characterized by CIFC with their respective resistance genotypes: CIFC HT 1343/269 (S_H6), CIFC H 420/2 (S_H5.8), CIFC H 419/20 (S_H5,6,9), CIFC H 420/10 (S_H5,6,7,9) were used, as well as Catuaí Vermelho (S_H5). Some derivatives of HT like clones CIFC 832/1 and CIFC 832/2 and their derivatives, coffee trees of cv. IAC 125 RN, cv. Obatã IAC 1669-20, H13439-4 were also inoculated. Twelve discs taken from expanded leaves (one disc per leaf) of each genetic material were inoculated with the rust urediniospores obtained from cv. Obatã and race II (v5) were used as control. The evaluation of the disease was performed after 35 days after inoculation using a scale of scores from 0 to 4 points (Conceição et al., 2005).

RESULTS

The inoculation of cv. Obatã urediniospores caused the disease in all coffee differential clones and in Catuaí, indicating that it was a XXIX race new rust with virulence genotype v5,6,7,8,9. It was found that all known HT alleles (SH5 to SH9) were superseded by the new breed. In field conditions, the pathogen behavior is less aggressive. Even in years that were more favorable to the fungus, it was observed, at most, a moderate susceptibility. The HT 832/1 and 832/2 coffee trees and their derivatives analyzed were all resistant to the XXIX race. Race II, used as a control, caused the susceptibility reaction in Catuaí.

CONCLUSIONS & PERSPECTIVES

In the genetic improvement program targeting the long-term resistance to diseases, it is important to know the variability of the pathogen and the permanent resistance allele. An example is the SH3 allele that keeps coffee trees immune to all rust races even after five decades of the first race was detected in Brazil. The transfer of the SH3 allele to cultivars derived from HT may be of great importance for obtaining cultivars that present more durable resistance to coffee rust.

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S2-P-31

First report of *Fusarium solani* causing coffee black berry disease in China

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RATIONALE

Coffee black fruit is a common symptom in Arabica coffee producing areas, which seriously affects the yield and quality. There are many reasons for black fruit. Here, we report the identification of the pathogens on a suspected coffee black berry disease sample based on morphology and molecular phylogenetic data.

METHODS

Typical diseased black fruit were collected for pathogen isolating, and the obtained single fungal colonies were observed for conidia morphology under the microscopy. The healthy coffee fruit were inoculated with conidia suspension of the single colony isolates, and the pathogenicity was observed 7 days later. Total genomic DNA was extracted from fungal mycelia. The ribosomal internal transcribed spacer (ITS) was amplified by using primers ITS1 and ITS4, β-tubulin gene by Bt2a and Bt2b, translation elongation factor (TEF-1a) by EF1-526F and EF1-1567R, 28S rDNA by LROR and LR5, and subcloned as recombinant plasmids for sequencing. The ITS, TEF, tubulin, and 28S rDNA single gene sequence tree and combined ITS- TEF gene sequence tree were constructed using MEGA 6.0.

RESULTS

The fungal isolation had a round, felt-like colony on PDA medium, the mycelium was off-white, the surface was sparse, and light yellow pigment appeared on the back. The conidia had 1~8 septums, its length was 6.08~65.3 µm and width was 2.76~9.03 µm. Small conidia were kidney-shaped and large conidia were sickle-shaped. The pathogenicity test showed that the infected fruits the symptoms similar typical symptoms as observed from the diseased fruits under natural conditions. Koch's postulates were fulfilled by re-isolating the fungus and verifying its colony and morphological characters. Molecular identification results showed that ITS, β-tubulin, TEF, 28S rDNA, four single gene clustering tree, and ITS-TEF gene sequence clustering results were consistent, indicating all that CPE5 and CPE12 belong to *Fusarium solani*. Therefore, the pathogen was confirmed as *Fusarium solani* by morphological and molecular identification.

CONCLUSIONS & PERSPECTIVES

To the best of our knowledge, this is the first reported the case of *Fusarium solani* infection on fruit of coffee in China.

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POSTERS

Session 3: Farm management

S3-P-02

Visual determination of Leaf Area Index (LAI) in coffee fields

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RATIONALE

The Leaf Area Index (LAI) is a useful parameter in the study of plant canopies, and is defined as the leaf area covering the corresponding soil area. In coffee cultivation (*Coffea arabica* L.), this variable has a marked influence on aspects such as productivity, evapotranspiration and the provision of ecological services. In addition, it could be used for the calibration of foliar applications or for the precise determination of crop irrigation water requirements.

METHODS

Four successive cycles of LAI determination were conducted by defoliation, two instruments (LAI 2200-C and Accupar LP-80) and the visual estimates by five field workers at Finca La Hilda, Poás, Costa Rica. In each cycle, 20 sampling points, defined as three continuous plants, were chosen. Workers visually estimated the LAI within defined ranges, on two occasions separated by 15 days. The LAI was measured with the instruments at the same points. Subsequently the plants were defoliated, their leaf fresh weight was measured and the LAI was calculated from the plant density and the leaf specific fresh weight. After each cycle a feedback session was given to the evaluators about their performance. A repeatability and reproducibility analysis of the visual estimates was performed, the fit to the linear regression model was tested and the coefficient of determination R^2 was calculated for each case.

RESULTS

The visual estimation method showed high repeatability and reproducibility through the cycles, as the variance attributable to the estimator and to the estimation event was very low. Visual estimates were adjusted to the linear regression model in most cases (with $\alpha = 5\%$). Furthermore, the evaluators improved their predictive capacity of the LAI throughout the cycles, as the value of R^2 increased in all cycles for 4 of the 5 persons. This results contrast with what was seen in the evaluation with instruments, since the R^2 values remained between 0.5 and 0.6 during all cycles. In two out of four cycles, the estimates of the instruments were not adjusted to the linear regression model because of an important underestimation bias.

CONCLUSIONS

This methodology has a high potential for the rapid, simple and reliable visual determination of the LAI in coffee fields, and opens up the possibility of using this parameter for decision-making in crop management.

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S3-P-05

Influence of biochar and poultry manure on weed infestation and growth of arabica coffee *(Coffea arabica)* seedlings

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Rational: Young coffee plants at nursery particularly after transplanting are very sensitive to weed infestation. Therefore, timely weeding and adequate nutrition is necessary to boost seedling vegetative growth. A pot experiment was conducted from 2017-18 at IRAD, Foumbot multipurpose research station, Cameroon. The main objective was to assess the influence of biochar and poultry manure on weed infestation and growth of arabica coffee seedlings.
Methods: The biochar was produced using an Elsa pyrolysis barrel at 450 0C with 58 min carbonization time from corncobs. The biochar were milled to <2mm and mixed at the rate of 20, 30 and 40t/ha-1 with 40t/ha-1 poultry manure and soil before applying to 0.01 m2 polythene bags with five replications.
Results: The 20t/ha-1 biochar + 40t/ha-1 poultry manure treatment significantly ($P < 0.05$) increased plant height, stem girth, number of leaves, and leaf area compared to control (poultry manure only). Treatments with 30t/ha-1 and 40t/ha-1 biochar had the lowest weed fresh weight and dry weight. Cyperus rotundus, Oxalis cornoculata and Cynodon nlemfuensis were most economically important weeds scored for their abundance and persistence. Overall, weed control efficiency was lowest in sole 40t/ha-1 poultry manure and 20t/ha-1 biochar treatment with 18% and 20% compared to 40t/ha-1 and 30t/ha-1 biochar treatment with 35% and 24% respectively.
Conclusions and perspective: The results demonstrated that combined application of poultry manure and biochar appears essential for a sustainable coffee seedling production in the Western Highlands of Cameroon. However, to enhance coffee seedling growth using biochar, the use of recommended doses is paramount.

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S3-P-06

On-farm performance of Arabica F1 Hybrids in the western highlands of Cameroon

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RATIONAL

Benefits of the use of Arabica coffee F1 hybrids in agroforestry (high productivity, cup quality, resistance to drought, pest and diseases) have dominated scientific debates in efforts to promote the use of such hybrids over conventional varieties. However, arabica F1 hybrids are relatively new to most farmers, especially those in the Western highlands of Cameroon, a major arabica coffee growing area, where the plant is mostly grown in association with food and fruit trees. Selecting shade trees is also important for maximising ecosystem services.

METHODS

From July 2018, a multi-site trial was conducted in six localities of different altitudes (950 to 1,400 m asl) in the western highlands of Cameroon to compare the H1 and Starmaya F1 hybrids with conventional cultivars (Marsellesa and Java). The experiment was laid out in a complete randomised design with eight varietal plots of 12 trees each of the H1, Starmaya hybrid and the control cultivars (Marcellesa and Java). The coffee plants were planted at 3.0 m spacing between rows and 1.5 m spacing between trees along a row, giving a total of 96 coffee plants per variety per site. The study incorporated a semi participatory methodology involving farmers to gain an on-farm assessment of the challenges, environmental feasibility, economic profitability and sociocultural acceptance of Arabica coffee hybrids. Shade tree advice tool, tree inventories and household interviews will be conducted to characterize Arabica coffee farms and farmers ecological knowledge on the provision of ecosystems services by associated shade trees.

RESULTS

First phenotyping data showed that the growth of the Starmaya and H1 hybrids variety are earlier and superior to the control varieties Java and Marsellesa. Both hybrids were also more stable and resistant to drought compared to pure line varieties in all altitudes. We also found that the use of shade tree advice tool could guide farmers in selecting appropriate trees that best meet their needs and provide essential ecosystem services.

CONCLUSION & PERSPECTIVES

Farmers appreciate and support the arabica hybrids due to their fast growth, production and drought resistance. The first true harvest is scheduled for October 2021. A national wide sensitization on Arabica coffee F1 hybrids could create awareness, generate a huge leap in livelihoods as well as get the attention of the government for policy drive. The overall impact of the study could be ensured through implementation of business driven- Agroforestry coffee clusters and innovation platforms.

Keywords: Agroforestry coffee clusters, Cameroon, F1 Hybrids, Starmaya, Shade tree advice

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S3-P-07

Effects of different substrate formulations on coffee seedlings production and growth

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RATIONALE

The nursery production of coffee seedlings affects not only the plant behavior just after transplant but also the potential productivity and the final quality of coffee. There is a need for more studies to optimize the nursery phase introducing the updated knowledge on this field. The aim of this trial was to study the influence of different substrate formulations on main seedling quality descriptors. Particular attention was paid on lateral (plagiotropic) branches emission. In fact, the earlier development of plagiotropic branches is strictly related to early production (Coste, 1992).

METHODS

The trial was carried out in 2018 on young plants of *Coffea arabica* L. cv. Laurina. Eight treatments were studied: 1) a common substrate fertilization (CSF); 2) CSF + mycorrhizae (M) (Aegis - Italpollina); 3) CSF + biostimulant (B) (Actiwave G® - Valagro); 4) CSF+M+B; 5) modified substrate fertilization (MSF); 6) MSF + M; 7) MSF+B; 8) MSF+M+B. Mycorrhizae was based on two fungal strains of *Rhizophagus irregularis* (previously known as *Glomus intraradices*), *Glomus mosseae* and bacteria of the rhizosphere. On seedlings, different vegetative parameters were evaluated. At the end of trial, other parameters (including dry weight, dry matter of canopy and rooting capacity) were taken into consideration.

RESULTS

After six months from the starting of trial, the substrate fertilization formula containing the highest amount of phosphorous influenced markedly the main morphological parameters measured, stimulating the earliest emission of plagiotropic branches, on which flowers and fruits develop. Contrary to expectations, the substrate mycorrhization did not affect the final results, probably due to their negligible substrate colonization. The use of biostimulant positively influenced the root growth and slightly increased the number of internodes, despite of reducing the chlorophyll content and delayed the early emission of lateral branches. On the contrary, the combined use of biostimulant and mycorrhizae did not provide clear advantages, in spite of the highest cost of substrate.

CONCLUSIONS & PERSPECTIVES

Based on these results, the substrate which provided the best results at lowest cost was the one containing the common fertilizing formula with the highest content in phosphorous. Further investigation are required in order to optimize the nutritional composition of the substrate, so as to support the development of the plant in the early stages. and to evaluate the functionality of applied wetting agents to the substrate. It will also be newsworthy to evaluate the effectiveness of biostimulants obtained from new matrices.

S3-P-09

Determinants of Coffee Production: The Case of Kogi State, Nigeria

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RATIONALE

Irrespective of the rising demand for coffee globally, Nigerian farmers seem not to be tapping the potential as the produce has seen a significant dip in its production. Many farms have been abandoned and some coffee plantations replanted to other crops.

As the world coffee output continues to increase, Nigeria's production continues to dwindle, and there had been fluctuations in global demand and prices in the last six years, but coffee demand is on the rise. This study determined the factors that boost production of coffee in Kogi state, Nigeria a state that has high comparative advantage for the production of coffee.

METHODOLOGY

Using well structured questionnaire, information were elicited from 120 coffee farmers in Kogi state using the multistage sampling technique. The first stage was the selection of two local government areas (LGAs) Kabba/bunu and Ijumu while second stage was selection of coffee farming villages/ communities from each of the LGAs and the third stage was random selection of coffee farmers from the existing Agricultural Development Programme (ADP's) list of coffee farmers. This selection was done proportionate to the size of the village population. The study used the descriptive analysis and the multiple regression methods to achieve set objectives.

RESULTS

The result shows that socio-economic characteristics and economic variables are determinants of production of coffee in the state; of all the coffee farmers interviewed, 67% of the farmers were male with 33% female. The mean age of the farmers was 60 ±5.96 years, mean household size of 8 ±2 persons; majority of the coffee farmers are smallholders with the average farm size of 1.5 ±0.8ha of farmland with over 30years of coffee farming experience. The result of the regression analysis shows that six variables: age of farmer(x1), age of coffee bush(x2), coffee farming experience(x3), market access/channel(x4), coffee price (x5) and farm size (x6) were significant at 1% level on the production ability of coffee farmers in Kogi State, Nigeria.

CONCLUSIONS & PERSPECTIVES

It was concluded that coffee production is viable in the area. Based on findings, this study recommends that more women and youths be encouraged to take up coffee cultivation as business in the state. Also, based on the findings that poor pricing and marketing channel contribute mainly to the abandonment of coffee farms which has multiplier effects on the production; It is recommended that government should intervene in the crisis of the coffee sector by creating appropriate marketing channel and putting in place price control system which will encourage new entrants and help old ones remain in business.

