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Impact of environmental conditions on plant health and leaf rust incidence in *Coffea arabica*

PA 101

Lucile Toniutti[1,2], Jean-Christophe Breitler[2], Hervé Etienne[2], Claudine Campa[2] , Sylvie Doulbeau[2] , Eveline Dechamp[2], Charles Lambot[1], Juan-Carlos Herrera Pinilla[1] and Benoît Bertrand[2]

[1] Nestlé R&D Tours, 2 UMR Interaction Plante Microorganismes et environnement , Montpellier, France. Email: lucile.toniutti@ird.fr

Abstract

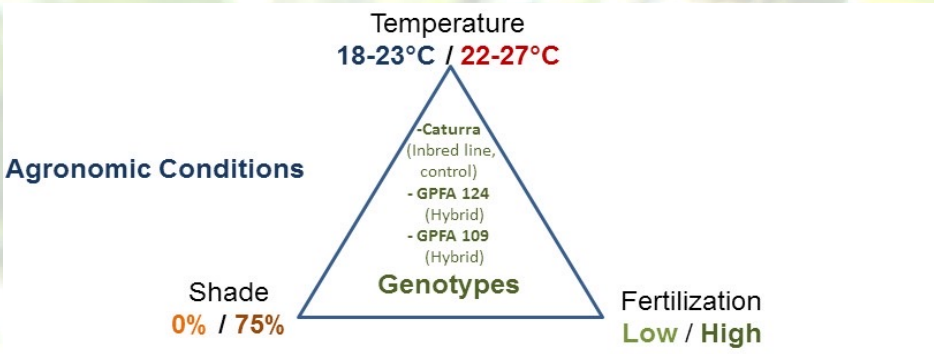
Between 2008 and 2013, some coffee producing countries in South and Central America, were confronted to severe epidemics of the coffee leaf rust (CLR), having important economic losses as well as social implications for the coffee farmers. The on-going climate change is one factor contributing to this outbreak. The main objective of this project is to give insights about how the physiological status of the coffee tree (*Coffea arabica*) can modify the leaf rust disease incidence. Three genotypes were compared using a combination of multiple stress conditions (temperature, fertilization, shade), close to the real situation in the field, before being inoculated with the rust fungus *Hemileia vastatrix*. We showed that light intensity and nitrogen fertilization levels have an effect on rust incidence. Furthermore, regardless of the stress condition applied, the rust incidence was significantly lower in the hybrids than in the inbreed lines.

The C/NH4 ratio of the leaves before inoculation, indicator of resources availability for growth and defense, was well correlated to the rust incidence.

Introduction

Climatic events between 2008 and 2013 within the inter-tropical region have favored *Hemileia vastatrix* development but also modified the physiological status of coffee trees (Avelino et al., 2015). Physiological status of the coffee tree influences the rust incidence (Avelino et al., 2005).The genetic resistance to rust exhibited for most of the cultivated coffee varieties and based on major SH genes, is becoming less durable through time (Van der vossen et al.,2015). Consequently, in the current global warming context, CLR epidemics are expected to become more severe and more frequent, and therefore appropriate actions need to be implemented in a short term by the breeders to rationally address this issue.

Experimental design: Nursery plants were grown in pots with horticultural substrate in small phytotron.A. Experimental facility. B. Rust inoculation by spraying the. C. Rust harvest



Assessment of the physiological status before inoculation
(Phenotyping, Metabolomics study, photosynthetic efficiency measure)

Macroscopic monitoring of the infestation

Results Light intensity and nitrogen fertilization levels have an effect on the quantity of rust produced regardless of the variety.

Regardless of the thermal regime, high light intensity and low nitrogen fertilization lead to the highest quantity of rust produced. Whatever the conditions, rust quantity is lower for hybrids than for inbred lines (Figure 1).

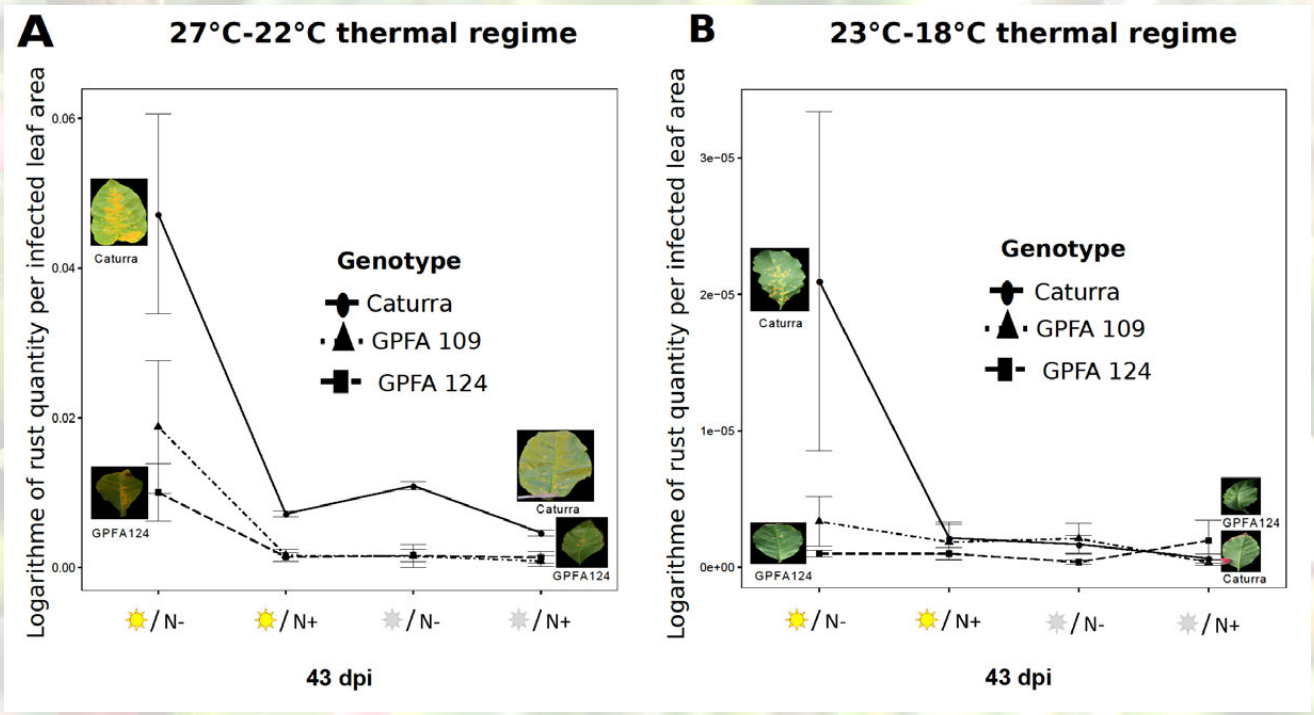


Figure 1: Rust quantity per infected leaf area produced by hemileia vastatrix depending on the environmental conditions in three different genotypes. A. 27° C-22° C thermal regime. B.23° C-18° C thermal regime.

Mineral element **Primary metabolism** **Secondary metabolism**

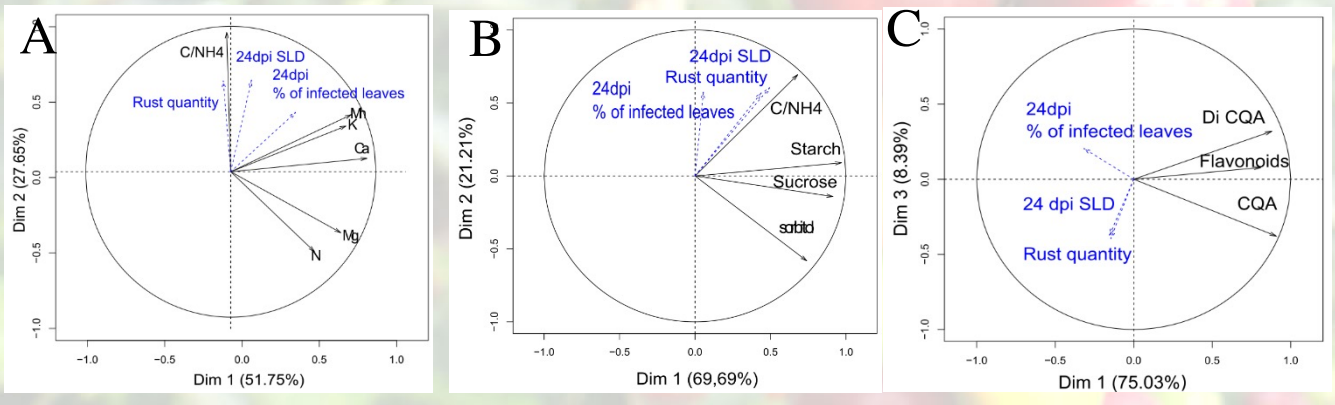


Figure 2:Principal component analysis. A/ Mineral element & B/ Compound involved in primary metabolism .The leaves C/NH4 ratio is significantly correlated to the rust infestation. C and NH4 being mineral element but also involved in amino acid biosynthesis, the C/NH4 ratio is represented on mineral and primary metabolism ACP. C. Secondary metabolism. No significant correlations have been found between secondary metabolism compounds and rust infestation.

Conclusion and perspectives

Light intensity and nitrogen fertilization levels have an effect on rust incidence. Furthermore, regardless of the stress condition applied, the rust incidence was significantly lower in the hybrids than in the inbreed lines. The C/NH4 ratio of the leaves before inoculation, indicator of resources availability for growth and defense, was well correlated to the rust incidence. To investigate the possible mechanisms behind these differences of incidence, transcriptomic analyses are also being carried out during the kinetic of infestation. In the long run, our objective is to propose a model explaining how a favorable physiological status can limit the leaf rust incidence in cultivated coffee trees.

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Generating a soil fertility database for coffee growing areas in Tanzania

PA109

Maro, Godsteven P., Mbwambo, Suzana G

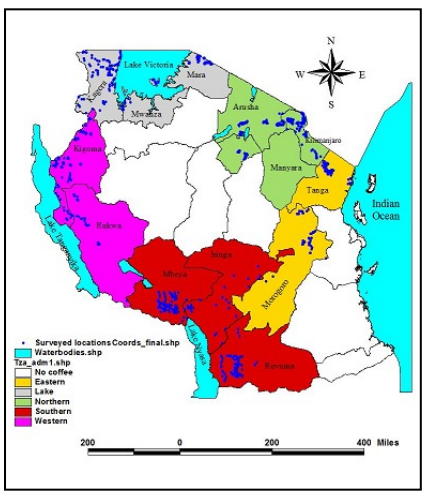
Tanzania Coffee Research Institute, P.O.Box 3004, Moshi, TANZANIA

Abstract

Soil fertility information from coffee growing areas in Tanzania has been scanty and incoherent. This paper describes an effort to build a sound soil fertility database for the country. Soil survey was done from August 2010 to June 2014, covering 41 districts in 5 zones. Samples were analyzed for pH-water, OC, total N, available P, CEC and exchangeable cations. Clustered data per zone were descriptively and spatially compared in terms of pH, CEC and OC; and the NPK supply potential was calculated from pH, OC, N, P and K. Southern Zone had high OC, N and P, while Northern Zone had high pH and K; and Lake Zone had high CEC. The NPK supply potential was in the order Lake > South > North > West > East. The database generated in this work has profound contribution to the Coffee Sector Development Strategy through highlighting potential areas for coffee intensification and expansion. It is also useful to coffee research, extension machinery, policy makers and even input stockists.