S3-P-10

Applying Scientific Data to Calibrate the Management of Coffee Farms

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BACKGROUND

Coffee has become one of Taiwan's most important beverage crops and its production is at an all-time high of 1,100 ha. Since majority farmers in Taiwan are small holders who suffer from higher costs of productions, most coffee tend to be higher-quality and suitable for domestic specialty market. This project aims to combine sensory evaluation with soil analysis, plant tissue analysis to create a scientific basis for farm management to increase the quality and production of Taiwan-grown coffee.

METHODOLOGY

Three experimental plots were selected in Taiwan (NU-A , NU-B and NU-C) and divided each into a test group and a control group. Each test group was calibrate-fertilized according to the result of soil and plant tissue analysis. The fertilizer was prepared with soybean pomace, phosphate guano, and pearl ash. Analysis of soil nutrients, plant tissues (in terms of nitrogen, phosphor, potassium), and the chlorogenic acid within green coffee is recorded. The coffee quality was also evaluated through SCA's cupping protocols.

RESULTS

Before treatment, data shows that the nitrogen(N) levels within green coffee from NU-A, NU-B and NU-C were 1.61%, 1.56% and 1.83% respectively. The phosphor(P) levels were 0.01, 1.20 and 0.02 g/ kg respectively. The potassium(K) levels were 0.87, 8.51 and 0.83 g/ kg respectively. Comparison with the reference value (2.07% N, 1.61 g/ kg P, 9.60 g/ kg K) shows green coffee produced from NU-A was 20% less N, 99% less P, and 90% less K. Green coffee from NU-B and NU-C were also 25%, 25%, 11% and 12%, 99%, 90% less N, P, K, respectively. After fertilization, all three test groups produce green coffee with lower chlorogenic acid levels than their respective control groups. The green coffee N levels found at NU-A, NU-B, and NU-C were 1.90%, 1.68%, and 1.63% respectively. Phosphor levels were 0.66, 1.36, and 0.02g/ kg and potassium levels were 4.63, 9.27, and 0.87 g/ kg respectively. Except for NU-C, green coffee of both test groups from NU-A and NU-B produce higher levels of N, P, K than the control groups. Higher sensory evaluation scores were also recorded from test groups of NU-A and NU-B .

CONCLUSIONS & PERSPECTIVES

In Taiwan, nutrient content within coffee varies greatly by region. Quality is severely affected if the insufficient nutrient is applied. The project therefore recommends combining sensory evaluation with soil and plant tissue analysis to establish a scientific dataset for the management of coffee farms. A well-calibrated fertilization system can greatly increase coffee production and coffee quality.

References:

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S3-P-15

Microclimate of an intercropped system of *Coffea canephora* and *Carica papaya*

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RATIONALE

Global warming might threat the production and thereafter the supply of coffee in the near future (Bunn et al., 2015), including Robusta type of coffee, namely as concerned the highly cropped *Coffea canephora cv. Conilon*. In this view, coffee crop management with shade trees might improve environmental conditions for coffee production (Partelli et al., 2014). This study assessed the microclimate close to Conilon trees under full Sun exposure or intercropped with papaya trees (*Carica papaya*).

METHODS

Two *Coffea canephora* farming systems were compared, 1) intercropped with *Carica papaya* trees; 2) coffee monoculture under full Sun exposure, both in Espírito Santo, Brazil. The micro-environmental air conditions were monitored as regards irradiance, temperature and relative humidity (RH), using external data loggers (HOBO U12, Onset HOBO Data Loggers) during daytime in three seasons throughout the year. The study was supported by Fapes, Capes and Cnpq.

RESULTS

The intercropped system of coffee and papaya trees showed decreased irradiance and temperature, and higher relative humidity in relation to the monoculture system of Conilon coffee in all monitored periods. In the warmer periods during the year, mean irradiance in the intercropped system was reduced up to 42% as compared with the monoculture coffee management system. The mean temperature in the intercropped system was reduced by 2.2 °C, but with reductions up to 8.3 °C in maximal values in relation to the monoculture system. These better microclimate conditions in the coffee-papaya intercropped system were in line with the finding with the intercropped system of Conilon coffee and *Hevea brasiliensis* (Partelli et al., 2014) and *Musa spp*. (Araújo et al., 2015).

CONCLUSIONS & PERSPECTIVES

The intercropped management system (Conilon coffee - papaya trees) provided better environmental conditions for coffee plant development, and showed a potential to be used as a preferential farming system to mitigate climate change and global warming impacts.

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S3-P-17

An initiative of GTA to establish coffee plantation in Eastern Himalayas with a vision to make 'Kalimpong Coffee' a brand like 'Darjeeling Tea'

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RATIONALE

Darjeeling lies between 27º31'05" and 26º27'10" N latitude and 88º53'00" and 87º59'30" E longitude occupying an area of 3303.98 sq km and is an integral part of the Eastern Himalayas. Based on the encouraging result of the coffee plantation in Govt Cinchona Plantation which was started in 2014, GTA had taken an initiative to establish Arabica coffee plantation in 2018. All technical guidance was provided by the Coffee Board of India. Initially, 1193 farmers were selected covering an area of 349.07 acres, which was divided into four clusters *viz* Bhalukhop-Sangsey, Algarah, Gitdabling and Lolay each with a 'Cluster Leader'. Project implementing govt officials kept in touch with the farmers through these Cluster Leaders. 0.72 million seedlings of ***Chandragiri*** variety of Arabica coffee were procured from Chickmangulur, Karnataka located 2652 km from Kalimpong. Farmers were briefed and trained on planting and plantation management.

METHODS

An analysis was carried out by the administration with the farmers and the project implementing group. Field visits were conducted to see the extent of the project implementation in the villages within two years of the initiation. Seedlings were transported in lorries for cheaper, better handling and faster movement. The seedlings were with soils in poly pots. Simple statistical analysis methods were used for data analysis.

RESULTS

After two years, we ascertained that 57% of the total coffee plants had survived in the field. The reason for the low rate of plant establishment was excessive time taken for transportation of seedlings. It took an average of 6 days for the seedlings to arrive Kalimpong and even though two vehic

les were involved in the process, took a total of 19 and 27 days for all the seedlings to reach Kalimpong. More than 90% of those seedlings dried up after planting. There was also an indefinite strike organized by All Indian Truckers' Association that lasted for 21 days attributing to poor plant survivability. Approximately 1% of the plants started fruiting in the second year itself. We realized that drip irrigation system is the need, without which it is extremely difficult to maintain the plantation.

CONCLUSION & PERSPECTIVES

Considering the quality, with attention to the aroma, flavour and taste of the coffee produced here and the demands from various places, it appears that there is a huge potential for coffee to become a thriving industry. After 185 years of establishment of tea industry in Darjeeling by the English (Malley 1907), Kalimpong Coffee could be yet another industry to delight people across the globe and we can help its aroma float to every nook and corner of the world.

References:

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S3-P-18

Evaluation of different weed control methods to reduce the use of Paraquat on coffee plantations on Costa Rica

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RATIONALE

Paraquat has been used as an herbicide since 1962 and is one of the most sell agrochemicals around the world, however, there is evidence of the high acute toxicity for human health. In this moment, paraquat is prohibited by Europe Union and restricted in other countries, including Costa Rica. Paraquat is highly used in coffee plantations.

METHODS

In 2019 two field trials were conducted; A) Chemical control alternatives: it was established with nine treatments, in an unrestricted random design with three repetitions. The treatments were: paraquat (300 g ai/ha), glufosinate ammonium (300 g ai/ha) and fluazifop-p-butyl (125 g ai/ha) these two herbicides in mixture with carfentrazone (24 g ai/ha), saflufenacil (35 g ai/ha) and oxyfluorfen (480 g ai/ha) and a control without herbicides. The variables evaluated were damage percentage, using a scale from 0 to 100 (De la Cruz, 1987) at 3, 7, 14, 28 and 42 days after the application (DAA) and fresh and dry weight of an established area (0,25 m2), 42 DDA. B) Use of propane: a field trial was carried using an unrestricted random design, with three dosages of propane (25, 50 and 75 kg/ha), paraquat (300 g ai/ha) and a control, without propane and paraquat. Each treatment was repeated four times; the evaluations were done at 1,3,7,14 and 21 DAA. The evaluations included the damage percentage, green color percentage, using the software ImageJ, and fresh and dry weight 7 DDA and 22 DDA.

RESULTS

The weeds sprayed with paraquat were the most damaged (88%) 7 DDA. Nevertheless, since this evaluation damage started to decrease in the treatment using paraquat. Since 14 DDA to 42 DDA the mixture that worked better was glufosinate ammonium + saflufenacil. In the case of narrow leaf weeds, they were affected the most by paraquat. Glufosinate ammonium by itself and in mixture with other molecules, was statistically similar in fresh weight at 42 DDA.

The use of propane generated high damage of weeds during the first seven days, however, from this day on, some weeds began to recover, specifically *Commelina diffusa*. Higher doses of propane were the most effective for weed control, but not exceeding the damage caused by paraquat.

CONCLUSIONS & PERSPECTIVES

Some herbicides mixtures such as glufosinate ammonium + saflufenacil could be used instead of paraquat, due to a possible ban of the molecule. Propane for weed control is not as effective as paraquat, but some improves can be done to make this technology more efficient, for example, improving gas output, to make the application faster and more homogeneous.

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S3-P-22

Efficiency in the use of phosphorus in Brazilian arabica coffee genotypes

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RATIONALE

Nutritional efficiency is a term used to characterize plants in their ability to absorb and use nutrients, and is related to the efficiency of absorption, translocation and use of nutrients. Different coffee genotypes are expected to show variability in their nutritional efficiency. This work aimed to evaluate the nutritional efficiency of phosphorus in arabica coffee trees.

METHODS

The experiment was conducted in a greenhouse of the Agricultural Research Corporation of Minas Gerais (EPAMIG), located in the municipality of Lavras, Minas Gerais, Brazil. The experimental design was a randomized block, in a 10x2 factorial scheme (10 genotypes of arabica coffee trees and two dosages of phosphate fertilization), with four replications. The genotypes were classified as: efficient and responsive (ER), efficient and non-responsive (ENR), non-efficient and responsive (NER) and non-efficient and non-responsive.

RESULTS

The cultivars Bourbon Amarelo IAC J10 and MGS Aranãs were classified as efficient and responsive, that is, they are efficient in the production of dry matter under conditions of low phosphorus supply and respond in increment of dry matter if phosphate fertilizer is provided. The cultivars Paraíso MG H 419-1, Topázio MG 1190 and MGS Paraíso 2 were classified as efficient and non-responsive, that is, they are efficient in the production of dry matter under conditions of low phosphorus supply, but do not respond in increment of dry mass. if phosphate fertilizer is provided. Progeny H 6-47-10 pl. 3 and the cultivar Catuaí Vermelho IAC 144 were classified as non-efficient and responsive, that is, they are not efficient in the production of dry matter under conditions of low phosphorus supply, but respond in increment of dry matter if phosphate fertilizer is provided. The cultivars Catiguá MG2, MGS Ametista and Sarchimor MG 8840 were classified as non-efficient and non-responsive, that is, they are not efficient in the production of dry matter under conditions of low phosphorus supply and do not respond in increment of dry mass if fertilization is provided phosphated.

CONCLUSIONS & PERSPECTIVES

The cultivars Catiguá MG2, MGS Ametista and Sarchimor MG 8840 are neither efficient nor responsive to phosphate fertilization. Progeny H 6-47-10 pl. 3 and the cultivar Catuaí Vermelho IAC 144 are not efficient, but they are responsive to phosphate fertilization. The cultivars Paraíso MG H 419-1, Topázio MG 1190 and MGS Paraíso 2 are efficient, but are not responsive to phosphate fertilization. The cultivars Bourbon Amarelo IAC J10 and MGS Aranãs are efficient and responsive to phosphate fertilization.

POSTERS

Session 4: Green coffee processing

S4-P-01

Technology of Refermentation to Increase Quality of Coffee Beans

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RATIONALE

One important step in order to improve the quality of coffee is the fermentation stage, the main purpose of coffee fermentation is to decompose the mucilage attached to the skin of the coffee horn so that it is easy to clean when washed. The treatment of fermentation in coffee products, especially in Arabica coffee is very important to improve the quality of coffee, especially in terms of flavor, in research. However, so far many coffee farmers do not do the fermentation process, even for arabica coffee. Therefore, it is necessary to make a referral so that the resulting coffee product becomes better, namely the type of specialty coffee.

METHODS

The refermentation technique is a fermentation technique on dried coffee beans using a starter consisting of yeast *Saccharomyces Cerevisiae* and Lactic acid bacteria (*Leoconostoc* and *Streptococcus thermopylus*). The steps of the refermentation technique are soaking coffee beans to 60% water content, fermentation process using a starter with a ratio of 20% for 18 hours, then washing, and drying up to 12% moisture content.

RESULTS

from organoleptic test, this technology can increase score until 5 point or have score more than 80 (specialty coffee). This technology can enhance the pleasant aroma until 23 compounds in 37 °C for 18 hours. Some compounds group including of acid, alcohol, aldehyde and acetate groups were contributed to acidy, fruity, nutty, and caramel aroma.

CONCLUSION

Technology of refermentation can increase quality of coffee bean to be specialty coffee. The implementation of refermentation techniques is certainly easier for the community because they can get good quality beans from dried coffee beans that are easily obtained.

S4-P-04

Maintain seed vigor to ensure complete transformation from precursors to flavor components

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RATIONALE

It has been reported that during processing the coffee seeds initiated germinating related metabolism that could result in different flavor. Flavor precursors can be completely transform to flavor components only if the seed vigor is maintained. So in this research we tried to manipulate the germination of the seeds during processing and see how it correlated with cup quality. We also developed a new method of processing that could highly maintain seed vigor.

METHODS

Coffea arabica L. samples were harvest at Chiayi, Taiwan. Fully mature cherries were de-pulped and fermented for 24 hours at 25°C and then washed with clean water. Washed coffee beans then divided into four batches, first batch directly dried under 40°C, the other third batches immersed in 100 ppm GA for 2 hours and kept moist for 3, 6, and 10 day to promote germination and then dried under 40°C until water content reached 10~12%. Soluble sugar and organic acids was analyzed using UPLC and HPLC. New post-harvest techniques: Fully mature coffee cherries were slowly dried under 10~15°C until water content reached 10~12%. Same batch of coffee cherries processed by farmers as control group. Viability test modified Hilst's method. (Hilst et al. 2016.) Coffee cupping followed SCA cupping Standards.