Introduction

Coffee is the second major export crop in Tanzania after tobacco, contributing 24% to the annual agricultural export earnings. Baffes (2003) pegged average coffee production at 45 000 – 52 000 metric tons, with smallholder productivity per tree ranging between 250 and 300 g of parchment which is very low. In the coffee stakeholders' forum 2009, soil fertility decline emerged as one of the most limiting factors. Semoka *et al.*, (2005) and Janssen, (2005) noted lack of coherent soil fertility information and that no detailed study of soil fertility has been done across the country in the recent decades. The objective of this study was to generate a soil fertility database for the Tanzanian coffee areas, and use it in soil fertility evaluation for coffee countrywide.



Study areas in blue dots

Methodology

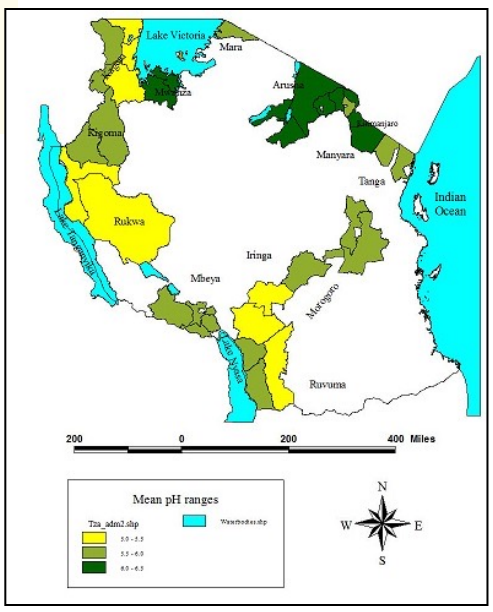
The study involved soil fertility survey of Class 2 (profiles) and Class 4 (augerings) in the coffee growing districts (five coffee growing zones, 14 regions and 41 districts). Important geographic information was collected with a GPS and later geocoded. Bulk soil samples were collected from natural horizons (profiles) and from fixed depths of 0-30cm, 30-60cm and 60-90 cm (augerings) for analysis..

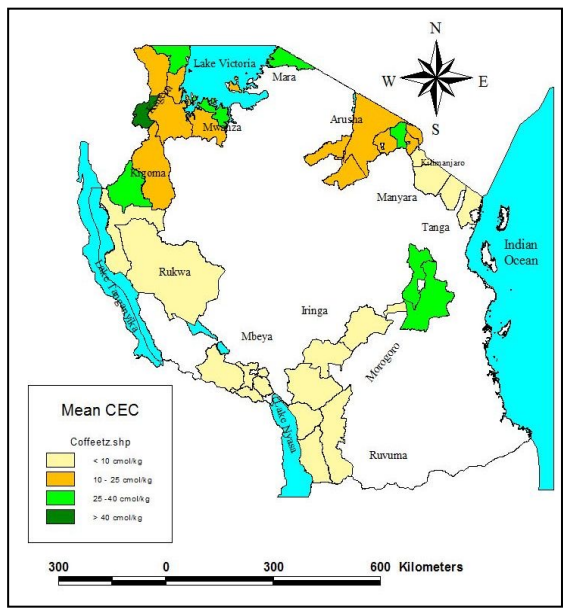
The soil samples were analyzed at TaCRI Lyamungu Soil Laboratory for pH-water (1:2.5), organic carbon (Walkley-Black method), total nitrogen (semi micro-Kjeldahl method), available Phosphorus (Bray 1), CEC (ammonium acetate pH 7), exchangeable cations by flame AAS and texture by the Bouyoucos Hydrometer method (Van Ranst *et al*, 1999). Zones were compared in terms of key soil fertility parameters pH, CEC and OC; and from these, plus total N, available P and exchangeable K, the NPK supply potential was calculated. The data were aggregated per district and mean values mapped by using ArcView GIS 3.2.

Results

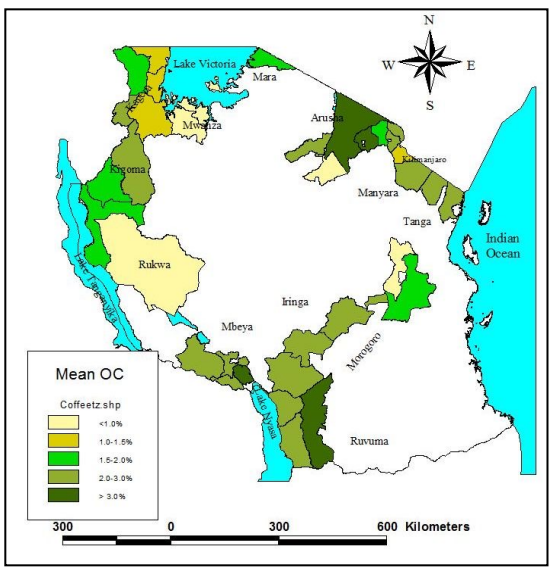
The results of this study was a rich soil fertility database, which gave the idea of the coffee production potential of the country. Zonal comparison of soil properties shows the lowest mean pH of 4.55 in the western zone (with absolute minima going as low as 3.5), and the highest mean pH of 6.85 in the lake zone (with absolute maxima going as far as 7.8). The grand mean comparison is in the order North > East > Lake > South > West. Mean CEC is in the order Lake > East > West > North > South, while those for OC and total N are in the order South > North > East > West > Lake and South > North > West > East > Lake respectively. Available P was in the order South > East > North > Lake > West; while exchangeable K was in the order North > Lake > South > West > East.

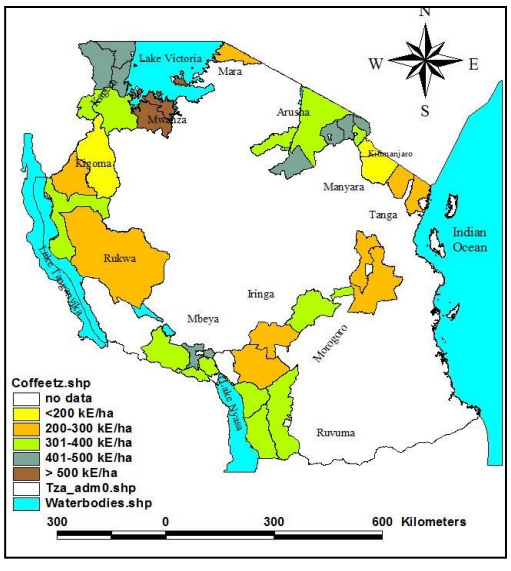
The NPK supply potentials were reclassified into five classes: <200, 200-300, 301-400, 401-500 and >500 kE ha^{-1}. Kibondo, Mwanga and Same had lowest potential, followed by nine other districts. At the high end we have the coffee districts of Mwanza Region (Sengerema, Geita and Ukerewe), followed by eight other districts. The most dominant category (12 districts) has potential of 301-400 kE ha^{-1}. The zonal order was Lake > South > North > West > East.





Distribution of pH and CEC per district





Distribution of OC and NPK supply potential per district

Conclusion

This work has generated a rich and consistent soil fertility database for the Tanzanian coffee areas, covering over 90% of the coffee growing districts. Spatial analysis showed higher fertility in the Northern and Lake Zones than the other zones. Districts that need special treatments like liming (West, South and Lake), phosphorus supplementation (East, South and West) and organic matter enrichment (Mwanga, Babati, Mpanda and Mvomero) have been pinpointed. Potential areas for coffee introduction and/or expansion have also been highlighted, thus contributing to the Coffee Sector Development Strategy. The database will be useful for ISFM research and extension planning, district councils (coffee subject matter specialists) and input stockists, among others.

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TaCRI TANZANIA COFFEE RESEARCH INSTITUTE

Relationship of growth and yield variables of hybrids of Coffee arabica variety KP423 and selected accessions of Ethiopian coffee

PA110

MTENGA, Damian*, REUBEN, Shazia**, KUSOLWA, Paul**
*Tanzania Coffee Research Institute, P.O.Box 3004 Moshi, Tanzania
** Sokoine University of Agriculture, P.O.Box 3005 Morogoro, Tanzania

Abstract

Molecular markers studies have revealed an extremely reduced genetic diversity in cultivated Coffea arabica L. while wild accessions from Ethiopian highlands have shown to constitute a valuable gene reservoir. Current study investigates the potential use of Ethiopian accessions in Tanzanian improved arabica coffee programme. Nine genotypes including six accessions from the Ethiopian collection, commercial variety KP423 were used in this study. Half-diallel mating design was used. The experiment was conducted at Lyamungu Tanzania in 2013. Data was recorded on the selected growth and yield variables at the end of the second year after field establishment and analysis was done using OPSTAT Statistical Package. Genotypic and phenotypic correlations for most of the growth and yield variables studied showed positive and significant correlation with each other indicating the possibility of simultaneous improvement of these variables. Path coefficient analysis showed that all variables were significantly contributing to the number of berries per node. Variables length of the longest primary (canopy radius), internode length and stem girth can be used as selection criteria in selection of superior coffee arabica varieties involving Ethiopian genotypes and commercial variety KP423.

Introduction

Currently improved arabica coffee varieties released by Tanzanian coffee research institute for commercial production have narrow genetic base. Broadening their genetic base using Ethiopian coffee arabica is anticipated to improve their performance. The estimation of genetic parameters allows the choice of suitable selection strategy; reducing the time required in cultivar development and release (Mistro et al., 2007). Study of interrelationship and association of characters provides an efficient tool to select the most important characters to be considered in breeding programmes. In coffee most of the agronomic characters are polygenic and they display different levels of interrelationships (Sureshkumar et al., 2013). Estimation of genetic parameters of the main selection traits guides selection in breeding programmes. In coffee, growth characters especially stem girth, plant height, inter-node length on stem and primaries, and canopy radius had a high repeatability (Walyaro, 1983). The objective of this study was to determine the combining ability, heritability and relationships of growth and yield variables of progenies of Tanzanian commercial variety KP423 and selected accessions of Ethiopian collection.

Materials and Methods

Nine genotypes were selected including six from Ethiopian collection maintained at the Institute, two from the germplasm and a commercial variety KP 423 were used in a half-diallel mating design. The trial was established at experimental field of Tanzania Coffee Research Institute, Lyamungu in 2013. Data were collected at Year 1 and 2 after establishment on: Stem girth, Plant height, Length of the longest primary (canopy radius), Number of primaries per plant, Number of berries (flower buds) per cluster, Internode length and Number of bearing primaries per plant. Analysis was done according to Sheoran et al; 1998 based on the fixed effect (model 1) method II by Griffing (1956) while Path coefficients analysis was performed according to Dewey and Lu (1959).