RESULTS

GA treated samples with 3 days of germination time had better cup quality (82.75); the control batch had the lowest cupping score (80.50); germinated for 6 days and 9 days both had the score of 81.50. Sucrose content dropped when germination begin, glucose and fructose reached highest level at fermentation stage and decreased with germination time. Malic acid content was highest at the third day of germination. Viability of coffee seed was significantly increased using low temperature drying method compare to seeds dried under conventional drying condition (35~40°C). The cup quality (84.25) was also better than control (81.25).

CONCLUSIONS & PERSPECTIVES

This results suggested that promoting germination at certain degrees would improve cup quality. In our results, the best timing was when glucose and fructose about to decreased in the seed, and Malic acid, which is an important intermediate of Krebs cycle, increased. So, the intensity of germination is related to coffee quality and it is important to keep the seed viable to ensure germination related metabolism can proceed during post-harvest processing. Our new techniques of processing can maintain seed viability and improve coffee flavor.

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S4-P-06

Yeasts and bacteria phenotyping on a coffee pulp fermentation simulation medium for the selection of new starters to improve coffee final quality

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RATIONALE

Microorganisms play a key role in coffee fermentation by degrading mucilage and consuming coffee pulp nutrients to produce aroma precursors that will contribute to organoleptic properties of the resulting coffee. In order to better control this crucial step, the use of starters – yeasts and/or bacteria – is of growing interest. To face the plurality of post-harvest processes, several yeast and bacteria strains were evaluated on coffee simulation medium to select appropriate candidates.

METHODS

Coffee fermentation simulation medium was formulated based on Pereira work (Pereira et al. 2014) and further implemented with minerals and nitrogen source (Avallone et al. 2001). Fermentation media were inoculated with 15 different *S. cerevisiae* and two lactic acid bacteria belonging to *O. oeni* and *L. helveticus* species. Fermentations were followed in 1L bioreactors by recording fermentation kinetics such as CO_2 release (g), microbial growth (CFU) or fermentative metabolites through HPLC. Volatile Organic Compounds (VOC) production of some trials was evaluated by using HS-SMPE/GC-TQMS to fine-tune selection of starters.

RESULTS

Most of the yeast strains presented short lag phase (< 12h) and were able to deplete sugars within 50h. These phenotypic traits could fairly be applied for wet coffee for instance. Moreover, yeast growth reached on average log 8 ± 0.16 and could be an asset to secure fermentation by occupying the medium and preventing other spoilage microorganism to grow. Acetic acid was produced in the range 0-0.449 g/L, as an illustration of the different genetic backgrounds of microorganisms. Metabolic properties were also discriminated by medium acidification, that occurred within 40h for yeast monocultures and 24h in the presence of bacteria, by dropping the pH by 1.5 unit for the later. This acidification can be desired on coffee fermentation to improve cup quality, choice of starter consortium being then important to drive coffee organoleptic profile. Finally, the diffusion of VOC has lately been highlighted in coffee (Hadj Salem et al. 2020) and as a matter of fact production by yeasts or bacteria was evaluated. Four of the studied yeast strains were tthen interesting for coffee application as 2-Phenylethanol and its ester acetate (rose), Ethyl decanoate (grape, pear) or Benzeneacetaldehyde (honey, rose) were widely produced over the fermentation.

CONCLUSIONS

Phenotyping led to the selection of four yeasts candidates further trialed on real coffee matrix. Further work is under study to better characterize enzymatic properties to better understand their mucilage degradation ability and fine-tune microorganism's selection for coffee fermentation.

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S4-P-07

Characterization of coffee flavor profiling through controlled symbiotic fermentation using *Saccharomyces cerevisiae* and lactic acid bacteria

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RATIONALE

Fermented coffee consumption has increased due to its unique aromas and rich flavors. However, a conventional process for coffee fermentation has limitations for consistent quality. To overcome these limitations, we conducted a controlled anaerobic fermentation process by inoculating the fermentation starter of plant origin. Then we performed a flavor profiling analysis to compare the characteristics of fermented coffee with traditionally produced coffee.

METHODS

Coffee cherries (El Paraiso, Columbia) were sorted according to ripening degree, sterilized with ozonated water and pulping. Fermentation of coffee bean was performed with *Saccharomyces cerevisiae* and a mixture of three lactic acid bacteria (LAB) species (*Lactobacillus brevis*, *L. curvatus*, and *L. plantarum)* isolated from Korean traditional fermented foods including *Nuruk* and *Kimchi*. Starter cultures were inoculated to initiate the anaerobic fermentation at 20°C for 24, 72 and 168 hours in anaerobically controlled fermentation tanks. After the fermentation process, flavor compounds for both green beans and roasted beans were analyzed using solid phase micro extraction- gas chromatography/mass spectrometry (SPME-GC/MS) (Agilent, Santa Clara, CA, USA).

RESULTS

The flavor analysis revealed that the ester compounds were only produced in fermentation process. Particularly, ethyl isovalerate, known as 'fruity and sweet odor' characteristic, was dramatically increased at the 72 hr fermentation mark. Whereas, isoamyl alcohol, the major end product of the fermentation process that gives its acrid odor, was detected only at the 168 hr mark. In roasted samples, acetoin and diacetyl (buttery odor) were produced at the highest amount at the 72 hr fermented coffee. Acetic acid and valeric acid (unpleasant odor) were produced solely at the 168 hr mark sample.

CONCLUSIONS & PERSPECTIVES

The flavor analysis and SCA cupping standard method for the fermented coffee revealed that the 72 hr fermentation process is (most) optimal for generating pleasant aromas and tastes.

By optimizing the anaerobic fermentation process, it is possible to acquire various coffee flavors and to produce high quality coffee from coffee beans of the same origin. Since coffee fermentation has yet to be well characterized at an industrial level, microbial culture studies of coffee fermentation will be needed for production of high quality coffee in a consistent and reliable manner.

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POSTERS

Session 5: Sustainability, climate change & labels

S5-P-02

Mexican public-private policy related to coffee sector- working together

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RATIONAL

The orange coffee rust caused, from 2012, strong economic and social damage with losses for the sector as production fell in the 2015/2016 cycle by more than 50 percent. This disaster was due, among other factors, to low investment in assets for productivity, little innovation and technology transfer to the small producer and the low genetic diversity in coffee plantations because approximately 90% of the area was still cultivated with varieties susceptible to rust (Zamarripa,2018).

METHODS

The public policy of coffee in Mexico (PIAC - PROCAFE), was based on productivity, resilience and adoption of high-tech (Arguello,2018). For the first time in Mexican coffee growing, the use of certified seed of improved varieties was adopted by farmers. Also, technicians and nursery manager were trained in the production and verification of plant with high genetic, physiological and phytosanitary quality according to the Production Guide prepared with international criteria. The transfer and innovation were carried out with the support of more than 400 extension agents in a public private coordination.

RESULTS

Around 150 thousand hectares were renovated with 550 million plants of a “soup” of varieties resistant to rust and high organoleptic quality for 3 years (2016 - 2018). With the intelligent renovation of coffee plantations and the application of best agricultural practices, the recovery of production was achieved, going from 2.2 to 4.5 million bags of 60 kg each produced in the 2015/2016 and 2019/2020 cycles, respectively, while producers decreased their production cost and improved the consistency of their quality.

Whit Committee on Sustaninability Assessment (COSA) support focus on to build smart programs oriented in efficiency and concrete results, it was measured ROI based on the concepts subsidized: certified nurseries, resilient plants, agrotechnology packages, tech assistance and how those investments impact at the incomes, resilience and employees. The conclusion at 4th year of impact was the ROI is at least 500 % with the adoption of high and resilient tech while the well being of producer’s families and employees are incremented substantially.

CONCLUSION & PERSPECTIVES

The design of public policy promoted investment in long-term assets and encouraged the adoption of technology such as the planting of resistant varieties and the higher density of plants per hectare, thus achieving greater sustainable coffee offer of México. If this productive approach continues mainly with resilient varieties and hybrids, it is estimated to double production in up to 8 million bags by 2024, when the current administration will conclude

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S5-P-05

Influence of the high atmospheric CO_2 concentration ↑[CO_2] and water deficit on leaf secondary metabolites concentrations in *Coffea arabica* L.

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RATIONALE

The increased atmospheric CO_2 concentration (↑ [CO_2] atm) is not an isolated effect, being accompanied by increases in air temperature and changes in precipitation patterns. In coffee producing regions, drought is considered the main factor affecting growth and coffee production. Coffee has a broad chemical composition, highlighting caffeine and 5-chlorogenic acid (5-CQA), which act in plant defense responses and are important secondary compounds in the final beverage quality. The aim of this work was to evaluate the interaction between ↑[CO_2] and water deficit on photosynthesis and caffeine and 5-CQA in Arabica coffee.

METHODS

C. arabica L. cv. Catuaí IAC 144 plants were grown in an Open Top Chamber facility at the Instituto de Botânica, SP, Brazil, under ambient (≅400ppm – CO_{2amb}) and high (≅800ppm – CO_{2high}) atmospheric CO_2 concentration. Leaf water potential (Ψwf) and gas exchange were measured using a pressure bomb type Scholander and a portable Infra-Red Gas Analyzer respectively. 5-CQA and caffeine were analyzed using a High Performance Liquid Chromatography (HPLC/UV-DAD) system.

RESULTS

Photosynthesis was greater in coffee plants grown under CO_{2high}, even under water deficit and when compared to other treatments. Decreases in Ψwf were about 42% and 56% in treatments under water deficit compared to treatments under daily watering. 5-CQA and caffeine were increased on combined ↑[CO_2] and water deficit over the 40 days. 5-CQA concentrations also increased under ↑[CO_2] and daily watered conditions, but decreased significantly under CO_{2amb} and water deficit treatment.

CONCLUSIONS & PERSPECTIVES

Higher [CO_2] had a positive effect on photosynthetic rates mitigating the possible effects of drought. Water deficit and ↑[CO_2] interaction influenced the accumulation of 5-CQA and caffeine, playing an important role in the contents of these compounds. Field experiments will be carried out to evaluate the influence of these changes on coffee berries composition.

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S5-P-06

Densification of by products coffee for energy uses

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The pulp of the wet industrial coffee process is obtained between 80-85% moisture (wet base or WB) from the pulping process. We determines that the pressing process with a helical press, reduces water content by 10% (wet base), in the pulp but also removes mucilage and some dissolved and suspended solids. This pretreatment implies a significant reduction in mass rather than moisture, but the most important aspect is that it seems to have an effect on fiber breakage of the pulp. The research summarizes the results of pelletizing densification tests and the results of caloric power of the product obtained for energy use about by products coffee.

Pressing number of times 2
Lost mass (% m lost / m initial) 5-6
Pellets productions by dry base (kg/h) 157,0
Flow biomass by number of time of (kg time press/h) 314,0
Moisture in (% WB) 30-50 30-50
Moisture out (% WB) 25-45 25-45
Power average pellets consumption (kW) 19.5

2.3.2 **Photographic record of pelletizing by-products coffee**

Figura 2.3.1. **Pellets from 100% pulp coffee**

Figura 2.3.2. **Pellets from 70% pulp coffee and 30% husk coffee**

Final results of pellets

References:

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S5-P-08

A comprehensive analysis of operations and mass flows in postharvest processing of washed coffee

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RATIONALE

Although coffee is one of the most valuable and widely traded agricultural commodities in the world ($83 billion USD in 2017 revenue), little information exists in the scientific literature regarding coffee bean postharvest processing. In particular, sustainability analyses require information on the coffee bean mass and property changes during processing, from harvest to final consumption.

METHODS

In this study, a detailed analysis of the washed or wet-processed method for coffee postharvest processing is provided. Mass flow data were collected through industry-scale site visits, surveys, laboratory measurements, and interviews with coffee wet and dry mill operators in several countries throughout Central America and Mexico, as well as roasters and cafés in the United States, to establish representative mass flow rates and process flow diagrams from harvest to cup.

RESULTS

Results indicate that 100 kg of harvested coffee cherries will on average yield 2.6 kg of mass consumed by humans as exported coffee, equivalent to approximately 839 metric cups (250 ml) of drip brew coffee *or* 897 metric shots (30 ml) of espresso.

CONCLUSIONS & PERSPECTIVES

The remaining 97.4 kg provide opportunities for development of alternative products, and other economic uses. Importantly, the data suggests that more mass is lost during depulping in practice than previously indicated by laboratory measurements. This study provides a foundation for further investigations in the fields of equipment improvement, byproduct utilization, and environmental and economic sustainability of the coffee processing and distribution chain.

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S5-P-09

Hours and Misfortunes of the Geographical Indications (GI) of Kintamani Bali Arabica coffee: What possibilities to reactivate them by reconsidering traceability

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RATIONALE

The Geographical Indications (GI) can play an important role in the coffee sector on the recognition of a specific quality due to a *terroir* effect. However, GI development appears as a path full of pitfalls.

METHODS

The GI "Kintamani Bali Arabica coffee" has been the first registered GI in Indonesia in 2008.

RESULTS

By this study concerns the difficulties of the GI development in the coffee sectoras (i) the low level of prices paid to coffee farmers, notably induced by the strength position of middle-men, (ii) the competition of other crops and (iii) the difficulties of maintaining cooperative strategies. Under the Ministry of Co-operative & SMEs of Republic of Indonesia, the Coop Coffee Project aimed since 2015 to relaunch a GI dynamic. The project has re- established a processing unit and the quality produced has interested Starbucks with an efficient traceability system, which links data on farmers' group, finance, and market developed by Bext360.

CONCLUSIONS & PERSPECTIVES

The lessons learned from the GI's trajectories in different countries during the last decades and the technology currently available bring new possibilities for GI's development.

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S5-P-10

Using δ13C and SLA to infer intrinsic water use efficiency of coffee along elevational gradients and forest canopy shade in humid tropics of Ethiopia

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RATIONALE

The current and future predicted changes in environmental conditions reinforce the need to improve water use efficiency (WUE) in order to provide an environmentally friend and sustainable coffee production. WUEi reflects a balance between gains (carbon assimilation) and costs (water consumed by transpiration). At the leaf level, WUE responses to plant water status can be easily measured using gas exchange measurements for short time intervals; however, to qualify the responses in a wider time scale, a detailed experiment like the leaf δ13C analysis would be of particular research interest. Thus, one way to assess how environmental changes affect coffee is via studying leaf traits, most notably stable carbon isotope composition (δ13C) and specific leaf area (SLA) to determine intrinsic water use efficiency.