Results

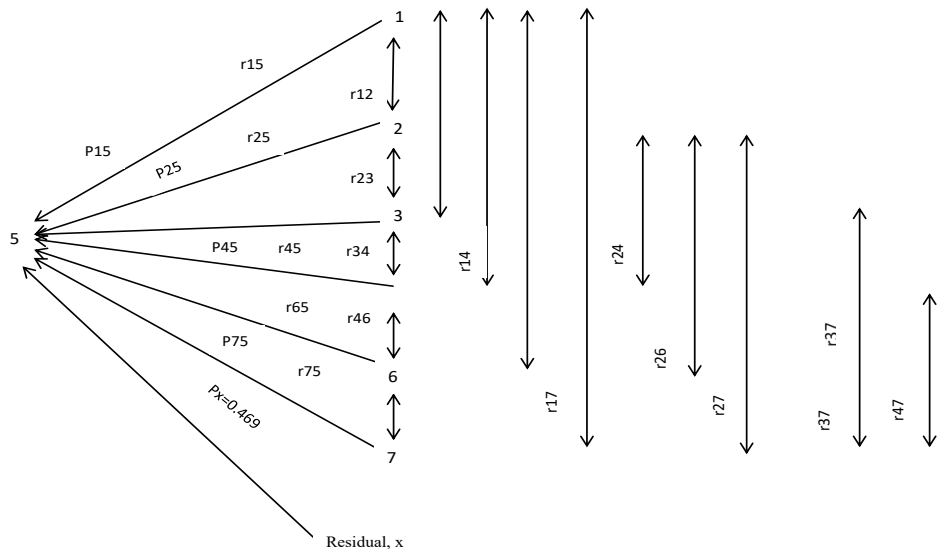

Figure 1: Path diagram and coefficients of factors influencing number of berries per node of coffee. P's are the direct effects; r's are the genotypic correlation coefficients

Table 1: Genotypic and phenotypic correlation coefficients for growth and yield variables of coffee

Variable		1	2	3	4	5	6	7
1. SG								
2. PH	rG	0.679**						
	rP	0.684**						
3. LLP	rG	0.687**	0.922**					
	rP	0.787**	0.857**					
4. NPP	rG	0.841**	0.354**	0.327**				
	rP	0.731**	0.515**	0.535**				
5. BPN	rG	0.538**	0.403**	0.583**	0.389**			
	rP	0.558**	0.413**	0.602**	0.431**			
6. IL	rG	0.543**	1.006**	1.070**	0.197*	0.483**		
	rP	0.458**	0.606**	0.645**	0.151NS	0.284**		
7. NBPP	rG	0.648**	0.306**	0.343**	0.792**	0.248**	0.110NS	
	rP	0.678**	0.433**	0.537**	0.751**	0.451**	0.256**	

Genotypic coefficient of correlation (r_G) is shown on the top and the phenotypic correlation coefficient (r_P) is shown on the bottom of each cell corresponding to the variables in a row. SG = stem girth, PH = plant height, LLP = length of the longest primary, NPP = number of primaries per plant, BPN = berries per node, IL = internode length, NBPP = number of bearing primaries per plant.
*Significantly different at P = 0.05 ** significantly different at P = 0.01

Discussion

Genotypic correlation coefficients (r) were found to be positive and highly significant for all studied variables. Variables length of the longest primary (LLP) (0.583), stem girth (SG) (0.538) and internode length (IL) (0.483) were found to contribute mostly in berries per node. The direct effect due to plant height (-1.17) was found contributing most negatively to berries per node comparing with other variables studied.

Conclusion

Variables length of the longest primary (canopy radius), internode length and stem girth may be used as an important criteria in selection for superior coffee arabica varieties involving Ethiopian genotypes and commercial variety KP423.

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Climate change in the Mount Kilimanjaro ecosystem and its impact on Coffee quality

PA111

Mbwambo, Suzana G ., Maro, Godsteven P

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

Abstract

Fifty-year climatic trends in the Mount Kilimanjaro coffee ecosystem were assessed for evidence of climate change and its possible effect on coffee quality. A section of the climatic data matching a 12 -year period (2003-2014) whereby coffee quality data were available, was linearly regressed against coffee bean size, cup quality and a combination of the two, under STATISTICA V7 software. Over the half century, rainfall has been decreasing slightly with the number of dry months per year also decreasing, implying better rainfall distribution. Mean maximum and minimum temperatures have been increasing by 0.9ºC and 1.6ºC respectively, an evidence of warming. The study has shown that climate is one of the important factors of coffee quality but, in the context of the study area, not in itself a decisive factor. Factors such as soil properties and crop management also play a role, so they can be properly manipulated through the climate-smart GAPs, to ensure that coffee quality is not compromised by the changing climate

Introduction

Coffee quality is the set of attributes of a coffee lot that determines its market value. Such attributes include botanical variety, topographical conditions, weather conditions and the care taken during growing, harvesting, storage and transport (Stanculescu *et al.*, 2011). Therefore distortion of weather trends over time that leads to change in temperature and rainfall patterns, as well as extreme weather events, can impact production cycles and negatively affect coffee production (Maro and Teri, 2008). It has not been established whether, and to what extent, climate change affects coffee quality. The study therefore aimed at assessing the climatic trends in the Mount Kilimanjaro coffee ecosystem for evidence of climate change, and determining whether this change has any effect on coffee quality

Methodology

The study area comprised of the southern slopes of Mount Kilimanjaro. Its reference weather station is located at TaCRI Lyamungu (37º15' East, 03º14' South, altitude 1258 masl). A half-century climate data set (1965-2014) was extracted from old files and descriptively analyzed, Coffee quality data 2003-2014 were collected from TCB and assessed in terms of bean size (Q_{BS}), cup quality (Q_C) expressed as the percentage of grades 4 to 6 to the total amount, and a combination of the two (Q_{BSC}). The three coffee quality parameters were separately and linearly regressed against matching climatic parameters using the STATISTICA V7 software to check if the climatic parameters have any significant influence on coffee quality.

Results and Discussion

Over the half-century, there has been an increase of mean T_{max} by 0.9ºC and mean T_{min} by 1.6ºC (an evidence of warming).This is in agreement with Craparo *et al*. (2015) and Maro and Teri (2008) who noted that T_{min} has been increasing at a higher rate than the other climatic parameters, and Laderach *et al*. (2012) who estimated the mean increase to be 1.3ºC. Total annual rainfall and the number of dry months showed a slight decrease with time (Figure 2). Coffee quality trends over 12 seasons showed two distinct periods (Figure 3). The period 2003-2010 is characterized by relatively high Q_{BS} but surprisingly low Q_C. The period 2011-2014 shows a sharp drop in Q_{BS}, from 50% to about 20%, and also the highest and most consistent Q_C. The gradual increase in Q_C may be partially attributed to the adoption of new improved coffee varieties with excellent cup quality (Teri *et al.*, 2004). On the other hand, Q_{BS} depends on a variety of factors; climatic, edaphic, agronomic and post harvest handling.

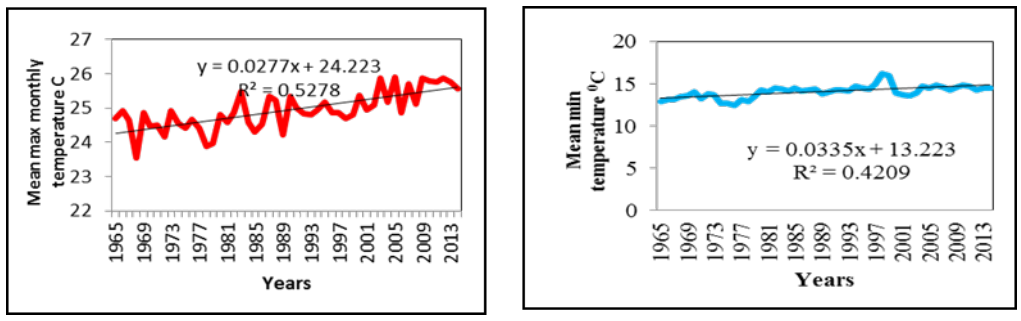

Figure 1: Mean monthly temperature (left) and Mean min temperature (right) in the study area

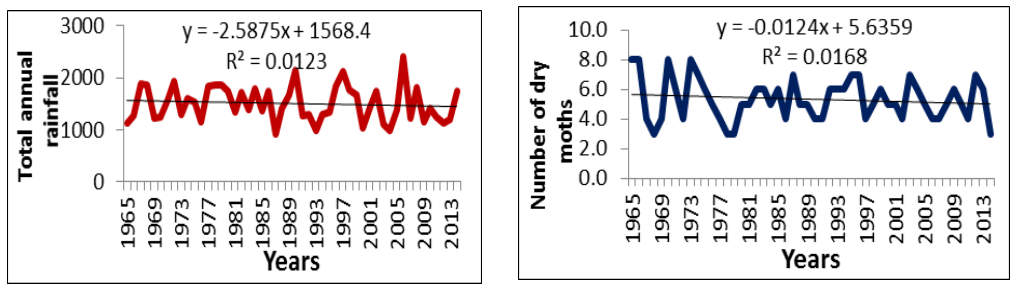

Figure 2: Total rainfall (left) and distribution of the number of dry months (right) in the study area

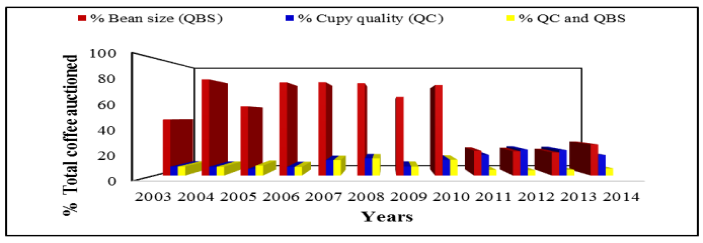

Figure 3: Coffee quality trend for the 12 year period (2003-2004)

The regression analysis (table 1), showed that, Absolute T_{max} influenced Q_{BS} positively and the rest negatively, while absolute T_{min} influenced Q_{BS} negatively and the rest positively. Mean T_{min} showed positive influence on bean size and negative influence on the rest. None of such influences were statistically significant, as p values were all above 0.05. The lack of significance ($p>0.05$) in all the selected climatic predictors of coffee quality implies that coffee quality is influenced by an aggregate of factors, climate included. Therefore if the climatic factors such as rainfall distribution can be managed through planned irrigation, all the good agricultural practices (GAPs) are adhered to, emphasis is put in central pulping, the specialty coffee with best attributes can still be produced even at the current rate of climate change.