METHODS

A total of 59 coffee farms were studied along elevational gradients (1506 m - 2159 m asl) and canopy shade (open - dense shade), out of which data were collected from 177 coffee shrubs in southwest Ethiopia. Forest canopy shade was measured using densiometer and hemispherical photography whereas soil moisture content was measured using moisture meter and gravimetrically. Leaf isotopic analysis (δ13C) was performed at the Stable Isotope Laboratory of Ghent University, Belgium. Specific leaf area was determined as a ratio leaf area/oven dry weight. A linear mixed-effect model was used to analyze the data.

RESULTS

The overall findings of our study showed that, the interaction effects of elevation and canopy shade had a strong impact on WUEi values. Smoothing curve from loess regression showed that, significantly higher values of WUEi were observed at elevations near 1934 m a.s.l. at around 43-50% shade level, suggesting that coffee plants growing around this zone can assimilate more CO2 with minimum water loss. At the scale of our study (653 m elevational difference), SLA showed a sharp decline with elevation. Higher SLA values at lower elevations could imply reduced drought stress. Linear relationships were observed between δ13C, ratios of intercellular to ambient CO2 fraction (Ci/Ca), and WUE. SLA negatively correlated with WUEi, implying that a decrease in SLA may be a disadvantage for CO2 uptake in that thicker leaves have a greater demand for CO2 per unit area due to a higher stomatal density.

CONCLUSIONS & PERSPECTIVES

Results from this study confirmed that elevation near 1934 m at intermediate shade level could be the optimum zone for efficient water use and reduced drought stress. Hence, the findings are crucial to adjust degree of shading for better performance of coffee plant at a given elevation under the changing climate.

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S5-P-11

Coffee, the Crucified Saviour of Kenyan Economy

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RATIONALE

The Kenyan coffee sector has an enormous potential in the country's economic sustenance. However, in the last three decades, coffee production in Kenya has maintained an alarming downward trend. If this trend is not reversed, Kenyan coffee will vanish out of the international market in a very near future.

METHODS

This paper provides a professional diagnosis of the Kenyan ailing coffee sector and provides feasible immediate solutions to the tumbling production trend. The three-decade coffee production trend has been carefully examined and the malady identified. The neglected role(s) of key stakeholders have been identified and sustainable strategies are recommended for adoption. The available production resources have been analysed and production potential has been estimated from a multi-disciplinary point of view.

RESULTS

Kenyan coffee production increased rapidly after independence from 43,778 tonnes in 1963–64 to 128,941 tonnes in 1983–84 but fell to below 50,000 tonnes after 2000. The downward trend has continued in ripples sometimes falling close to 30,000 tonnes. The area under coffee has also declined by 32% from 170,000 ha in 1998 to the current 115,000 ha. Kenya is endowed with wonderful production resources including climatic and edaphic conditions that supports production of one of the best quality and highly sought-after Arabica coffee in the world. Kenyan coffee sector alone has a potential of contributing more than Kes 128 billion (US$ 1.3 billion) in Gross Domestic Product annually. Unfortunately, the sector has not enjoyed the necessary support from the government since independence. This has left the farmers at the mercy of poorly managed cooperative societies and the gluttonous private coffee millers, marketers and middlemen. Consequently, coffee farming remains one of the most unproductive enterprises while coffee trade remains one of the most lucrative businesses in Kenya.

CONCLUSIONS & PERSPECTIVES

The sector has a high potential of revamping back mainly assured by the naturally occurring production resources and the resilient nature of the farmers. However, the Kenyan government must play the leading role of revitalizing the sector by adopting and enforcing recommended strategies.

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S5-P-14

Strengthening extension services to support the rejuvenation of coffee industry in Tanzania

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RATIONALE

The Tanzania Coffee Research Institute (TaCRI) has released top hybrid coffee varieties that combine high yields and good beverage quality with resistance to the devastating diseases – coffee leaf rust (CLR) and coffee berry disease (CBD) for Arabica and coffee wilt disease (CWD) for Robusta. TaCRI has also packaged good agricultural practices (GAPs) for the new varieties including packages for the rehabilitation of old farms of the old varieties. Despite the above efforts to develop new varieties and packaging reserach recommendations, one of the challenges has been on how to speed up the dissemination of these achievements due to weak extensions services for delivery of clear extension messages to coffee growers.

METHODS

In addressing the problem of low productivity and production, TaCRI has put much empahsis on strengtheing the extension services for delivery of good agricultural practices (GAPs) to coffee growers. A number of extension methodologies have been well defined and applied to extension officers of the public and private sectors including lead farmers. Such extension methodologies that TaCRI is currently deploying in technolgy transfer include but not limited to provision of training courses to extension officers of all sectors, facilitatig the extension officers through provision of transport, conduct extension visits/study tours, developing and distribution of extesnion materials with clear extension messages in simple language. Further, we have adopted a farmer to farmer approach by training lead farmers including provision of motorcyles who then work with extension officers to train their fellow farmers on the best use of good agricultural practices (GAPs) and hybrid seedlings multiplication.

RESULTS

Notable achievements have been realized whereby 12,104 extension officers and 4,206 farmer promoters (lead farmers) trained in GAPs and hybrid seedlings multiplication thus improving access to planting materials of the improved hybrid varieties and application of GAPs by coffee growers. Yield increase and hybrid seedlings availability for farmers who accessed clear extension messages by extension officers and lead farmers.

CONCLUSION & PERSPECTIVES

This paper has outlined various innovative strategies used in strengthening the extension services to support the rejuvenation of coffee industry in Tanzania. Therefore, multi approach strategies in addressing weak extension services is a way forward to speed up the dissemination of research recommendations to coffee growers

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S5-P-15

The role of women in the multiplication of hyrid seedlings of coffee varieties in Tanzania

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RATIONALE

Women contribute significantly to agriculture and food security around the world, and gender equality and women's empowerment are critical to ensure sustainable development. Yet, women have continued to face unequal access to training, resources ownership, and opportunity to practice and adopt new agricultural technologies, grow their business and use their cash income to improve their livelihoods.

METHODS

The Tanzania Coffee Research Institute (TaCRI) has released hybrid coffee varieties that are high yields and good beverage quality with resistance to the devastating diseases – coffee leaf rust (CLR) and coffee berry disease (CBD) for Arabica and coffee wilt disease (CWD) for Robusta. The challenge has been how to meet the demand of the improved seedlings of the new varieties with the current demand far exceeding the supply. The new coffee varieties are hybrids that require more specialized multiplication and distribution system. The major focus has been on capacity building of community-based groups with 25 to 30 members most of who are women to multiply hybrid seedlings by clonal propagation, grafting and seed methods for the gradual replacement of the traditional coffee varieties with improved varieties but also producing selling thus making it additional source of income. We have been sensitizing women during agricultural/open days/coffee forums, during backstopping visits, conducting exchange/study visits, conducting structured visits, village based training, use of women lead farmers to actively participate in hybrid seedlings multiplication across the coffee growing zones thus improving accsss to hybrid seedlings for replanting programme.

RESULTS

Since the release of improved coffee varieties in 2003, notable achievements have been realized whereby 300 farmers' owned nurseries have been established producing up to 10 million seedlings annually thus improving access to planting materials of the improved hybrid varieties.

CONCLUSION & PERSPECTIVE

It was found that women play a big role in coffee hybrid seedlings multiplication, hence there urgent need to women representation in seedlings multiplication that will contribute to increased seedlings access to coffee growers. Further, research is needed to identify opportunities along coffee value chain to widen women participation that will contribute to increased income and livelihood improvement of women.

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S5-P-16

Influence of agroforestry system on restoration of Gorongosa rainforest and in the physical and chemical characteristics of Gorongosa Coffee

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RATIONALE

Deforestation has dramatically increased in the world in recent years, and some regions in sub-Saharan Africa have the highest deforestation rates, with high environmental negative impacts. The coffee production under agroforestry system (AFS) in the Gorongosa mountain in Mozambique, a region of rich biodiversity, aims at to reconcile coffee crop sustainability (1,2) with biodiversity recovery, local development, and increase the income of small holder farmers, reducing poverty and, consequently, the pressure on local natural resources. Thus, the impacts of coffee production under AFS on the recovery of degraded environment, and coffee bean quality were evaluated.

METHODS

A study compared areas with coffee AFS production and rainforest degraded areas. The identification of degraded areas was based on the observation of biological (vegetation cover and species composition) and physical (presence of crusts in the ground) parameters. Data of height and diameter of tree species in the two areas were collected and the number of individuals of tree species used to determine the richness, relative abundance, diversity of the tree species in the two areas. Additionally, mature fruits of *Coffea arabica* L. *cv*. Costa Rica, from plants cultivated at altitude of *ca*. 650, 825 and 935 m, under dense shade, moderate shade (promoted by native tree species), and full sun exposure, were harvested. Physical (weight of 100 grains and grain size, apparent density, color) and chemical (caffeine, trigonelline, pH, and chlorogenic, caffeic, *p*-coumaric and ferulic acids) parameters were assessed.

RESULTS

Globally, the diameter and height of the species did not show large variations among areas, but species richness, diversity and abundance were higher in areas of coffee AFS production than in degraded areas. Regarding the quality of coffee, the weight of 100 grains, density, caffeine content and total phenols tended to increase with altitude. The green bean color suggested an improvement in quality with increasing altitude. Trigonelline, caffeic, *p*-coumaric and ferulic acids, and soluble solids contents were influenced by both shade and altitude, with significant increases with shade at 935 m.

CONCLUSIONS & PERISPECTIVES

The coffee AFS production is helping reforestation of degraded areas of Gorongosa mountain. Bean quality tend to increase with altitude, with some contribution of shade, although further analysis is being performed.

Acknowledgements: funding from Camões, I.P., Portugal (project *GorongosaCafé*), Agência Brasileira de Cooperação (Brazil), and Fundação para a Ciência e a Tecnologia, Portugal (units UIDB/00239/2020; UID/04129/2020; UID/04035/2020).

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Electrogenic H^+-pumps activities of Coffea spp. grown under low ultraviolet radiation levels

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RATIONALE

The role of UVs on the cellular functioning in *Coffea* spp. is quite unknown. In particular, electrogenic H^+ pump couples ATP hydrolysis to proton transport out of the cell, and so establishes an electrochemical gradient across the plasma membrane, which is used to couple secondary transport de ions and metabolites. In this context, this work aimed at evaluating the impacts of reduced ultraviolet radiation (UV-A and UV-B) levels on three electrogenic H^+ pumps activities, the membrane and vacuolar type H^+ ATPases (P-ATPase and V-ATPase, respectively) and membrane type H^+-pyrophosphatase (PPase) of *Coffea* spp. genotypes.

METHODS

Nine month-old *C. arabica cv*. Catuaí Amarelo IAC 62 and *C. canephora cv*. LB1 plants, representing the two most widely cultivated coffee species, were grown in 32-L pots inside a greenhouse covered with either glass or polycarbonate, exposing the plants to UV-A (13.85 and 3.92 W m^{-2}) and UV-B (3.82 and 0.38 W m^{-2}), respectively, in northern Rio de Janeiro State, Brazil. The microsomal fraction from completely mature leaves was isolated by means of differential centrifugation (1). The hydrolytic activity of the H^+ pumps (P-ATPase, V-ATPase and PPase) was colorimetrically determined by measuring the release of Pi (2).

RESULTS

Regardlless the UV treatment, Catuaí showed higher P-ATPase and V-ATPase activities than LB1. Under reduced UV conditions the P-ATPase activity increased in both genotypes, while V-ATPase tended to increase in a species-dependent manner, *i.e.*, only in Catuaí. On the other hand, LB1 had higher PPase activity than Catuaí, and low UV levels decreased its activity in both genotypes. Therefore, under higher UV levels plants usually, presented lower activities of H^+-ATPases (P-ATPase and V-ATPase) due to the lower availability of ATP. However, ions homeostasis across the vacuolar membrane is maintained by the PPases activity, which use pyrophosphate as substrate, which is abundant as a product of various cellular reactions in stress conditions. Thus, it is consistent that we have observed an increase in PPase activity in both genotypes under higher UV levels.

CONCLUSIONS & PERSPECTIVES

Our results indicate a better metabolic adjustment in coffee plants grown under low UV levels, probably associated with greater efficiency of P-ATPase activity (and V-ATPase only in Catuaí), what might be implicated in the greater plant growth under these conditions.

Acknowledgements: funding support by CNPq, CAPES, and FAPERJ (Brazil), as well by Fundação para a Ciência e a Tecnologia (Portugal) through the units UID/04129/2020 (LEAF) and UIDP/04035/2020 (GeoBioTec).

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S5-P-22

Evaluation of genotype – environment interactions of new *Coffea arabica* F1 hybrids planted in North-West provinces of Vietnam

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RATIONALE

The H2020 BREEDCAFS (http://www.breedcafs.eu) project aims to test new F1 hybrids of *Coffea arabica* (high yielding, stress resistant and adapted to agroforestry) in coffee producing countries, such as Vietnam. In 2018, these hybrids were planted in "demoplots" on-farms, along an altitudinal gradient in the North-West Vietnam and monitored in the subsequent years (2019-2021). Yields were also estimated in 2020. The results of these multilocation trials are presented and discussed.

METHODS

The tested F1 hybrids of *C. arabica* were Starmaya (male sterile CIR-SM01 x Marsellesa), and Centroamericano H1 (Sarchimor T5296 x Rume Sudan Ethiopia) [1-3]. The pure lines local Catimor (provided by NOMAFSI) and Marsellesa (father of Starmaya) were used as controls. In each "demoplot", these accessions were tested in two repetition blocks of 50 plants (*e.g.* 5 lanes of 10 plants). Eleven "demoplots" were set up in smallholder farms located at various altitudes (from 600 to 1100 m.a.s.l.) and under various agroforestry systems in Son La and Dien Bien provinces. The climate conditions were registered in each plot. These trials were planted in June 2018 and monitored (height, basal trunk diameter, number of plagiotropic branches…) in May 2019 and 2020. Yield components (fruiting nodes/tree, fruits/node, seed-fruit ratio…) were also measured along the production cycle and yield obtained in late 2020 (first harvest). Beans were processed, graded and cup tasted.

RESULTS

In all "demoplots", the hybrids Starmaya and H1 were always the most vigorous with the highest height and trunk width. On the other hand, the highest numbers of primary (plagiotropic) branches were mainly observed for the Marsellesa and H1 hybrid. In all "demoplots", local Catimor yields were consistently lower compared to other accessions. In addition, yield of all accessions was higher at high altitude. First results of cup-quality will be also presented.

CONCLUSIONS & PERSPECTIVES

Beyond the BREEDCAFS project (ending in September 2021), plant phenotyping will continue in 2021 as well as yield and quality monitoring which will be also evaluated in 2021 and 2022. With the help of the local Vietnamese partners, work is ongoing to set up large-scale trials necessary for the accreditation process of these accessions in Vietnam.

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POSTERS

Session 6: Biochemistry & biotechnology & composition of green coffee

S6-P-01

Biochemical composition and mineral content of Ethiopian green arabica coffee beans

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Green coffee beans are composed of several volatile and nonvolatile compounds that are responsible for the aroma and flavor of coffee beverage and whose compositions are influenced by various factors along the coffee value-chain - from farm to cup. The most abundant classes of volatile compounds include alcohols, esters, hydrocarbons and aldehydes and that of nonvolatiles are caffeine, trigonelline, chlorogenic acids, soluble fiber, diterpenes (fats), sucrose, protein, free amino acids and peptides. This paper is based on own data and published sources (Habte et al., 2016; Mehari et al., 2016a, 2016b, 2016c; others), and mainly focused on caffeine, trigonelline, chlorogenic acids, sucrose and mineral contents of green arabica coffee beans from the four major coffee-growing regions of Ethiopia (Harar, Southeastern, Southwestern and Northwestern Ethiopia), as the main aroma and flavor precursors and bioactive compounds. Except some study reports on mineral contents, the average contents of caffeine, trigonelline, chlorogenic acids (CGA), sucrose and minerals reported for Ethiopian coffee from four coffee regions are generally comparable to those values reported for arabica coffee from elsewhere in the world. The four regional coffees and the coffee types (i.e., *Harar*, *Sidamo*, *Yirgacheffe*, *Jimma*, *Kaffa*, *Wellega* and *Gojam coffees*) have different levels of caffeine, trigonelline, individual CGAs (except 5-*p*CoQA) and minerals. For example, caffeine content was significantly higher in *Gojam coffee* than in the other coffee types. *Harar coffee* was found to contain lower levels of caffeine; however, the difference is significant only compared to *Jimma*, *Wellega* and *Gojam coffees*. Similarly, *Kaffa coffee* was found to have a significantly lower content of trigonelline than the other coffee types, except for *Jimma* and *Wellega coffees*. The southeastern coffee contained higher amounts of Cu, Fe, Mg, Mn, P, Si and S than the southwestern and Harar coffees. There were, however, statistically similar levels of total CGA across the four regional coffees. In general, these findings indicate Ethiopian coffee potential for quality profile mapping and geographic origin indication.

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S6-P-02

Biochemical analysis of coffee wilt disease-resistant Robusta coffee varieties with prospect of use in herbal formulations

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RATIONALE

Coffee is rich in various ethno-pharmacological compounds that exhibit anti-oxidant properties and are important in wound healing, anti-inflammatory, anti-aging, anti-cancer and protection of body cells from ultraviolet irradiation-induced apoptosis. These compounds exist in, and can be extracted from various parts of the coffee plant, cherry and the accrued byproducts. Thus, coffee is ingredient to various industrial products including beverages, confectioneries, pharmaceuticals and body-care products. Therefore, it is important to determine the suitable developmental stages of coffee and the appropriate drying methods that exhibit/retain optimal quantities of these bioactive compounds.

METHODS

The study included seven Coffee wilt disease-resistant (CWD-r) Robusta coffee varieties, established in an RCBD at the National Coffee Research Institute, 800-1160msl. Samples of green unripe and red ripe cherries were picked fresh and analysed for key biochemical compounds. A sample of the red cherries was divided into 2 portions and dried by either sun or solar until a moisture content of 11.5-12.5%. The dried samples were hulled and graded to obtain a green bean retention size 15 and above. The graded samples were milled, extracted with 15% methanol and analysed for caffeine, chrologenic acids and trigonelline using HPLC, equipped with a diode array detector. B vitamins were extracted with water and analysed using an Agilent LC-qTOF system with MassHunter software.

RESULTS

Caffeine, chlororgenic acids and trigonelline ranged from 2.6-4.2%, 6.6-10.4% and 1.1-1.3% respectively among the tested varieties. Variation in these biocompounds was not significant ($P \leq 0.05$) among the varieties and the drying methods. A strong positive correlation was observed between caffeine and chlorogenic acids ($r = 0.91$, $P < 0.05$), while a negative correlation was observed between caffeine and trigonelline ($r = -0.48$, $P < 0.05$) and between trigonelline and chlorogenic acids ($r = -0.51$, $P < 0.05$). Amongst the B-vitamins, riboflavin (67.1 ± 1.1 µg/Kg) and niacin (52.0 ± 2.1 µg/Kg) were observed in the fresh unprocessed coffee and their concentration did not vary significantly between the unripe and ripe coffees. Upon drying, riboflavin retention was $31.5 \pm 7\%$ under sun-drying and $12.0 \pm 13.4\%$ under solar-drying while niacin retention was $94.2 \pm 4.3\%$ in sun-drying and $36.3 \pm 6.8\%$ in solar-dried coffee.

CONCLUSIONS AND PERSPECTIVES

Results point out to low biochemical variability among the CWD-r varieties. Drying of coffee at lower temperatures should preserve bioactive compounds, with prospect of use in organic formulations for such applications as pharmaceutical and body-care.

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S6-P-04

Coffee (*Coffea arabica* L.) Bean Transcriptome affected under Rust (*Hemileia vastatrix* Berk. & Br) and Yield Stresses

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Stress is one of the major problems induced by coffee leaf rust (CLR), which is caused by *Hemileia vastatrix* Berk. et Br. This study evaluated the effect of CLR control and fruit thinning treatments on the gene expression of immature and mature coffee beans in two CLR susceptible cultivars of *Coffea arabica*. All differentially expressed genes (DEGs) were grouped into gene ontology (GO) functional categories related to the treatments, maturity stages, and cultivars. The enriched metabolic pathways related to the DEGs revealed differences between the management practices and the physiology of the plant by genotype. A higher number of DEGs were found in the immature stage where synthesis of fatty acids and carbohydrates were most active. Structural modifications and accumulation of metabolites in the cell wall differed between treatments, revealing activation of metabolism caused by stress. Stress changed both gene expression and volatile profiles in pathways especially related to unsaturated fatty acid metabolism. The overall interaction of rust control and fruit thinning management showed that stress influences the bean's defense response and the chemical composition in a cultivar dependent manner.

S6-P-06

Effective DNA extraction method for coffee leaves and other high phenolic contaminant plant tissues

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RATIONALE

Isolation of DNA from plant tissues which have high phenolic content is often difficult. The extraction of DNA from coffee leaf sample by general CTAB DNA extraction method is often resulted in low DNA quality with high phenolic contaminants that interfere with the subsequent manipulation.

METHODS

The combinations of two extraction buffers containing CTAB, SDS and NaCl (1, 2), as main ingredients, in the first two extraction steps were found to successfully reduce phenolic contamination. The high quality and amplifiable DNA from the fully expanded leaf tissue of coffee (*Coffea Arabica* L.) were successfully obtained.

RESULTS

The quality of the resulting DNAs detected by A260/280 ratio was in the range of 1.79 to 1.86 indicating low protein and ethanol contamination. The DNA yields were ranged from 400 to 2000 ng per 1 µl from the 0.2 g fresh leaf extract. Agarose gel electrophoresis showed clear intact genomic DNA. The PCR reaction performed by SSR primer showed clear and fully amplifiable products indicting low interference from possible contaminations.

CONCLUSIONS & PERSPECTIVES

This extraction protocol is suitable for DNA extraction from coffee leaf sample. Moreover, this extraction technique can also be applied for DNA extraction of other problematic leaf sample containing high phenolic compounds.

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S6-P-07

Substances with physiological effects in several tissues of different coffee species - Part 2 chlorogenic acids

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RATIONALE

The beans of the two economically most important coffee species *Coffea arabica* and *Coffea canephora* differ in their chlorogenic acid content [Clifford; Kuhnert 2019]. The aim of our studies was to examine in addition to the beans also the leaves, roots, branches, pulps, and blossoms of various Arabica varieties and other *Coffea* species with regard to their chlorogenic acid content. This should provide an insight into the occurrence and the respective distribution so that certain parts of the plant could be used for commercial exploitation, all the more since the diterpenes and alkaloids of the same samples were also analyzed.

METHODS

The plant material was made available by the Coffee Research Foundation Ruiru, Kenya, the greenhouse for tropical crops Witzenhausen, University of Kassel and the Dicafé company, Mexico City. Each sample was freeze-dried. According to Kölling-Speer 2005, the chlorogenic acids were extracted with methanol/water (ASE/USB) and analyzed with HPLC and PDA. Three isomers of caffeoylquinic acid (CQA), three isomers of di-caffeoylquinic acid (diCQA) and 5-feruylquinic acid (5-FQA) which formed the main compounds, were evaluated quantitatively.

RESULTS

In most parts of the plant, 5-CQA was the dominant compound; the blossoms contained up to 19.5 g/100 g, whereas the beans only showed contents up to 10.6 g/100 g. For leaves, between 0.3 g/100 g - 4.3 g/100 g and for pulps, between 1.2 - 3.1 g/100 g were analyzed. The branches and roots generally contained very low levels. The most important representative of the diesters was 3,5-diCQA. It was noticeable that the proportion of 3,5-diCQA in the leaves, blossoms, and branches of Robusta was higher than that of 5-CQA. In general, a decrease in the total content was observed from the tip of the plant to the root, with the young, tender plant parts such as blossoms exhibiting the highest chlorogenic acid content, which is probably due to the importance of this for the plant as a protective and defensive substance.

For the contents of diterpenes and alkaloids, see Poster part 1 and 3.

S6-P-08

Substances with physiological effects in several tissues of different coffee species - Part 3 caffeine and other alkaloids

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RATIONALE

The beans of the two economically most important coffee species *Coffea arabica* and *Coffea canephora* differ in their caffeine content. The aim of our studies was to examine, in addition to the beans, the leaves, roots, branches, pulps, and blossoms of various Arabica varieties and other *Coffea* species with regard to their caffeine, theobromine, theophylline, and trigonelline content. This should provide an insight into the occurrence and the respective distribution so that certain parts of the plant might be used for commercial exploitation, all the more since the diterpenes and chlorogenic acids were analyzed from the same samples.

METHODS

The plant material was made available by the Coffee Research Foundation Ruiru, Kenya, the greenhouse for tropical crops Witzenhausen, University of Kassel and the Dicafé company, Mexico City. Each sample was freeze-dried. Caffeine was determined according to DIN method 10777-2. In addition, the levels were compared with those from the chlorogenic acid determination. In addition, theobromine, theophylline, and trigonelline were determined using these methods.

RESULTS

The highest caffeine content was determined to be 2.03 - 3.24 g/100 g in the blossoms. Contents of 0.45 - 1.23 g/100 g were found in leaves and pulps of various types. Branches and roots were generally poor in caffeine; leaves of the varieties Excelsa, Liberica, and Eugenioides did not contain caffeine. The relationship between caffeine and chlorogenic acids known for beans has now been confirmed for pulps and blossoms. A high caffeine content was also found in parts of plants and varieties with a high chlorogenic acid content. The contents of the other alkaloids are also discussed.
For the contents of diterpenes and chlorogenic acids, see Poster part 1 and 2.

S6-P-13

Transcriptome of *Coffea eugenioides* reveals genes differentially expressed in leaves and fruits

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RATIONALE

Coffee is one of the main agricultural commodities in Brazil. *Coffea arabica* represent 70% of coffee worldwide production and was originated from a recent and natural hybridization between *C. canephora* and *C. eugenioides*. The majority of coffee genetic studies have been focusing on the specie with greater economic importance, the alotetraploid *C. arabica* and its diploid ancestral *C. canephora*. However, *C. eugenioides* is still poorly studied. One way to improve our knowledge on *C. eugenioides* is to perform an RNA-seq analysis. In this study, we re-analyzed a previous RNA-seq data using a reference genome to improve trancriptmome assembly and analysis.

METHODS

Two RNA-Seq libraries of *C. eugenioides* (leaf and fruit) were aligned to a *C. eugenioides* reference genome using HISAT2 and StringTie softwares. We used the *C. eugenioides* genome reference from the Arabica Coffee Genome Consortium (ACGC). Transcripts were built by Kallisto and the annotation process were performed using Blast2GO software, BlastX tool against Genbank (NCBI-nr) and UniProtKB databases. We validate our in silico gene expression profile using RT-qPCR analysis.

RESULTS

Our transcriptome assembly using a reference genome resulted in a total of 16,743 transcripts, where 322 were considered as new transcripts for *C. eugenioides*, and 36 transcripts were not yet described in *Coffea* genus. We observed 416 and 507 genes up-regulated in leaves and fruits, respectively. RT-qPCR validated RNA-seq in silico expression profile of all candidate genes, they were highly expressed in fruits compared to leaves.

CONCLUSIONS & PERSPECTIVES

The use of reference genome improved our initial transcriptome work with more than 300 new genes being identified. Our results increase our knowledge about the genetic basis of one *C. arabica* ancestor, *C. eugenioides*, and will help future coffee breeding program to develop new cultivars with desirable diterpene content.

References:

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POSTERS

Session 7: Roasted coffee technology & processing

In-silico espresso coffee: formulation, test and future perspectives

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RATIONALE

Properly controlled extraction processes are able to improve the organoleptic and nutritional properties of espresso coffee (EC), with a consequent enhancement of coffee market services and sustainability. We propose a physico-chemical model of the coffee extraction process that opens the way to implement the aforementioned control strategy.

METHODS

The extraction model is based on fluid-dynamics laws (Bear, J. e *media*, 2012) to fully describe the main aspects of water percolation in the coffee powder. In particular, different parts of the model focus on the dissolution and transport processes for different chemical compounds. Therefore, a finite element procedure for the numerical solution of this model allows the computation of the chemical compounds in the EC cup, knowing the physico-chemical properties of the coffee powder and the extraction parameters. The reliability of the model is assessed on an experimental basis: the numerical outcome is compared with the chemical laboratory analyses performed by HPLC-VWD on EC samples of two coffee varieties (Arabica, Robusta), having different granulometries and extracted under different conditions (water temperature and pressure).