Table 1: Regression summaries for bean size, cup quality and their combination

Model	Effects on QBSC		Effects on Q_C		Effects on QBS_C	
	β	sig	β	sig	β	sig
Constant		0.465		0.748		0.534
Total rainfall	-0.201	0.759	-0.2	0.741	-0.105	0.897
No. of dry months	-0.157	0.819	0.696	0.299	0.063	0.941
Absolute temp max	0.081	0.882	-0.057	0.909	-0.193	0.777
Absolute temp min	-0.167	0.796	1.29	0.07	0.462	0.57
Temperature max	-0.683	0.367	-0.341	0.614	-0.373	0.683
Temperature min	0.279	0.565	-0.453	0.327	-0.313	0.603

β -Is a measure of how strongly each predictor variable influences the dependent variable

Conclusion

The study has shown that climate is one of the important factors which influence coffee quality but, in the context of the study area, not in itself a decisive factor. Other factors such as soil properties and crop management also play a role. It is therefore recommended that this subject should be explored further by using longer-term coffee quality data. On the other hand, climate-smart GAPs such as farm sanitation, mulching and storm water trenches for moisture conservation, integrated soil fertility management (ISFM) and pest management (IPM), should be emphasized in order to ensure that coffee quality is properly and sustainably generated despite the climate change.

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TaCRI TANZANIA COFFEE RESEARCH INSTITUTE

ECONOMIC RETURNS AND BENEFITS TO SMALLHOLDERS FROM INVESTMENTS TO PRODUCE CERTIFIED QUALITY COFFEES

PA112

Abstract

The study to analyze economic returns and benefits to smallholder's farmers from investing to produce certified quality coffees was conducted in Muleba district. A total of 120 smallholders (members of certified and non-certified cooperatives or individual farmers) were random selected in the study area. The coffee yields, costs of production and net revenue gained by farmers were the main indicators used to measure the economic return for farmer investing in coffee certification. The results indicates that, the certified farmers gain substantial profit as opposed to non-certified farmer as discussed in this paper.

Introduction

Coffee certification and verification was introduced to help farmer improve quality and access niche market (Solidaridad & Rijsbergen 2014). Tanzania implemented a five years project of Building Capacity in Coffee Certification and Verification for Specialty Coffee Farmers in AFCA Countries (CFC/ICO/45). The goal of this project was to equip farmers with skills to meet certification and verification standards (ICO, 2014). Coffee farmers invested in producing certified coffee (Ibanez, 2015). This study therefore assess the economic benefit gained certified coffee farmers.

Methodology

The study was conducted in Muleba district. Purposive sampling technique was used to select 23 primary co-operatives certified by organic, fair trade and Uts certification schemes. The study also employed simple random sampling technique to select 120 respondents in the study area. Primary data were collected by using structured questionnaire and focus group discussion (FGD) with co-operative leaders. Descriptive statistical and quantitative methods were used to analyze the data collected including coffee yields, prices and net revenue gained by farmers. The result were presented in graphs and tables.

Result and discussion

- The level of production for certified was observed to be higher than non-certified farmers. This was due to proper record keeping at cooperative where they sales their coffee.
- Production for non certified farmers were low due to poor record keeping and also poor marketing system which allow farmer to sale their coffee while the crops are in the farm.

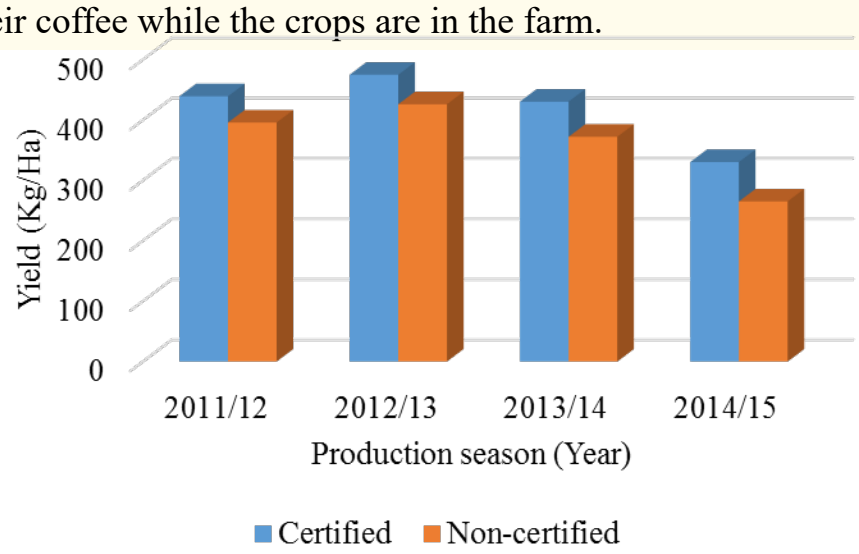

Figure 1: Trend of production for certified and non-certified

- The cost of coffee production were high for non-certified farmers due to poor farming practices and high price of inputs such as agrochemicals to control coffee disease and pests
- Environmental benefits were noted as the impact of minimum use of agrochemicals.

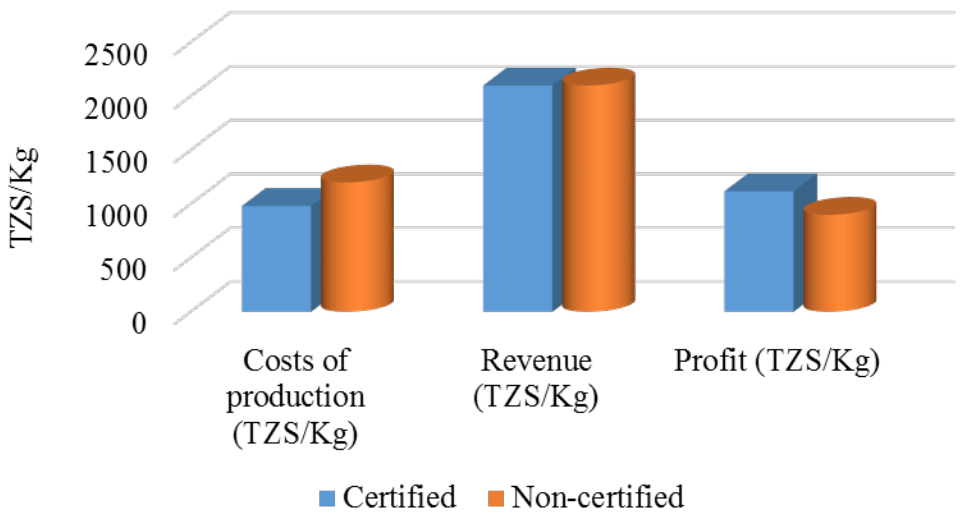

Figure 2: Profit and loss analysis

Conclusion

From the analysis above the study conclude that, coffee yield is low for all certified and non-certified farmer which implies that investing in certification doesn't imply increase in coffee yield this is because the potential for well managed coffee tree is 2 kg per tree of clean coffee). Therefore for certified coffee farmer to get profit they need to invest to increase coffee production and quality. Government support is required so as to encourage farmers to invest more on application of good agricultural practices including use of recommended fertilizers and agrochemicals to control coffee disease and pests. Also access to niche market which offer high price for quality coffee. The impact of this intervention will boost yield and also get more profit.

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Effects of shading on incidence and infestation level of key coffee pests in Kilimanjaro region, Tanzania

PA113

MAGINA, F. L., MARO, G. P., MAERERE, A. P., SIBUGA, K. P., KYAMANYWA, S., ERBAUGH, J. M.

Abstract

A study was conducted from 2011 to 2014 on station at TaCRI, Lyamungu, Moshi to assess the severity of key coffee pests; **insects:** *Antestiopsis* spp, *Hypothenemus hampei* and *Monochamus leuconatus*, **diseases:** *Colletotrichum kahawae*, *Hemileia vastatri*x: **weeds**: *Cynodon dactylon*, *Digitaria scalarum.*, and *Commelina benghalensis* on coffee grown under shade of banana under different spacing of coffee and banana (1:1, 2:1, 3:1) and unshaded coffee. Results showed that the severity of *Antestiopsis* spp was significantly (P≤0.05) higher under shaded coffee as compared to un-shaded ones. *M. leuconatus* was significantly higher (P≤0.05) at unshaded as compared to shaded coffee. The severity of *H. hampei* was significantly higher at un-shaded and shaded coffee. However *C. kahawae* was significantly higher at shaded coffee and banana (3:1 and 2:1). But no significant difference was observed for *H. vastatrix* in shaded and unshaded conditions. The severity of *D. scalarum* was significantly (P≤0.05) higher in shaded coffee while *C. benghalisis* no significant difference (P>0.05) was observed in shaded and unshaded coffee. This shows that shading in coffee ecosystem has an effect on severity of pests which varied with different pests' species. Farmers are therefore encouraged to plant shade trees where *M. leuconatus. C. kahawae, H. vastatri*x, ***C****. benghalensis* and *D. scalarum* occur in order to culturally manage them or maintain them below their respective economical injury levels.

Introduction

Coffee in Tanzania is traditionally shaded with banana or the indigenous shade trees like *Albizia maranguasis. A. gummifera, A. schimperana.* The primary reason for shading is to reduce the effect of global warming such as excessively high leaf temperatures (Mugo, 2013). Also the benefits to adopting this system versus a pure coffee system, it offers higher returns to the smallholder farmers (Chipungahelo, 2004). Shade also plays an important role in maintaining long-term coffee productivity, soil conservation, water and biodiversity, and improvement of coffee quality. Despite all these, shading produces a micro-climate that enhances or impend pests through ecological balance, both in favour or against the crop. The objective of this study is to assess the effects of shade on incidence and infestation levels of pests in existing plantations with trees shades and purposely established plantations intercropped with banana at various planting spacing commonly practiced by coffee farmers in Tanzania.

Methodology

Different shading level of coffee and banana in the trial area were differentiated by measuring with a Light Intensity Meter (LIM) instrument.

Insect pests: The number of white coffee stem borer (*Monochamus leuconatus*) was estimated by examining the lower trunk, up to 0.6 m above the collar level for any signs of stem girdling or boring by white coffee stem borer. In each hole with emission of fruss we assumed one larva and the adult on the tree was counted. Population density for Antestia bugs (*Antestiopsis* spp) was estimated by examining the trees for the presence of the pest without disturbing the tree canopy. The total number of adults and nymphs per bush were counted and population density of coffee berry borer (*Hypothenemus hampei*) was determined by randomly selecting one berry primary branch in the middle third of the bearing head and examined for the presence of the pest and recorded every month.

Diseases: The incidence of coffee berry disease (*Colletotrichum. kahawae*) and leaf rust (*Hemileia vastatris*) were determined by observation of the entire tree canopy and severity of infestation were recorded on a scale of 0 to 4 (0 = No infestation and 4 = acute infestation) and recorded every month.

Weeds: Densities of star grass (*Cynodon dactylon,*), couch grass (*Digitaria scalarum*) and wondering jew (*Commelina benghalensis*) were counted in quadrants measuring 2 m x 2 m in shaded and unshaded coffee plots replicated three times and recorded weekly. All of the collected data were summarized and means calculated and subjected to ANOVA. The GenStat statistical package release 12.1 software was used.

Results and discussion

Results showed that shading of coffee with banana and shade tree differently affected the severity of different coffee insect pests, diseases and weeds. The severity of *Antestiopsis* spp was significantly (P≤0.05) higher under shaded coffee as compared to un-shaded ones. This study agreed with Mugo, *et al.,* 2013 who found that where coffee is un-pruned promotes rapid multiplication of *Antestiopsis* spp and low parasitism level. *M. leuconatus* was significantly higher (P≤0.05) in unshaded as compared to shaded coffee. This findings are in line with Murphy *et al*., 2008 who observed that coffee plants in open patches are more prone to *M. leuconatus* infestation and normally the attack starts to such points. Severity *of H. hampei* was significantly higher (P≤0.05) at un-shaded and light shaded coffee (Figure 1). The study agreed with Wrigley 1988 that more infestation with *H. hampei* occurs where the coffee is grown under heavy shade like in the study area. However severity of *D. scalarum* was significantly (P≤0.05) higher in unshaded as compared to shaded coffee. This agreed with CAB, 2007 who found that *D. scararum* is more troublesome in unshaded than shaded plantations. No significant difference (P>0.05) was observed for *C. benghalensis* in shaded and unshaded coffee (Figure 2). This agreed with CABI, 2007 who indicated that the weed establishes well in both shaded and unshaded areas in arable and plantation crops. No infestation by *C. dactyron* weed was observed in the study area. *C. kahawae* was significantly higher (P≤0.05) in shaded coffee and banana (3:1 and 2:1). But no significant difference (P>0.05) was observed for *H. vastatrix* in shaded and unshaded conditions. This agreed with Manuel *at el*., 2010 who indicated that *C. kahawae* has a high incidence of occurring in highland regions.

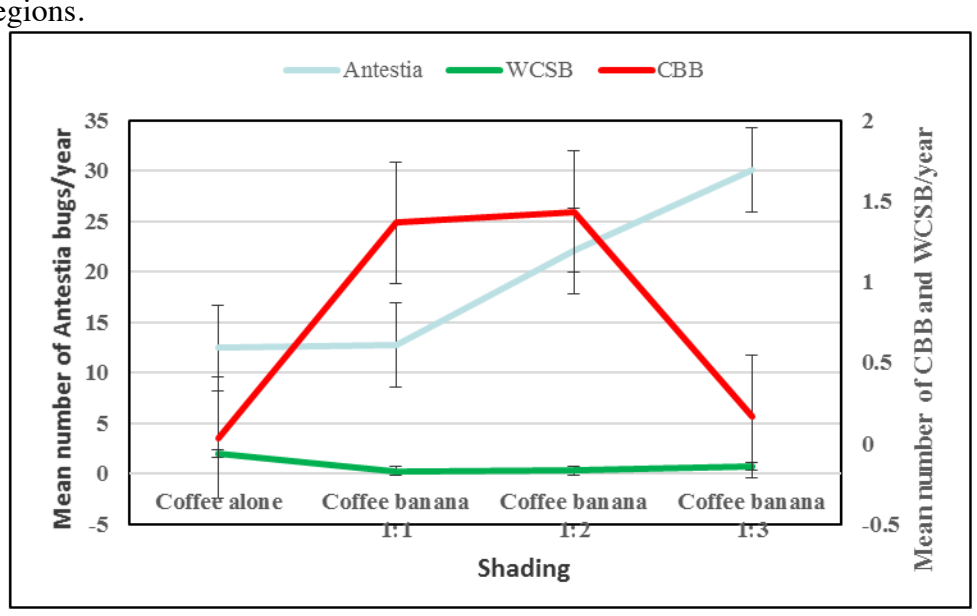

Figure 1: Mean number of Antestia bugs, WCSB and CBB per year for four years (2011 – 2014)

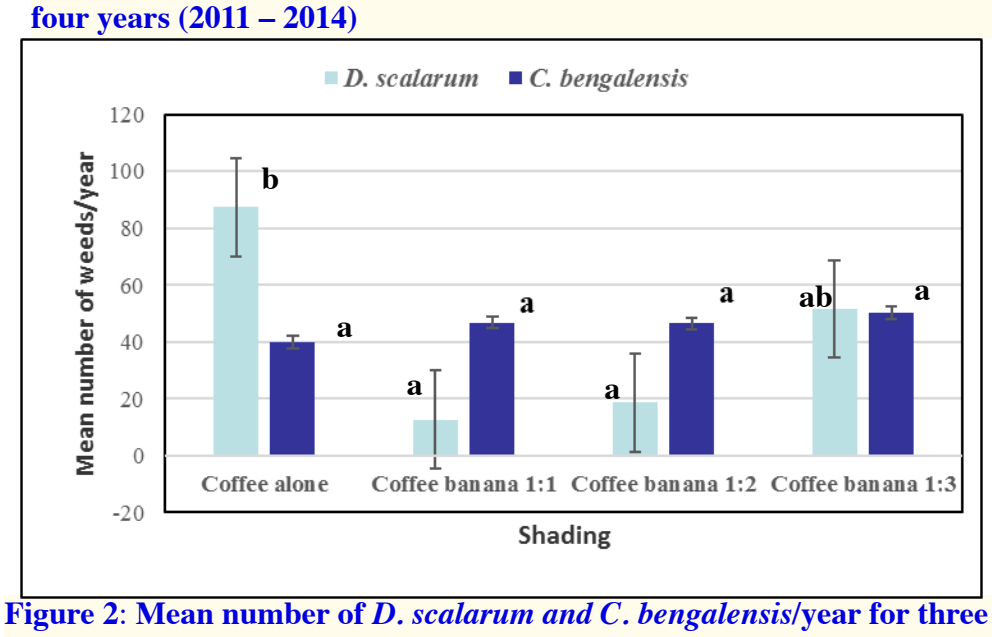

Figure 2: Mean number of *D. scalarum and C. bengalensis*/year for three years (2012 – 2014)

Note: Means followed by the same letters are not significantly different at 5% according to DMR test

Conclusion

Shaded coffee significantly lowered the severity of *M. leuconatus, H. vastatris* and *C. kahawae* while that of *Antestiopsis* spp, *H. hampei* and *C. benghalensis* was significantly increased. Farmers are therefore encouraged to plant shade trees where *M. leuconatus. C. kahawae, H. vastatris*, ***C****. benghalisis* and *D. escularum* occur in order to culturally manage them or maintain them below their respective economical injury levels.

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PA114

TaCRI innovative IPM strategies developed for control of coffee insect pests in Tanzania

***MAGINA, F. L, *KILAMBO, D. L, **MAERERE, A. P & *TERI, J. M**

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

**Department of Crop Science, Sokoine University of Agriculture (SUA), P.O Box 3005, Morogoro, Tanzania

Abstract

Coffee insect pests are one of the major factors which affect coffee production and quality in Tanzania. For a period of 15 years TaCRI has been developing ecologically and environmentally sustainable coffee insect-pests control measures which include; use of bio pesticides, traps, parasites, attractants and biological agents. Some of these innovative practices and their impacts on management of coffee insect pests are discussed in this paper.

Introduction

Coffee is an important cash and export crop in Tanzania which accounts for 25% of the crops exports value (BOT, 2013). The crop suffers heavy yield losses caused by wide range of insect pests namely; *Monochamus leuconatus* (> 25%), *Hypothenemus hampei* (50-90%), *Antestiopsis* spp (45%), *Leucoptera* spp, *Coccus* spp (50%) and *Planococcus kenyae.* Minor important pests including *Dirphya nigricornis, Diarthrothrips coffeae* and *Prophantis smaragdina* (Magina, 2011). For decades industrial chemicals have been used to control coffee insect-pests. However, for economic and environmental reasons, TaCRI has been evaluating and developing ecologically sound coffee insect-pests control measures for 15 years which include; use of bio-pesticides (botanicals), coloured sticky traps, entopathogenic fungus, attractants (locally made brews & sticky traps) and biological agents (predators and parasitoids). The technologies developed are progressively disseminated to farmers through participating agricultural shows, open days, demonstration plots, backstopping programmes and publications (leaflets & posters).

Technologies developed and their impacts

(a) Alcohol traps for management of CBB.

TaCRI has modified the Brocap trap developed by CIRAD by use of plastic bottle (Figure 1), by use of methylated spirit and local brews (e.g. "mbege", "lubisi" and "dengelua"). The lures have the capacity of reducing the infestation by 80-85% per year by catching 300 – 500 adult CBB per week (depending on the infestation levels). The trap has been adopted and is used by small holder farmers in the Northern zone and some coffee estates like Burka Coffee Estate, Kilimanjaro Plantations Limited etc.

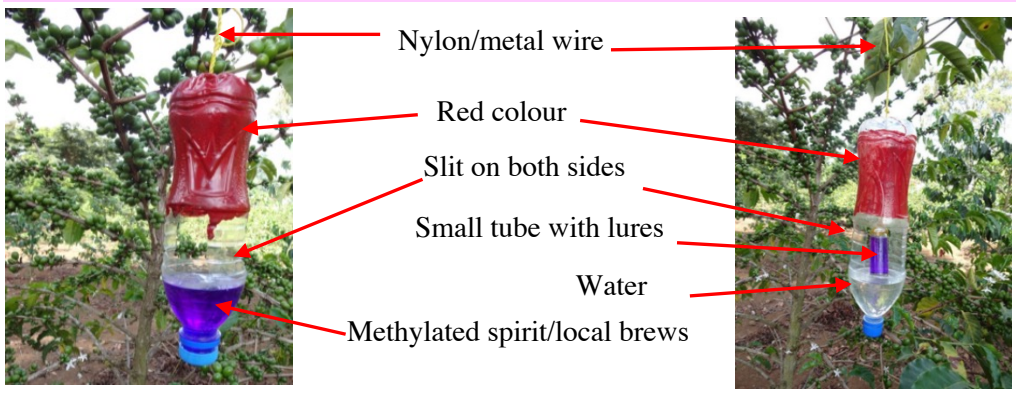

Figure 1: CBB trap made of plastic bottle (modification from CIRAD-Brocap trap)

Figure 2: *Tephrosia vogelii* plant

(b) Use of biopesticides (*Tephrosia vogelii*) for management of Antestia bugs

TaCRI has collected and established 11 botanicals with insecticidal or/and repellent properties. Extract of *Tephrosia vogelii* at a rate 1.2 Kg/1 litre of water has been successful in managing Antestia bugs in the field as does Chlorpyrifos (industrial chemical)(Magina *et al.,* 2012a) (Figure 2). The botanical is now used in the Northern zone (Kilimanjaro, Arusha and Tanga) when botanical gardens has been established by farmer groups. The way forward is to evaluate the active ingredient (AI) and commercialize for farmers use.

(c) Use of parasitoids for CBB management.