RESULTS

This is an ambitious extension of a preliminary study published in (Giacomini, J. e*h.Flow*, 2020), where we have considered only caffeine and chlorogenic acids in a lower number of extractions. This introductory study revealed a good agreement between numerical and laboratory results with a low percentage error. Results of a wider calibration work will be shown, by including in the model other relevant chemical species for coffee taste, e.g., lipids, citric acid, malic acid and by considering a wider grid of extraction settings. Sugars are not included since their presence in EC is not detectable with standard analyses, being under the instrumental sensitivity and therefore under the human taste sensitivity (Batali, M. E. e*Agric.*, 2020).

CONCLUSIONS & PERSPECTIVES

The obtained results are strongly promising and show that the proposed model gives a reliable approximation of the main physico-chemical processes occurring during the EC extraction. The next step is the implementation of a control procedure of espresso machines, where the proposed model is the core element. This opens fascinating perspectives for coffee world, like the customisation of the coffee beverage, directly based on customer's preferences and health needs, and the optimisation of the extraction process in terms of coffee powder used, which in turn will increase the sustainability of the coffee market.

References:

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S7-P-02

Variability of espresso brewing in capsule systems

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RATIONALE
In practice espresso coffee brews are variable. There are multiple causes of this both due to the brewing system and the physics of flow through a packed bed of coffee grains, Melrose et al (2018). This paper will show how modelling can be used to help quantify the relative contributions of different effects.
METHODS
Data from a home On-Demand commercial brewer, experimental rig and multi-scale modelling are used to give insight into the variability of brewing from compact beds of coffee particles.
RESULTS
For commercial brewers it is important to recognise variability both of the pump characteristic and the resistance to hydrodynamic flow from the capsules with its coffee bed. Data for the time evolution of bed resistance will be reported. In some capsule systems variability in the piercing of an aluminium film at the outlet can strongly affect the flow resistance – this will be quantified by modelling and experiment. Comparison of data for dilute batch and bed brewing combined with modelling, will show that a generic feature of coffee grains packed in beds is a suppression of yield at early time. This is found to be greater than that estimated to be just due to concentration build up in the bed pore space. Possible reasons for this will be discussed.
CONCLUSIONS AND PERSPECTIVES
Effects leading to the variability of coffee brews can be modelled and quantified. It is hoped that by combining with extraction modelling of molecules the relationship between brew compositions and variability can also be quantified.

References:
- Melrose, J.R., Andrews R. J., Harpley, P. J., Graaff, G. K., 2018a. Capsule, System and use of the system for preparing double beverages like a double espresso, double Lungo and a double ristretto. Patent Application WO 2018/026280 A1.

POSTERS

Session 8: Coffee chemistry & sensory sciences

S8-P-01

Brew temperature at fixed brew strength has little impact on the sensory profile of drip brew coffee

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RATIONALE

For 70 years, industry has relied on the Coffee Brewing Control chart as a guide for coffee quality[1]. This chart relates total dissolved solids (TDS) and percent extraction (PE) to the acceptability of the coffee. The language on the chart lacks detail, and pre-dates modern sensory methodology. Additionally, the Coffee Brewer's Handbook indicates that a water extraction temperature of 92-96 °C is necessary for proper extraction to occur, despite the absence of published sensory data establishing the validity of this range.

METHODS

Coffees were brewed to three levels of TDS (1%, 1.25%, 1.5%) and three levels of PE (16%, 20%, 24%). These 9 target brews were also prepared with three different water brewing temperatures (87 °C, 90 °C, 93 °C), adjusting grind size and brew time as necessary to achieve the desired TDS and PE values, yielding 27 total samples for evaluation by established sensory methodology[2,3]. Results were then mapped versus TDS and PE using Response Surface Methodology to visualize how attributes vary with brew index.

RESULTS

Of the three tested variables, TDS strongly affected attribute intensity variation, PE affected fewer attributes, and brew temperature had no significant effect according to multivariate analysis of variance and minimal significance at univariate level. We found that bitterness, astringency, viscosity (body) and rubber flavor increase with TDS alone. Sourness, dark green, fermented, citrus, berry, and smoky flavors increased with TDS but decreased with PE. Ashy and brown roast flavors increased with both TDS and PE. Black Tea decreased with TDS and increased with PE. Temperature had a small effect on nutty flavor, and some attributes (fermented, earthy and brown spice) did have a small temperature:TDS or temperature:PE interaction.

CONCLUSIONS & PERSPECTIVES

These results help expand the coffee brewing control chart with much richer insight on how to extract specific desired flavors. Results indicate that TDS plays the most important role in the flavor profile, with PE impacting fewer attributes but still making a noticeable difference overall. Our results indicate that brews prepared to the same TDS and PE are not appreciably different when prepared with different brew temperatures, at least over the range tested, calling into question many current industry standards for the temperature required for a drip brew machine.

References:

- Lingle TR. Specialty Coffee Association of America; 1996.
- Batali ME, Frost SC, Lebrilla CB, Ristenpart WD, Guinard JX. J Sci Food Agric. 2020 Feb 7;jsfa.10323
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S8-P-02

The effect of post-brew holding time on the sensory quality of drip brew coffee

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RATIONALE

After brewing, chemical reactions in coffee continue to increase the acidity, and as such, many shops choose to throw out brewed coffee after 30-120 minutes. However, there are few systematic studies documenting the sensory perception of this change. There are multiple ways to store coffee and we expect that sensory quality over time will be dependent on storage conditions.

METHODS

Trained sensory panels evaluated light, medium, and dark roast coffee held at 6 time points between 15 and 180 minutes in three different carafe types (glass on hot plate, thermal jacket, and vacuum). Titratable acidity and pH were measured. Additionally, a large consumer panel of coffee professionals evaluated fresh and held coffee in a blind paired preference test.

RESULTS

Overall, the differences over time were lower than expected, and did not occur quickly. In the paired preference test, a significant majority of the coffee industry professionals preferred held coffee over fresh. For the descriptive panel, chemically, the light roast was fairly stable, with minimal increase in titratable acidity or decrease in pH. Only in the vacuum carafe was there an increase in perceived sourness, vinegar flavor, and citrus flavor, and in the thermal jacketed carafe a slight increase in roasted flavor over time in light roast coffee. For dark roast, there was a noticeable increase in acidity, but that was not reflected in perceived sourness, with no significant increase in any attribute intensity over holding time for dark roast. Over time, intensities in aroma, taste, and mouthfeel decreased for attributes such as chocolate and roasted flavor, bitter taste, and astringent mouthfeel. Medium roasted coffee had the most noticeable sensory variation over time. Titratable acidity increased, and sourness increased with it, as well as astringent mouthfeel and citrus flavor. Burnt, ashy, and roasted flavors decreased over time for the vacuum and thermal jacketed carafes but not for the glass carafe. The thermal jacketed carafe alone increased in medicinal flavor. Generally, for all conditions, the changes that did occur were not significant until 90 minutes post brew.

CONCLUSIONS & PERSPECTIVES

Drip brew coffee can likely be held longer post-brewing without suffering adverse sensory degradation than current guidelines recommend, potentially increasing sustainability and profitability of retail coffee operations. Insulation appears to retain original flavors better than heated storage.

S8-P-03

Titratable acidity and perceived sourness in drip brewed coffee

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RATIONALE

Recent sensory experiments have revealed that perceived sourness in drip brew coffee is extremely sensitive to changes in total dissolved solids (TDS) and percent extraction (PE)[1,2]. As such, determining simple chemical measures to predict sourness would be of value to the coffee industry.

There is little data in the literature about the relationship between pH, titratable acidity (TA), and different brewing parameters like TDS and PE. Here we correlate these acidity measures and brewing metrics with detailed sensory descriptive analysis data for different roast levels and brew temperatures.

METHODS

Multiple sensory studies were performed on drip brew coffee across the range of the classic Coffee Brewing Control Chart, using a trained expert panel and a hybrid of Spectrum Method and QDA to evaluate multiple sensory attributes, including sourness. Concurrent with sensory evaluations, pH, TA, TDS, and PE were measured for every sample.

RESULTS

We found that titratable acidity was linearly correlated with TDS over all brewing conditions and roast levels examined, with regression coefficients always exceeding 0.96 regardless of roast level. In contrast, titratable acidity had no statistically significant correlation with PE. However, perceived sour intensity increased strongly with TDS and decreased strongly with PE. The pH exhibited a strong roast level dependence but little variation among brews.

CONCLUSIONS & PERSPECTIVES

Titratable acidity is a good but imperfect proxy for perceived sourness, while pH may not be. Future work is needed to understand the chemical basis for sour perception in brewed coffee in terms of the impact of extractable compounds that mitigate sourness at higher PE.

References:

- Frost, SC, et al. Journal of Food Science, 2020,2530-43.
- Batali, ME, et al. Scientific Reports, 2020, 16450.

S8-P-04

Specialty Coffee Association's Cupping Protocol: Global study demonstrates diverse perceptions and applications of coffee's common language

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RATIONALE

The Specialty Coffee Association's Cupping Protocol is a globally recognized industry tool used by many stakeholders across the globe to assess coffee quality. Though used by thousands of coffee professionals daily, there has been little effort to understand how the Protocol is being used and its efficacy. A research project was performed to better understand users' current applications of the protocol and its perceived strengths and weaknesses.

METHODS

Data was collected from an online survey and semi-structured interviews. Surveys were disseminated in English, Spanish, Korean, Traditional Chinese, and Simplified Chinese via the Specialty Coffee Association's social media network. Semi-structured interviews were conducted via video meetings with key specialty coffee stakeholders.

RESULTS

Data from 1575 survey respondents and 47 semi-structured interviews revealed that results (score and descriptor) from the SCA Cupping Protocol are frequently used in contracts and price-setting negotiations in coffee transactions, though it was clear that factors outside cupping results influence a coffee's value. Technical aspects of the protocol that were commonly cited as problematic include: Sweetness, reference words, overall, clean cup, body, and complexity. Though there was strong variance in the reported level of its objectivity, the SCA Cupping Protocol was rated as a strong tool in creating a common language inside the coffee community (unlike for consumers).

ANOVA indicates the frequency of use of the Protocol is the most strongly correlated factor with positive perceptions of Protocol, including efficacy as a negotiation tool, objectiveness as an evaluation method, and as means of communication. The most "negative perception" role in coffee is consultant, followed by "other", importer and roaster, while the most "positive perception" roles are cooperative, followed by processor, producer and exporter.

CONCLUSIONS & PERSPECTIVES

Results reveal a high level of adoption by the industry of the SCA Cupping Protocol, which has become a key element in specialty coffee trading. Future improvements of a specialty coffee appraisal system should include the key recommendations and take into account other attributes besides intrinsic quality.

S8-P-05

Coffee silverskin as a source of antioxidant dietary fiber in chocolate cakes

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RATIONALE

Coffee silverskin, CS, corresponds to the integument that covers the raw coffee bean, and is a solid residue generated in coffee roasting, where it is detached from the beans and carried away by the heating air and collected from the exhaust gas by cyclone separation. This residue has been reported to present high contents of dietary fiber as well as high antioxidant potential and prebiotic activity, and thus a few recent studies have evaluated its potential as an ingredient in food products such as breads and cakes. In view of the aforementioned, the aim of this study was to confirm the potential of coffee silverskin as a source of antioxidant dietary fibers in cakes. Given that previous studies have shown that water treatment of the fiber decreases the antioxidant potential (Ateş & Elmacı, 2018), untreated CS were added to a commercial chocolate-flavored cake formulation. Furthermore, although the antioxidant potential of CS has been previously evaluated, this is the first study that evaluates its potential as a source of macroantioxidants.

METHODS

CS (Arabica variety) were donated by a local roaster (Luz, MG, Brazil), ground, sieved (D < 0.5 mm) and used without further processing (CS). Chocolate-flavored cake formulations were purchased from a local market in Belo Horizonte, MG, Brazil. The cakes were prepared using a commercially available cake mixture, with the mixture being increasingly replaced by CS, resulting in four formulations: F0 (control), F1 (2.6% CS), F2 (3.6% CS) and F3 (4.6% CS). The prepared cakes were evaluated in terms of color, extractable and non-extratable (macroantioxidants) phenolics, and total antioxidant capacity according to methodologies described in the literature (Resende et al., 2019) as well as sensory analysis. The evaluated sensory parameters were acceptance of color, smell, taste, texture and overall impression using a 9-point hedonic scale, and intention to buy using a 5-point scale.

RESULTS

Addition of CS increased total dietary fiber content from 2.7 (F0) to 6.3 g/100g (F3), and FRAP-based antioxidant capacity from 2.8 (F0) to 6.5 µMFeSO4/g (F3). The amount of macroantioxidants increased from 8.9 (F0) to 16.1 mg/100g (F3). No significant differences were observed in terms of color, texture and aroma of the cakes. The cakes with lower amounts of CS (F1 and F2) presented the same acceptance level.

CONCLUSIONS & PERSPECTIVES

CS are an interesting source of dietary fiber with antioxidant potential that can be used in the preparation of chocolate cakes.

References:

- Gizem Ateş & Elmacı, 2018. LWT. DOI: 10.1016/j.lwt.2018.01.003.
- Resende et al., 2019. Food Chemistry. DOI: 10.1016/j.foodchem.2018.07.079.

S8-P-07

COFFEE CUALITY™ - A New Method for the Assessment of Coffee Sensory Quality by Experts

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RATIONALE

There currently are a number of ways to assess coffee sensory quality with experts, most of which produce an overall quality score on a 100-point scale, with little to no justification nor information on the actual sensory profile of the coffee.

Inspired from the methodology we use in our consumer research to justify, explain and then act on the overall liking score a consumer gives to a product, we have created a new method for the sensory evaluation of coffee quality which includes both analytical and holistic components and is designed for use by trained experts at every stage of the coffee chain - from coffee valuation at origin to coffee marketing at retail.

METHODS

The COFFEE CUALITY™ Method combines (1) the rating of the sensory quality of the coffee on a 100-point scale, with (2) the rating of key sensory attributes on just-about-right (JAR) scales, (3) the selection of relevant sensory descriptors from a Check-All-That-Apply (CATA) list and (4) brief open comments. The data is then analyzed with a combination of (5) Analysis of Variance, (6) Internal and External Quality Mapping (Delgado and Guinard, 2012), (7) Penalty Analysis, (8) Correspondence Analysis, (9) Word Cloud Analysis and (10) Penalty-Lift Analysis.