TaCRI has investigated two potential parasitoids for CBB (*Cephalonomia stephanoderis* and *Prorops nasuta*) (Figure 3) which are available naturally in coffee farmers in Kilimanjaro region (Magina *et al*. 2012b). We are building capacity for mass multiplication and release in the field with CBB problem.

Cephalonomia stephanoderis

Prorops nasuta

Figure 3: Potential parasitoids for coffee berry borer (CBB) management

(d) Predators (ladybird beetles) for mealy bugs & green scale management.

Figure 4: Ladybird beetles

TaCRI has discovered 4 predators (ladybird beetles) namely; *Exochomus aethiops*, *Cheilomenes propinqua, Cheilomenes lunata,* and *Chilocorus circumdata* (Figure 4) which are effective against mealy bugs & green scales. The next step is mass multiplication and release in the coffee fields infested with the pest(s).

(e) Oil/animal fats for management of African white coffee stem borer (WCSB) & yellow headed borer.

TaCRI has evaluated cooking oils and animal fats as an attractants of ants which consumes the larva of WCSB in the coffee fields (Figure 5). The technology is being applied in the Southern highlands (Ruvuma region) and some parts in the Northern zone of Tanzania.

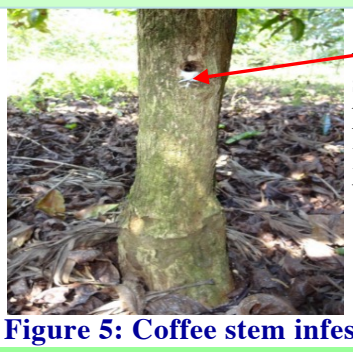

Figure 5: Coffee stem infested with WCSB

(f) Entomopathogenic fungi (*Metarhizium anisopliae*) for management of CBB.

The effectiveness of *M. anisopliae* for management of adult CBB have been evaluated in the laboratory at TaCRI and considered to perform well (Figure 6). Also mass multiplication of the fungus was possible by use of rice grain in the laboratory. The way forward is to evaluate the effectiveness of the fungus for management of the pest when applied in the coffee fields.

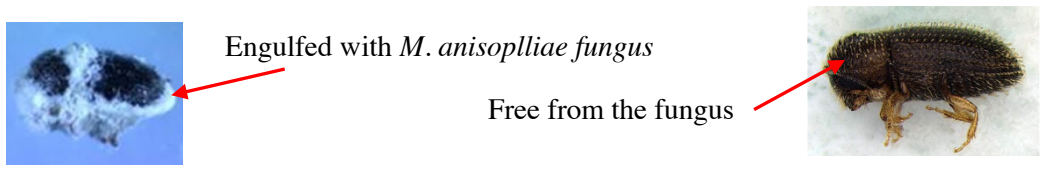

Figure 6: *M. anisopliae* engulfs adult coffee berry borer (CBB)

(g) Coloured sticky traps for management of thrips & leaf miner.

TaCRI has modified the yellow sticky traps made of special papers by use of plywood (30 cm x 30 cm) painted yellow and spread with petroleum jelly or used motor oil on yellow painted plywood's (Figure 7) for trapping thrips, leaf miner and other flying insects like aphids, whitefly, moths and fruit fly in coffee fields.

Figure 7: Yellow sticky traps made of plywood

Conclusion

Research is needed to study the biological control and the use of natural products for pest management. In so doing we will have helped the coffee industry in the country to penetrate the lucrative, but restrictive, niche coffee markets in some countries which have set the maximum residue levels (MRL).

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Anatomy Root of Coffee Plants in Phosphorus Overdoses

PA120

SOUZA, Antonio J.J.[1], SANTOS, Adailson F.J.[2], ALMEIDA JUNIOR, Osmar[3], CASTRO, Evaristo, M.[4], GUIMARÃES, Rubens J.[5], RESENDE, Mário L.V.[6]

[1,2] Universidade do Estado da Bahia, BRA, [3,4,5,6] Universidade Federal de Lavras, BRA.

Abstract

Recent studies have found that the coffee plants requires more phosphorus supply.

Introduction

Coffee plants requires more phosphorus supply. Scalco et al. (2014) found that applying more water for irrigation, the greater the phosphorus root absorption and greater plant growth. However, studies on root anatomy of coffee plants are scarce in the literature when grown in larger doses of phosphorus. Objective is thus to evaluate coffee roots anatomy grown with phosphorus overdoses (P_2O_5) and irrigation levels.

METHODS

In Inovacafé agency - UFLA, Coffee plants were grown in pots in design of randomized blocks. With a factorial 4 x 4, with four phosphorus levels (0, 80, 240 and 720 g P_2O_5 per pot) and four irrigation levels (25%, 50%, 75% and 100% of field capacity) with three replications.

At 180 days of implantation were collected roots and determined the epidermis in µm and xylem area in µm².

RESULTS

With increasing levels of phosphorus was a linear decrease in the thickness of root epidermis. At high phosphorus concentrations, epidermal cells become smaller, with smaller diameter. It is believed that phosphorus exerts synergistic and antagonistic in the root absorption process (ÁVILA et al., 2012), this effect has a sharp expression in epidermal cells because they are the first contact with the soil solution.

To decrease the water levels in the amount of available water caused a linear increase in cross-sectional area occupied by the root xylem. As the plant is subjected to drought stress conditions is increase in the number of vessels at the root, decreased diameter. This modification is a variation of the plant to accommodate the increased water stress and the highest negative pressure avoiding the risk of embolisms and therefore cavitation. Similar data were found in the works of (OGASA; MIKI; YOSHIKAWA, 2010).

Conclusion

The increase in phosphor application and irrigation reduces the thickness of the epidermis and xylem area.

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INFLUENCE OF CHERRY POSITIONS IN CANOPY ON COFFEE QUALITY

PA122

Cheng B.; Smyth, H.E.; Furtado, A.; Henry R.| Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, QLD, AUSTRALIA|b.cheng@uq.edu.au

Abstract

RATIONALE

Coffee is one of the most valuable commodities exported worldwide. An increasing awareness of quality is bringing growing demand for high quality and specialty coffees. Quality attributes, like caffeine accounts for coffee bitterness, while trigonelline and sucrose are aroma precursors contributing to key volatiles, like pyrazines, furans, etc.[1] Environment, such as altitude, was found to influence coffee quality [2]. However, there is no published research on different canopy positions influencing coffee quality.

METHODS

This study investigated differences in key components of coffee beans harvested from the upper and lower tree. Caffeine, trigonelline and sucrose were analyzed using High-performance liquid chromatography. 13 Volatiles were detected by Gas chromatography–mass spectrometry. Sensory evaluation was conducted with a group of trained panel for description and 86 untrained panel for statistical analysis.

RESULTS

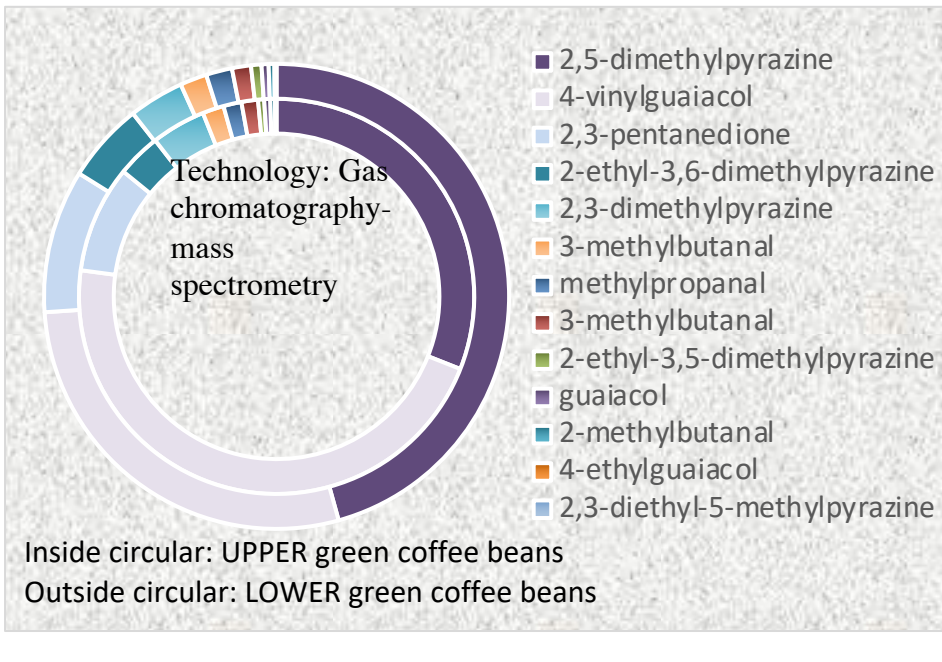
Caffeine, trigonelline, sucrose and 13 volatiles are significantly higher in coffee beans collected from the lower canopy. Consistently, sensory results indicate more significantly intense aroma in low canopy coffee beans.

CONCLUSIONS & PERSPECTIVES

The low canopy yields higher quality coffee beans. Higher caffeine and trigonelline from the low canopy is probably result from lower growing temperature that delays seed maturity and allows more bean filling. The sucrose pattern parallels with coffee leaves that results from more carbon assimilation. Results from this study when combines with future transcriptome analysis will explains how gene expression determines coffee quality in upper and lower canopy.

Introduction

This research targets an understanding of coffee quality by investigating upper and lower cherry canopy influences on key chemical components and sensory properties.



Inside circular: UPPER green coffee beans
Outside circular: LOWER green coffee beans

Figure 2: 13 Aromatic volatile components distribution in Arabica coffee beans from both UPPER and LOWER canopy

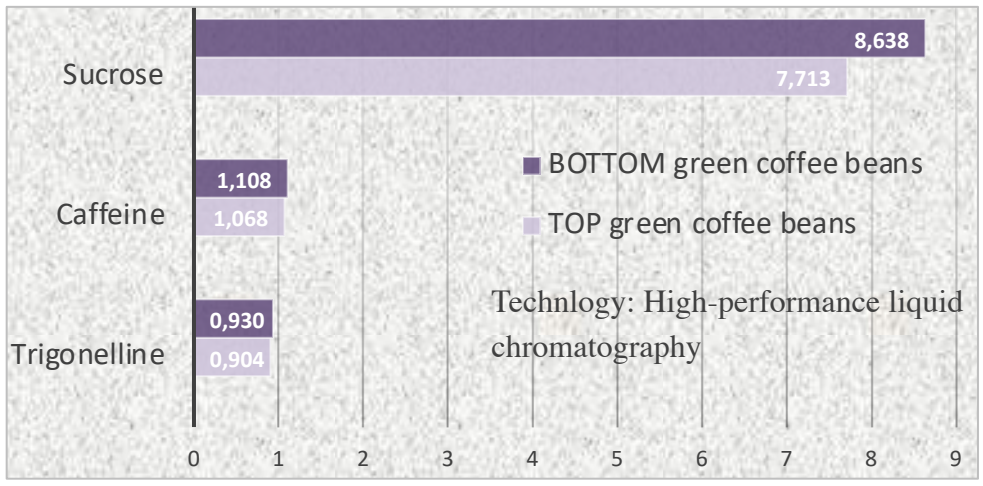


Figure 1: Sucrose, caffeine and trigonelline components in Arabica coffee beans from UPPER and LOWER canopy

Table 1 : Differences in sensory evaluation between UPPER and LOWER coffee beans

Difference between coffee from UPPER and LOWER canopy	Two sided		
	0.05	0.01	0.001
Coffee BEANS	*		
Coffee GROUND		**	
Treatment			***

Caffeine, trigonelline, sucrose and 13 volatiles are significantly higher in coffee beans collected from the LOWER canopy. Consistently, sensory results indicate more significantly intense aroma in the LOWER canopy coffee beans.