The outcome of the COFFEE CUALITY™ Method is a comprehensive, deconstructed, justified and validated assessment of the sensory quality of the coffee and of the expert's performance, i.e., (11) ability to discriminate, (12) reproducibility and, most importantly, (13) alignment with other experts on the concept of quality.

COFFEE CUALITY™ should be used with multiple experts so that the full complement of statistical tools may be applied. But if used by one single expert, results are displayed as the judge's completed scorecard.

RESULTS

The COFFEE CUALITY™ Method includes a sample identification and preparation protocol, a sensory evaluation protocol and scorecard, a statistics suite and a presentation and visualization dashboard.

COFFEE CUALITY™ variants have been developed for the main types of coffee beverages – coffee prepared for cupping, drip coffee, espresso and cold brew.

CONCLUSIONS & PERSPECTIVES

With proper training in data collection and data analysis for COFFEE CUALITY™, we foresee that coffee experts worldwide could provide a more comprehensive, actionable and valid assessment of the sensory quality of coffee, which in turn would improve coffee valuation at all stages of the production and marketing chain.

Reference:

Delgado, C. and Guinard, J.-X. Internal and external quality mapping as a new approach to the evaluation of sensory quality – a case study with olive oil. Journal of Sensory Studies, 27(5): 332-343, 2012.

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- Delgado, C. and Guinard, J.-X. Internal and external quality mapping as a new approach to the evaluation of sensory quality – a case study with olive oil. Journal of Sensory Studies, 2012, 27(5): 332-343.

S8-P-08

Soluble coffee sensory panel performance and training effectiveness assessment

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RATIONALE

Sensory quality is one of the key attributes in the soluble coffee industry. It can be evaluated via 8 attributes (Aroma, Bitterness, Acidity, Astringency, Sweetness, Body, Aftertaste, Roasting Sensation) by a Sensory Panel. This work aims to set an objective method to assess and assure their performance focusing on the Agreement among assessors, their abillity to Discriminate among samples and the Repeatability of their results.

METHODS

A 7-member sensory panel cupped 6 distinctive soluble coffees, randomly coded, with 1 repetition each, in 2 sessions. Assessors scored individually all attributes ranging from 0 (absence) to 5 (strong) without prior to having any or updated training. All cupping results were statistically analysed using PanelCheck software. Overall and individual's performances were checked via 2-way Anova; Agreement of assessors was verified via Tucker-1 plots; their Discrimination skills were analysed via F-plots; their Repeatability skills were calculated via MSE (mean square errors). Repeatability and Discrimination were also checked using p*MSE plots. By reviewing the results statistically, the panel was subsequently trained in 4 sessions, focusing on cupping profiles and specific sensory attributes. Afterwards, cupping sessions were equally repeated as before training and cupping scores were statistically compared to the data before training. All data was considered for 5-% significance level. Results were rated in an internally devised three-level performance scheme with mandatory levels of minimum 60% for the upper spectrum and maximum 10% for the lower spectrum; and optional minimum 30% in-between.

RESULTS

Prior to training, cupping results displayed that the panel distinguished 3 out of 8 attributes. Repeatability complied with the performance scheme, but Agreement (mainly on Body) and Discrimination (mainly on Acidity) did not. Performance scheme was met by 2 out of 7 panel members. We could identify 2 panel members with the poorest performance in Bitterness and Astringency. After the training, cupping results showed the panel's ability to distinguish 6 out of 8 attributes. Panel overall Repeatability, Agreement and Discrimination complied with the performance scheme. 6 out of 7 assessors met the individual's performance scheme.

CONCLUSIONS & PERSPECTIVES

PanelCheck software allowed us to assess statistically a soluble coffee sensory panel's performance. Focused cupping was effective to train the sensory panel to agree on sensory profiles, discriminate samples and repeat results, increasing the confidence on the QA and R&D sensory reports. Continuous panel training and bi-annual assessments are advised.

References:

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- Tomic, O., Luciano, G., Nilsen, A., Hyldig, G., Lorensen, K., Næs, T. (2009): Analysing sensory panel performance in proficiency tests using the PanelCheck software. European Food Research and Technology, 230 (3), 497-511, DOI 10.1007/s00217-009-1185-y.
- Tormod Næs, Paula Varela and Ingunn Berget (2018): Individual Differences in Sensory and Consumer Science – Experimentation, analysis and interpretation (1st edition). Cambridge, UK: Woodhead Publishing.

An Equilibrium Desorption Model for the Strength and Extraction Yield of Full Immersion Brewed Coffee

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RATIONALE

Brewing is the final step in preparation of beverage coffee, and it is well established that the sensory profile of the brew is highly correlated with the strength (i.e., the total dissolved solids, *TDS*) and the extraction yield *E* of the brew. Despite the importance of these two metrics to coffee, there are few theoretical models available to predict how different brewing parameters affect *TDS* and *E*, with extant models focusing on flow extractions like espresso or drip brew. An equally important class of brews involves full immersion, such as that found in classic French press brews or the traditional cupping method used by coffee professionals around the world. In this talk, we will derive and experimentally corroborate a pseudo-equilibrium desorption model for the *TDS* and *E* of full immersion brewed coffee.

METHODS

A predictive model for the TDS and *E* of full immersion brewed coffee was derived using a pseudo-equilibrium desorption approach assuming a single species-averaged equilibrium constant *K*. Coffee was brewed using full immersion method where coffee grounds were submerged in water over a wide range of brew ratios, brew temperatures, grind sizes, and roast levels. Experimentally measured TDS and *E* of full immersion coffee brewed at various conditions were analyzed and compared to the model predictions.

RESULTS

Our model yields theoretical predictions indicating that the TDS is approximately inversely proportional to the water/coffee mass brew ratio, while E is independent of the brew ratio. Our experimental results yield excellent agreement with both theoretical predictions, and further demonstrate that the species-average equilibrium constant is surprisingly insensitive to the major brewing parameters including grind size, roast level, and brew temperature over the range 80-99°C. An analysis of the standard oven-drying method for measuring E indicates that it yields significant underestimates of the true value at equilibrium, due to retained brew within the spent moist grounds.

CONCLUSIONS & PERSPECTIVES

In terms of practical implications, our results indicate that full immersion brewing offers precise control over the *TDS* by brew ratio but little control over *E*, so the relative simplicity of full immersion brewing is offset by a lack of flexibility in fine-tuning the desired sensory profile.

References:

- Liang, J., Chan, K.C., and Ristenpart, W.D., Scientific Reports, in review (2021). https://doi.org/10.21203/rs.3.rs-127037/v1

S8-P-12

Acid release during brewing.

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RATIONALE

A wide variety of organic acids are released in coffee brewing and compositions in grounds have been measured. There are some puzzles reconciling the data sets, the pH of coffee brews are far higher than the measured organic acid compositions would suggest. This was noted by Maier (1997) and argued that many of the acids are neutralised by bases both from the coffee particles themselves and from the brew water. In this paper the relative importance of these effects will be estimated by use of a new modelling tool for neutralisation in multi poly-acid solutions. Data for the kinetics of pH and Titratable acidity (TA) of coffee brews with time will be reported.

METHODS

Experiments were conducted on a Schaerer Art espresso coffee machine set to a coarse grind. Aliquots were collected and analysed for TA and pH over time. A modelling tool was developed to predict the pH and TA of solutions of mixtures of weak poly acids, and strong bases. It was used to model coffee brews from literature data sets of organic acid composition in coffee grounds.

RESULTS

It will be shown that equating the measured mineral content of coffee grounds to an effective base content is alone sufficient to account for the degree of neutralisation and the observed Ph and TA of coffee brews. However, the modelling suggests that there is likely an important contribution from acid-groups on larger macromolecules; but there is limited composition data on this. The model quantifies which organic acids are most influencing brew acidity (Acetic, Citric are two key ones). The experimental data for pH and TA over brew time give insight into to how the composition of released acids varies over time. It will suggest a key area still to understand is the role of acid groups on large macro-mols.

CONCLUSIONS AND PERSPECTIVES

Good progress has been made in developing a quantitative tool for relating the pH and TA of coffee brews to measured organic acid compositions. Despite the early work of Maier (1997) the role of neutralisation determined from the coffee grounds themselves seems to be little recognised, this paper supports its key role. There is a need to get further data on the possible role of acid-groups on macro molecules in brews.

References:

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S8-P-13

Assessing Guatemalan Coffee Bean Quality with E-Eye, E-Nose, and E-Tongue Systems

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RATIONALE

Sensory evaluation is performed for qualitatively judging flavors. Expertise is necessary for the sensory panels, and evaluation is focused to on ensuring reliability. Recently, electric sensor systems have been developed to analyze taste, smell, and the appearance of foods. Instead of subjective evaluation, we have objectively evaluated the quality of coffee beans using electronic sensing systems.

METHODS

Eleven samples were prepared for this study. Of these eleven samples five were Guatemalan SHB coffee with distinctive flavors that were prepared from branded coffee from eight regions in Guatemala, while the others were non-branded Guatemalan SHB coffee. The green coffee was analyzed by E-Eye (IRIS) that was used to obtain detailed visual assessments of the color parameters of the overall beans. The light-roasted coffee was evaluated using two Q graders with SCAA cupping methodology. This method involves scoring on 10 attributes, and the maximum score of each attribute is 10 points. Specialty coffee is defined as coffee that receives a score of ≥80 points. E-Nose (HERACLES-II) was used to analyze the head space vapor of roasted and ground coffee samples with salting-out water. E-Tongue (ASTREE) was used to analyze the compounds dissolved in the liquids. All the equipment for the E-Sensing system was supplied by Alpha MOS, France. Statistical analysis between the SCAA cupping scores and the E-sensing data was conducted using their software.

RESULTS

Partial least squares (PLS) regression analysis indicated that the E-Eye, E-Nose, and E-Tongue data from the Guatemalan coffees were well correlated with the SCAA cupping scores of the Q graders. On developing the predictive model, three attributes (uniformity, clean cup, and overall), which were identified to not have a relationship with aroma and taste, were reduced for analysis. The border score as a Specialty coffee is approximately 53.5 points (full score 70.0) based on the previous experience of Q graders. Moreover, the predictive model works well in verifying unknown Guatemalan SHB coffee.

CONCLUSIONS & PERSPECTIVES

Analysis of coffee beans via integrating E-sensing systems allowed the objective evaluation of specialty quality of the Guatemalan coffee beans. This rapid analysis must work effectively and facilitate reliable research and development, QC, and procurement even for a large number of samples.

S8-P-14

Quality control of *Coffea canephora* genotypes cultivated in the state of Espírito Santo (Brazil) by ESI-FT-ICR MS

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RATIONALE

The quality of coffee is related to good production practices in agriculture, among these factors are cloning. With genetic improvement, the objective is to obtain greater productivity and quality of beans. Cloning influences the final quality and according to the Brazilian Coffee Industry Association (ABIC) the consumer is the main target for the quality control of coffees, therefore, sensory analyzes are extremely important, and for this reason they are classified as standard method of analysis. The objective of this study was to analyze the results obtained by ESI(±)FT-ICR MS and compare it to the data from the sensory analysis.

METHODS

The 42 coffee genotypes from the northern state of Espirito Santo were sent for sensory analysis, performed by six tasters, all with Q-Graders certification. Posteriorly, the samples were also analyzed by ESI(±)FT-FT MS. Based on the results obtained, chemometric models were constructed by principal component analysis (PCA). Optimization of the pretreatment and cutting in relative intensity (IR) was performed in order to obtain the excellent models. The study was supported by Fapes, Capes and Cnpq.

RESULTS

Sensory analysis data resulted in low values for sample 8, 28 and 29. In contrast, sample 36 has the highest score in the assessment. The frequency graph shows that the grade range that characterizes most samples is 75-77.5. Through the analysis of the results of the optimization of the models, it was possible to notice that in ESI(-) and ESI(+) the cut in IR was 5 and 7, respectively, and the pretreatment in both was Center and Standard normal variate (SNV), taking into account the best separation of the samples and the explained variance values. The results of ESI(+) show that in PC1> 0 there is sample 36, while in PC1 <0, samples 28 and 29. However, by ESI(-) it was possible to notice better clusters. PC3 separated the samples with the lowest / highest scores in the sensory evaluation. At PC3 <0 the ions of *m/z* 255, 367, 353 and 361, respectively, palmitic acid, feruloilquinic acid, cafeoilquinic acid and internal standard, are the main ones according to loads.

CONCLUSIONS & PERSPECTIVES

The ESI-FT-ICR MS proved to be an appropriate technique for classification in the quality control of genetically modified Conilon coffees.

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S8-P-17

New DIN-/CEN-HPLC method for the determination of the diterpene 16-O-methylcafestol

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Of all coffee species, Coffea arabica and Coffea canephora have the greatest economic importance. Arabica coffee is grown in higher regions where the cherry grows and ripens slowly due to the low temperature. This affects the pleasant mild taste of the coffee beverage. In contrast, the Robusta plant can also thrive in lower regions with higher and fluctuating temperatures. The drink has an earthy dull note. On the world market, the Arabica bean is therefore traded at significantly higher prices than the Robusta bean. In 2019, in the course of «Operation OPSON VIII» in Germany, three cases were uncovered in which coffee declared as pure Arabica coffee had been mixed with the cheaper Robusta beans. The evidence of adulteration was provided by the 16-O-methylcafestol occurring in Robusta coffee, a diterpene which is also stable under roasting conditions so that the compound is recognized worldwide as a marker substance for Robusta. In Germany, an HPLC method for determining the content of 16-O-methylcafestol in green and roasted coffee was published by the German Institute for Standardization (DIN 10779) in 1999. Recently, a not yet certified NMR method has also been used which due to the simpler sample preparation delivers faster results nevertheless requiring the use of a very expensive analytical device. In 2019, the CEN working group «Food Authenticity» was founded. At their last meeting, it was decided to create CEN standards for the determination of 16-O-methylcafestol in green and roasted coffee using both NMR and HPLC. The established but time-consuming DIN 10799 is to be replaced within this context by a method developed in the working group and herewith presented. It allows for the analysis of up to eight samples per day with reduced solvent requirements. The method will be validated in an international round robin test in March/April 2021 under the leadership of the Federal Ministry for Consumer Protection and Food Safety (BVL). The internationally recognized CEN methods form the basis for a similar assessment by all institutions dealing with the authenticity of coffee and coffee products (coffee roasters, retailers, food monitoring) and also protect consumers from being misled and deceived.

S8-P-18

HS-SPME-GC-MS fingerprints for the "identitation" of the coffee oxidized note

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RATIONALE

The quality of coffee is related to its flavour and aroma developed in chemical reactions during roasting [1]. During storage, coffee undergoes a series of chemical modifications influenced, among others, by temperature, moisture and oxygen, modifying the aromatic fraction and, thereby, its quality. These phenomena affect the shelf-life of roasted coffee, mostly depending on its form (ground or whole beans) and packaging, because of the fast oxidation of some components [2].

METHODS

HS-SPME-GC-MS combined with PCA was used to analyse roasted ground coffees undergone to an accelerate aging. Different packages: standard caps and soft pack for moka coffee were analysed to highlight aroma changes and to identify components related to the oxidation processes. The information resulting from the chemical HS-SPME-GC-MS fingerprints were compared to those of the sensory panel.

RESULTS

The trend over time of HS-SPME-GC-MS analyses goes towards an increase in volatile acids for the two coffee packages analyzed. With aging, PCA showed that moka samples and standard caps lost in quality of aromatic bouquet with a contemporary growing in acidity. Among the two packs, moka showed the highest amount of acids due to the extended superficial area available to the oxidative processes. These trends were also confirmed by the pH values of the brews. Results from the panel test showed an increase of off-flavor oxidized and acid notes in all coffees that prevailed in moka pack compared to caps.

CONCLUSIONS & PERSPECTIVES

These preliminary results show that the HS-SPME-GC-MS fingerprints combined with chemometrics is promising to study chemicals involved in the changes of coffee aroma during its shelf life. The chemical fingerprints affords to identify and define a "chemical identity" of the oxidized note in compliance with sensory evaluation.

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S8-P-19

Study of preferable aromatic and chlorogenic acids-rich beverages from unroasted coffee aged via water heating without roasting

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RATIONALE

Green coffee is considerably aromatized by roasting, and based on its origin, it has a characteristic aromas. Green coffee has many components that are precursors of the aromas, and they are generated by heating. This study was undertaken to create a new aromatic beverage from green coffee via heating it in water, which results in the generation of aromas.

METHODS

Origins of green-coffee samples from all over the world including Arabica and Robusta varieties were treated by the washed and natural in post-harvest processes. A sealed container, such as a can, was filled with a solid–liquid mixture obtained by adding water to whole green coffee or ground green coffee. The container was placed into boiling water, and the heating time considered was up to approximately 12 h. Additionally, whole or ground green coffee with a water ratio of 5–15 times was immersed at 85°C to 100°C for 30 min to 60 min. This extract was filled in the container, and it was placed in to boiling water. After heating and cooling, the containers were opened and the residues were separated. The flavor of the liquor was evaluated, and the pH and chlorogenic-acids contents were analyzed.

RESULTS

In both the mixed state of green coffee with water and the extracts obtained from green coffee and hot water, some origins produced fruity or a black tea-like aroma in boiling water at 100°C for ≥5 h. Preferred origins of coffee beans were Natural Ethiopian Arabica and Robusta that were obtained from countries such as Indonesia. The washed beans, which typically demonstrated good aromas after adequate roasting, did not demonstrate good aromas. Moreover, when the temperature exceeded 120°C, cereal odor and the stuffy heat odor were observed that were not preferable with acidity. Furthermore, when the sealed container was not used, the volatile aroma components generated during the heat treatment were scattered. Although the chlorogenic acids content considerably decreased after roasting, the heated green coffee liquor retained approximately 80% of the original green coffee.

CONCLUSIONS & PERSPECTIVES

A high-quality extract could be obtained from coffee beans without roasting. This extract has potential applications as a new beverage, a natural fragrance, among others. Study conducted in the future will focus on how to scale-up production.

S8-P-20

Acids in Coffee: A Meta-Analysis of Chemical Composition

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RATIONALE

Coffee contains a variety of organic acids (OAs) and chlorogenic acids (CGAs) that contribute to overall sensory properties like sourness and bitterness. Although much work has been done to characterize acid concentrations, large variations in sample types and measurement methodologies complicate interpretation of general trends. Here we perform a systematic review and meta-analysis of the literature to elucidate the concentrations of OAs and CGAs in coffee, across two species of coffee, *C. arabica* and *C. canephora* (robusta), for both green coffee and roasted coffee at multiple roast levels.

METHODS

An extensive review of the scientific literature was conducted to identify peer-reviewed articles that reported experimental measurements of the concentration of any specific CGAs or OAs in coffee. Only publications in which the concentration of a specific type of acid was explicitly reported were included; publications listing the total acidity or the non-quantified presence were not. Each sample was characterized as green or roasted, with the roast level further qualitatively denoted as light, medium, or dark based on a selection rule informed by how the publication described or quantified their roast levels.

RESULTS

A total of 121 different publications were found to report concentration measurements for at least one of 24 different CGAs or 26 different OAs, yielding a total of 5,929 distinct acid concentration measurements. Analysis of the full data set reveals several trends. Most notably, darker roast levels in robusta coffees are associated with a very large increase in acetic acid concentration, such that acetic acid strongly dominates the total OA concentration for robusta dark roasts. As for CGAs, in both arabica and robusta coffee 5-CQA is the major component, and higher roast levels tended to sharply decrease the concentration of all CGAs. The total amount of CGA present was more dependent on roast level than the type of coffee (arabica vs. robusta).

CONCLUSIONS & PERSPECTIVES

The meta-analysis suggests that the increases in certain OAs with roast level might play more of a role in the sensory profile of dark roast coffees than previously suspected, especially acetic acid. Likewise, the CGA concentrations and decrease with roasting are more similar between robusta and Arabica than previously elucidated.

POSTERS

Session 9: Health & safety, consumption

S9-P-01

In vitro evaluation of the affinity of coffee fractions extracts as ACHE inhibitors, regulators of the cholinergic system and preventive factors in Alzheimer's disease

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RATIONALE

Isothermal titration calorimetry (ITC) is an efficient method to study ligand-protein interactions allowing the design of potential enzymes inhibitors. In this study, the most effective acetyl- (AChE) and butyrylcholinesterase (BChE) inhibitors were identified as isolated fractions of coffee bean extracts, rich in caffeine, monochlorogenic and dichlorogenic acids.

METHODS

Fractionated extracts from Robusta Cherry type (*Coffea canephora* L.) and Arabica Cerrado type (*Coffea arabica* L.) beans (roasted to a varying degrees: green, light, dark), were "digested" in a simulated "digestive" system. The system mimicked physiological conditions of the gastrointestinal tract including a mixture of selected probiotic bacteria. The evaluation of AChE and BChE inhibition by samples after "digestion" was done using ITC (Budryn et al., 2018).

RESULTS

Extracts from coffee fractions that underwent simulated in vitro "digestion" combined with AChE, BChE and acetylocholine (ACh) gave strong complexes. Binding at the active site of enzymes was competitive and resulted in a blocked ACh hydrolysis and increased content of free ACh. Extract from Robusta coffee fraction after "digestion" at the "gastric" stage showed the highest affinity to BChE and formed the most stable complexes with the enzyme. The study also showed that the addition of bacteria in the "digestive" system increased the affinity of bioactive compounds from "digested" coffee fraction extracts. This means the increased bioavailability of compounds from coffee caused by probiotic bacteria in the "digestive" tract.

CONCLUSIONS & PERSPECTIVES

The ITC analysis of the affinity of "digested" coffee fractions to AChE and BChE enzymes allowed to determine the most efficient cholinesterase inhibitors. The obtained results expanded the knowledge on the possibilities of using of the coffee fractions as an alternative to drinking coffee and on the bioavailability of bioactive coffee compounds in the context of the treatment of Alzheimer's disease (project No. UMO-2018/29/N/NZ9/01160).

References:

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S9-P-03

Coffee Consumption and Health Effects Studies in the Post Genomic Era: a brief review

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RATIONALE

Coffee is a complex mixture of bioactive compounds that play a myriad of effects in humans. Genome-wide associations studies (GWAS) of coffee consumption led to the identification of a series of genetic locus highly expressed within the coffee consumer population. In parallel, there are many epidemiologic studies pointing to beneficial health effects of daily coffee intake. Mendelian randomization (MR) is increasingly used to determine if the data obtained from GWAS and epidemiologic studies of coffee and caffeine consumption could provide support for a causal role of coffee and/or caffeine use on risk of various diseases.

METHODS

This review used the terms 'coffee consumption' and 'genome' to search within the PubMed database in the last 10 years. The search found 140 studies but only 31 used in their methods genetic or genomic techniques, such as genotyping and GWAS. Sixteen out of the 31 studies used genotyping and/or other genomic methods, while all the other 15 studies used MR methodology to investigate a causal role for coffee consumption and health benefits. Due to findings of an increased use of MR, another PUBMED search was made using the terms 'coffee' and 'MR' and resulted in a total of 46 studies.

RESULTS

As new gene loci are identified through GWAS, new areas of association studies such as, sleep quality and impact of perception of bitter taste started to be investigated as well. Seven out of the sixteen genetic/genomic studies associated with coffee/caffeine consumption were searching for gene loci related with metabolic syndrome. Only one out of fifteen MR studies, which examined the association of coffee and gout was able to provide a causal role for coffee drinking and gout. Most of the MR studies felt short due to confounders such as trait heterogeneity, pleiotropy, and collider bias.

CONCLUSIONS & PERSPECTIVES

All the MR studies used for their randomization GWAS from the Coffee and Caffeine Consortium. There is a need for new GWAS searching for loci independent of the caffeine content. Much could not be concluded from the MR studies due to factors associated with metabolism of caffeine and caffeine content in the coffee beverage. Nonetheless, more elaborated MR studies are being designed to allow the determination of the causal role of the daily coffee intake with data collected from epidemiological studies. As per the data reviewed here, it is getting closer to find answers for old questions still unanswered.

References:

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LIST OF POSTERS

Session 1: Plant science

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Magesa	Marco Jeremiah	The role of women in the multiplication of hyrid seedlings of coffee varieties in Tanzania	**S5-P-15**
Mangueze	Adilson	Influence of agroforestry system on restoration of Gorongosa rainforest and in the physical and chemical characteristics of Gorongosa Coffee	**S5-P-16**
Ramalho	José C.	Electrogenic H+-pumps activities of Coffea spp. grown under low ultraviolet radiation levels	**S5-P-21**
Rigal	Clément	Evaluation of genotype – environment interactions of new *Coffea arabica* F1 hybrids planted in North-West provinces of Vietnam	**S5-P-22**

Session 6: Biochemistry & biotechnology & composition of green coffee

Last name	First name	Title of poster	# poster
Adem	Mohammed Worku	Biochemical composition and mineral content of Ethiopian green arabica coffee beans	**S6-P-01**
Atwijukire	Evans	Biochemical analysis of coffee wilt disease-resistant Robusta coffee varieties with prospect of use in herbal formulations	**S6-P-02**
Echeverria-Beirute	Fabian	Coffee (*Coffea arabica* L.) Bean Transcriptome affected under Rust (*Hemileia vastatrix* Berk. & Br) and Yield Stresses	**S6-P-04**
Khomarwut	Chatnapa	Effective DNA extraction method for coffee leaves and other high phenolic contaminant plant tissues	**S6-P-06**
Kölling-Speer	Isabelle	Substances with physiological effects in several tissues of different coffee species - Part 2 chlorogenic acids	**S6-P-07**
Kölling-Speer	Isabelle	Substances with physiological effects in several tissues of different coffee species - Part 3 caffeine and other alkaloids	**S6-P-08**
Pereira	Luiz F. P.	Transcriptome of *Coffea eugenioides* reveals genes differentially expressed in leaves and fruits	**S6-P-13**

Session 7: Roasted coffee technology & processing

Last name	First name	Title of poster	# poster
Giacomini	Josephin	In-silico espresso coffee: formulation, test and future perspectives	**S7-P-01**
Melrose	John	Variability of espresso brewing in capsule systems	**S7-P-02**

Session 8: Coffee chemistry & sensory sciences

Last name	First name	Title of poster	# poster
Batali	Mackenzie	Brew temperature at fixed brew strength has little impact on the sensory profile of drip brew coffee	**S8-P-01**
Batali	Mackenzie	The effect of post-brew holding time on the sensory quality of drip brew coffee	**S8-P-02**
Batali	Mackenzie	Titratable acidity and perceived sourness in drip brewed coffee	**S8-P-03**
Fernandez-Alduenda	Mario	Specialty Coffee Association's Cupping Protocol: Global study demonstrates diverse perceptions and applications of coffee's common language	**S8-P-04**
Franca	Adriana	Coffee silverskin as a source of antioxidant dietary fiber in chocolate cakes	**S8-P-05**
Guinard	Jean-Xavier	COFFEE CUALITY™ - A New Method for the Assessment of Coffee Sensory Quality by Experts	**S8-P-07**
Hoang The	Ha	S8-P-08 - Soluble coffee sensory panel performance and training effectiveness assessment	**S8-P-08**
Liang	Jiexin	An Equilibrium Desorption Model for the Strength and Extraction Yield of Full Immersion Brewed Coffee	**S8-P-11**

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Melrose	John	Acid release during brewing	**S8-P-12**
Nakai	Mari	Assessing Guatemalan Coffee Bean Quality with E-Eye, E-Nose, and E-Tongue Systems	**S8-P-13**
Partelli	Fábio Luiz	Quality control of *Coffea canephora* genotypes cultivated in the state of Espírito Santo (Brazil) by ESI-FT-ICR MS	**S8-P-14**
Speer	Karl	New DIN-/CEN-HPLC method for the determination of the diterpene 16-O-methylcafestol	**S8-P-17**
Strocchi	Giulia	HS-SPME-GC-MS fingerprints for the "identitation" of the coffee oxidized note	**S8-P-18**
Sugiura	Motohiko	Study of preferable aromatic and chlorogenic acids-rich beverages from unroasted coffee aged via water heating without roasting	**S8-P-19**
Yeager	Sara E.	Acids in Coffee: A Meta-Analysis of Chemical Composition	**S8-P-20**

Session 9: Health & safety, consumption, quality & trends

Last name	First name	Title of poster	# poster
Grzelczyk	Joanna	In vitro evaluation of the affinity of coffee fractions extracts as ACHE inhibitors, regulators of the cholinergic system and preventive factors in Alzheimer's disease	**S9-P-01**
Santos	Roseane Maria	Coffee Consumption and Health Effects Studies in the Post Genomic Era: a brief review	**S9-P-03**

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