Conclusion

Higher quality from lower canopy probably results from lower growing temperatures and delayed cherry maturity resulting in more bean filling. Results from this study when associated with future transcriptome analysis will explain how gene expression determines coffee quality in the upper and lower canopy.

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THE UNIVERSITY OF QUEENSLAND AUSTRALIA

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IMPACT OF WATER AVAILABILITY ON FRUIT NITROGEN, PHOSPHORUS AND POTASSIUM IN CONILON COFFEE PLANTS

PA123

COVRE, André Monzoli*, BONOMO, Robson*, TOMAZ, Marcelo Antônio*, RAMALHO, José Cochicho**,***, PARTELLI, Fábio Luiz*#,

* Universidade Federal do Espírito Santo, São Mateus, Espírito Santo, Brazil. # Corresponding author: partelli@yahoo.com.br
** PlantStress&Biodiversity Group, DRAT, LEAF, ISA, Universidade de Lisboa, Portugal. *** GeoBioTec, FCT, UNL, Portugal.

INTRODUCTION

Understanding the dynamics of nutrients and their relationship with climate conditions is an important tool for determining the periods of higher plant nutrient demands and planning crop fertilization programs.

OBJECTIVE

The objective of the present study was to evaluate fruit nitrogen, phosphorus and potassium concentrations and accumulation over two years in irrigated and non-irrigated *Coffea canephora* cv. Conilon plants.

MATERIAL AND METHODS

The experiment was performed in two consecutive crop seasons in the municipality of Itabela, in southern Bahia state, Brazil. Three-year-old plants of *C. canephora* cv. Conilon 'genotype 02' [1] were used. A completely randomised split-plot experimental design was used, with subplots and 14 replicates. The main plot factor was irrigation vs. no irrigation of plants, and the sub-plot factor was different fruit and leaf collection times.

Fifty plagiotropic branches per plot, with 12 productive nodes and 24 fully developed leaves, in two years were analysed. Plant material was collected every 28 days, beginning from 10 days after flowering (DAF) until fruit ripening. Five branches were randomly collected from each treatment. The N, P and K concentrations in the fruits were quantified in triplicate [2]

IMPACT OF WATER AVAILABILITY ON FRUIT NITROGEN, PHOSPHORUS AND POTASSIUM IN CONILON COFFEE PLANTS

COVRE, André Monzoli*, BONOMO, Robson*, TOMAZ, Marcelo Antônio*, RAMALHO, José Cochicho**,***, PARTELLI, Fábio Luiz*

*Universidade Federal do Espírito Santo, UFES, São Mateus, ES, Brazil. **PlantStress&Biodiversity Group, DRAT, LEAF, ISA, Universidade de Lisboa, Portugal. *** GeoBioTec, FCT, UNL, Portugal. *Contact author: partelli@yahoo.com.br

RATIONALE
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RESULTS, CONCLUSIONS & PERSPECTIVES
The fitted curves for fruit N, P and K accumulation, obtained under irrigated and non-irrigated conditions displayed similar (sigmoid) patterns, on both crop seasons, but irrigated plants showed significantly higher N, P and K contents. All nutrients showed an initial phase of low accumulation rates (ca. 10 and 100 DAF), followed by a rapid expansion phase with the highest accumulation rates (ca. 100 and 250 DAF), and a final phase with lower rates at the end of fruit formation (ca. 250 and 300 DAF), what agrees with [3].
N was the most accumulated nutrient in the fruits at ripening, followed by K and P. These findings reflect the importance of N in fruit development, demonstrating the benefits of N fertilization of coffee plants during their reproductive phase. Since N and K are accumulated in similar amounts and close patterns, it seems likely that they should be applied together [4]. Fertilization should be parcelled to meet the different nutrient demands of plants in different phases of fruit development.

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RESULTS, DISCUSSION AND CONCLUSION

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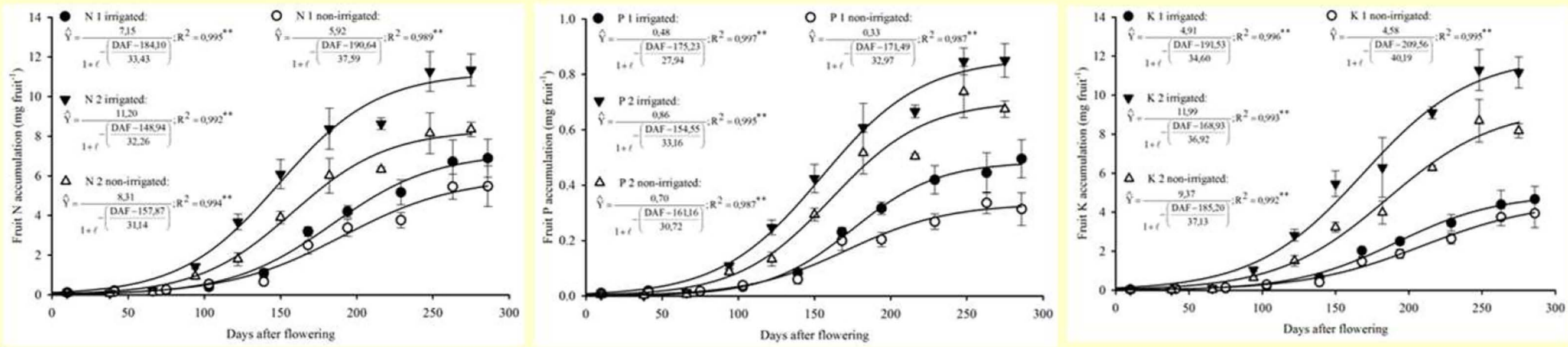

Fig.: Accumulation of Nitrogen, Phosphorus and Potassium in the fruits of irrigated and non-irrigated Conilon coffee plants, from flowering to fruit ripening in two consecutive crop seasons (Year 1 – K 1, Ca 1; Year 2 – K 2; Ca 2) in the Atlantic region of Bahia. Each data point represent the mean ± S.E. (n = 5). **Significant at $p<0.01$

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Support

•Farmers: Ademir Trevizani, Daniel Trevizani and Luiz Antônio Covre

ACCUMULATION OF CALCIUM, MAGNESIUM AND SULPHUR IN FRUITS OF *Coffea canephora* CV. CONILON PLANTS UNDER TWO WATER CONDITIONS

PA124

COVRE, André Monzoli*, BONOMO, Robson*, TOMAZ, Marcelo Antônio*, RAMALHO, José Cochicho**,***, PARTELLI, Fábio Luiz*#,

* Universidade Federal do Espírito Santo, São Mateus, Espírito Santo, Brazil. # Corresponding author: partelli@yahoo.com.br
** PlantStress&Biodiversity Group, DRAT, LEAF, ISA, Universidade de Lisboa, Portugal. *** GeoBioTec, FCT, UNL, Portugal.

INTRODUCTION

Climate changes, mostly related to global warming and reduced water availability, are important limiting factors to coffee production [1]. For these reasons, Conilon coffee is grown along the Brazilian Atlantic coast, mostly in irrigated areas.

OBJECTIVE

For that, it was evaluated fruit calcium, magnesium and sulphur accumulation and concentration throughout the year under irrigated and non-irrigated conditions in *Coffea canephora* cv. Conilon plants.

MATERIAL AND METHODS

The experiment was performed in two consecutive crop seasons in the municipality of Itabela, in southern Bahia state, Brazil. Three-year-old plants of *C. canephora* cv. Conilon 'genotype 02' [2] were used. A completely randomised split-plot experimental design was used, with subplots and 14 replicates. The main plot factor was irrigation vs. no irrigation of plants, and the sub-plot factor was different fruit and leaf collection times.

Fifty plagiotropic branches per plot, with 12 productive nodes and 24 fully developed leaves, in two years were analysed. Plant material was collected every 28 days, beginning from 10 days after flowering (DAF) until fruit ripening. Five branches were randomly collected from each treatment. The Ca, Mg and S concentrations in the fruits were quantified in triplicate.

ACCUMULATION OF CALCIUM, MAGNESIUM AND SULPHUR IN FRUITS OF *Coffea canephora* CV. CONILON PLANTS UNDER TWO WATER CONDITIONS

COVRE, André Monzoli*, BONOMO, Robson*, TOMAZ, Marcelo Antônio*, RAMALHO, José Cochicho**,***, PARTELLI, Fábio Luiz*
*Universidade Federal do Espírito Santo, UFES, São Mateus, ES, Brazil. **PlantStress&Biodiversity Group, DRAT, LEAF, ISA, Universidade de Lisboa, Portugal. *** GeoBioTec, FCT, UNL, Portugal.
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Climate changes, mostly related to global warming and reduced water availability, are important limiting factors to coffee production [1]. For these reasons, Conilon coffee is grown along the Brazilian Atlantic coast, mostly in irrigated areas. For that, it was evaluated fruit calcium, magnesium and sulphur accumulation and concentration throughout the year under irrigated and non-irrigated conditions in *C. canephora* cv. Conilon plants.

METHODS
The experiment was performed in two consecutive crop seasons in the municipality of Itabela, in southern Bahia state, Brazil. Three-year-old plants of *C. canephora* cv. Conilon 'genotype 02' [2] were used. A completely randomised split-plot experimental design was used, with subplots and 14 replicates. The main plot factor was irrigation vs. no irrigation of plants, and the sub-plot factor was different fruit and leaf collection times. Fifty plagiotropic branches per plot, with 12 productive nodes and 24 fully developed leaves, in two years were analysed. Plant material was collected every 28 days, beginning from 10 days after flowering (DAF) until fruit ripening. Five branches were randomly collected from each treatment. The Ca, Mg and S concentrations in the fruits were quantified in triplicate.

RESULTS, CONCLUSIONS & PERSPECTIVES
The highest fruit Ca and Mg accumulation rates were observed during the fruit rapid expansion, filling and ripening phases, indicating a strong Ca and Mg demand in these stages of fruit development. Accordingly, it was found that approximately 70% of the total Ca and Mg content required by fruits is accumulated these stages [3], suggesting a concomitant application of these nutrients. Sulphur accumulation rates followed a close pattern to that of Ca and Mg. The higher Ca, Mg and S concentrations occurred in the initial phase of fruit development.
As regards water availability, the plants submitted to water shortage showed a tendency to lower minerals accumulation in some parts of the year, possibly related to a lower translocation through the transpiration flow, likely related to a higher stomatal closure. If fact, nutrient translocation into fruits occurs through mass flow, resulting from high rates of water transport to fruits in the well-watered plants. Fertilization should be parcelled to meet the different nutrient demands of plants in different phases of fruit development.

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[3]Partelli FL, *et al.* (2014) *Revista Brasileira de Ciência do Solo*, 38(1):214-222. doi: DOI: 10.1590/S0100-06832014000100021.





RESULTS, DISCUSSION AND CONCLUSION

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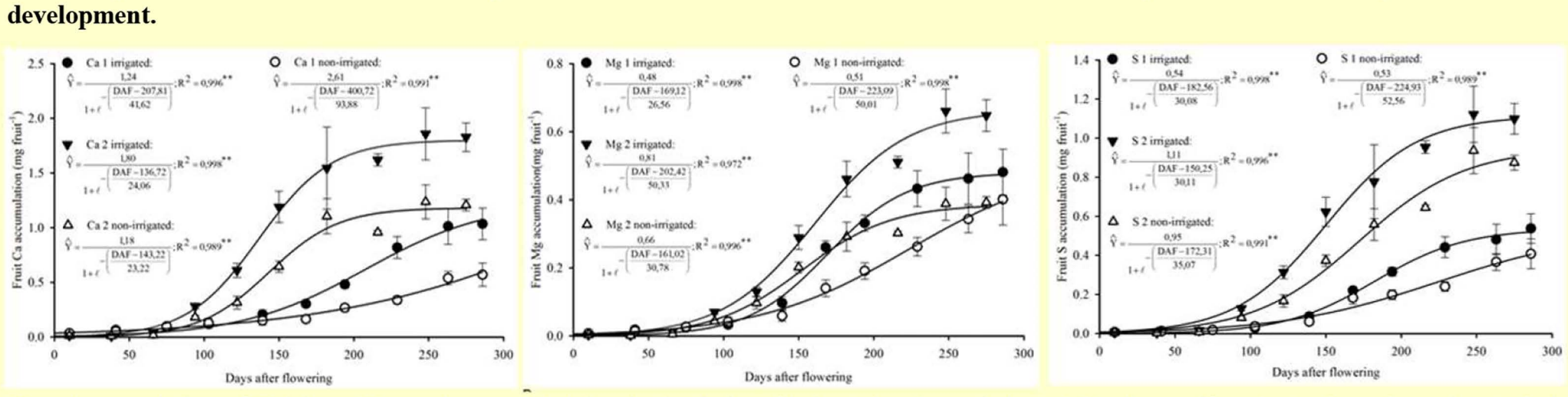

Fig.: Accumulation of Calcium, Magnesium and Sulphur in the fruits of irrigated and non-irrigated Conilon coffee plants, from flowering to fruit ripening in two consecutive crop seasons (Year 1 – K 1, Ca 1; Year 2 – K 2; Ca 2) in the Atlantic region of Bahia. Each data point represent the mean ± S.E. (n = 5). **Significant at $p<0.01$

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Support

•Farmers: Ademir Trevizani, Daniel Trevizani and Luiz Antônio Covre




TWENTY COFFEE CULTIVARS (COFFEA ARABICA L.) PLANT GROWTH AND ROOT MORPHOLOGY ANALYSIS

PA126

*DONG Yunping, HUANG Lifang, LIN XingJun, SUN Yan, WANG Xiaoyang, CHEN Peng, YAN Lin**

Spice and Beverage Research Institute, CATAS, Wanning, Hainan Province, China

Abstract: the seedling growth and root morphology of 20 arabica coffee germplasm resources were investigated with a pot experiment. the 20 arabica coffee germplasm resources were divided into three types: tall, medium and low, among which M13, M14 were chosen as standard tall type and CATUAI as standard low type, CATURRA, T8867 as medium.Most of the tested germplasm resources have slender and a larger number of lateral roots. M13 lateral root length, lateral root volume, lateral root surface area was significantly greater than CATUAI. Lateral-root diameter was found significantly negatively correlated with lateral-root length.

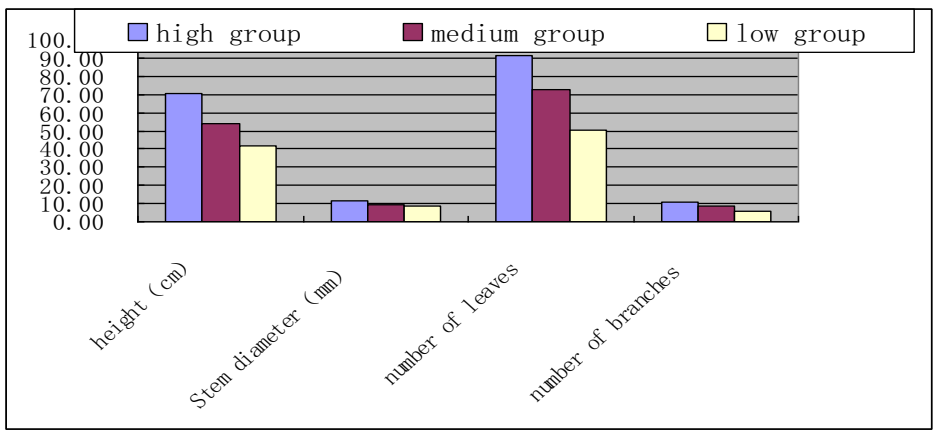

Figure 1: Growth parameters in 20 arabica coffee Seedlings

Introduction: Daniel[1] ,Burkhardt [2]research showed that Coffee Germplasm which had high transpiration rate were due to its well-developde root system.Taye Kufa[3] Studies on root growth of wild Coffea arabica populations in Ethiopia,He mentioned that the dry areas grown coffee trees have slender and a large number lateral roots than that of in humid region.Through analysis
the coffee Plant growth and root morphology at seedling st age,So as to gives a credible method for screening young coffee germplasm excellent traits.

Table 1 : Root growth parameters in 20 arabica coffee seedlings

group	lateral root length（cm）	lateral root volume（cm^3）	lateral root surface area (cm^2)
high group	20803.43a	30.51a	2873.31a
medium group	13812.30abc	20.51ab	1879.74ab
low group	6749.03c	12.82b	1037.14b

Main Text: The Coffee Cultivars growth were significant differences in height,number of leaves and number of branches.The average plant height was 70.49cm，including six germplasm such as M14,typica, Rume Sudan and so on.There were 8 Cultivars average plant height was 53.76cm,including bourbon, Caturra,CIFC 7963 and so on.The least average plant height was 41.90cm,was found in Catuai,Reyin2, Dtari 028 ,etc. The More number of leaves and branches Was found in 16 Cultivars such as Caturra,T8667,Reyin2, etc .
Most of the tested germplasm resources have slender and a large number of lateral roots.M13 lateral root length, lateral root volume, lateral root surface area was significantly greater than Catuai.

Conclusion: By observing 20 Cultivars characters, selected good variety such as Caturra,T8867,CIFC7963,Reyin2,DTARI 028.Wang kaixi,Lv yulan And zhou hua Research showed that these varieties were high or dwarf, much branched, high yield, short fruit Festival, is mainly cultivated in the production.It shows that the method suggested in this paper is reliable.Screening of excellent traits in the coffee seedling stage has the advantages of small occupation area, low labor intensity and short period.
Coffee seedling root formed with first-order lateral root,second-order lateral root and Third-order lateral root at the age of 15 months old.those with more second-order and Third-order lateral root have slender lateral root diameter.
M13 was found have most slender lateral roots,Its root length, lateral root volume, lateral root surface area was significantly greater than Other Cultivars.Study on drought resistance of this germplasm,Is expected to select a strong drought resistant varieties.

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PA127

Effect of altitude on biochemical composition and quality characteristics of green arabica coffee beans depends on shade and processing method

Mohammed Worku[a,b], Bruno de Meulenaer[b], Luc Duchateau[b], Pascal Boeckx[b]
[a] Jimma University, Ethiopia; [b] Ghent University, Belgium

Abstract

We studied altitude, shade, processing and their two-way interaction effects on biochemical compositions and quality attributes of green arabica coffee beans in southwestern Ethiopia by analyzing caffeine, total chlorogenic acids, trigonelline and sucrose compositions plus quality attributes of 108 coffee samples collected from with shade or without shade, in a range of altitudes and processed by wet or dry method. While altitude significantly affected caffeine, total chlorogenic acids and flavor; altitude-processing, altitude-shade and shade-processing interactions significantly affected trigonelline and sucrose, sucrose and acidity, and bean physical quality, respectively.

Introduction

Biochemical compositions and quality attributes of coffee beans may vary with genetics, edaphic and climatic conditions, agricultural practices and postharvest techniques. Several studies (e.g., Avelino et al., 2007; Vaast et al., 2006; Vaast et al., 2004) investigating altitude, shade and processing effects on these traits of coffee in various parts of the world reported different results. The objectives of our study were (1) to test the hypothesis that altitude and shade effects on biochemical compositions and quality attributes of green arabica coffee beans are site specific and can be dependent on postharvest processes, and (2) to study for the first time the effects of altitude, shade, processing and their two-way interactions on biochemical composition and quality of green arabica coffee beans in its region of origin, southwestern Ethiopia.

Results

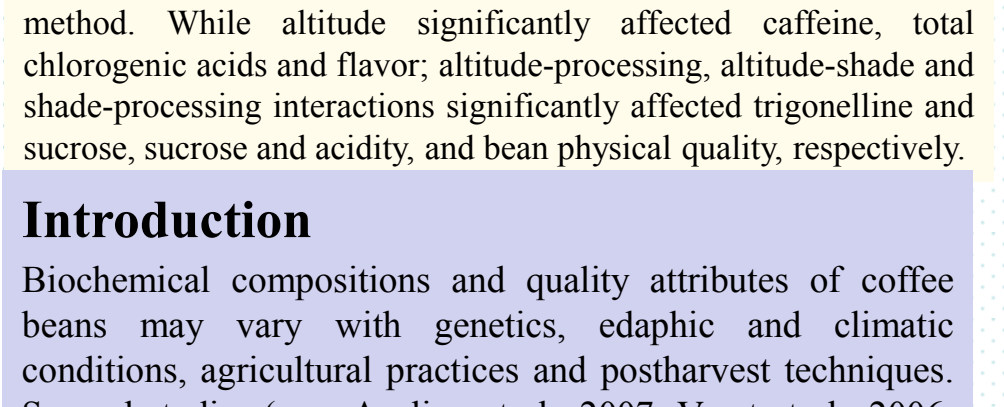

Fig. 1 Main effects of attitude on caffeine (mg/g DW) and total chlorogenic acids (TCGA) (mg/g DW).

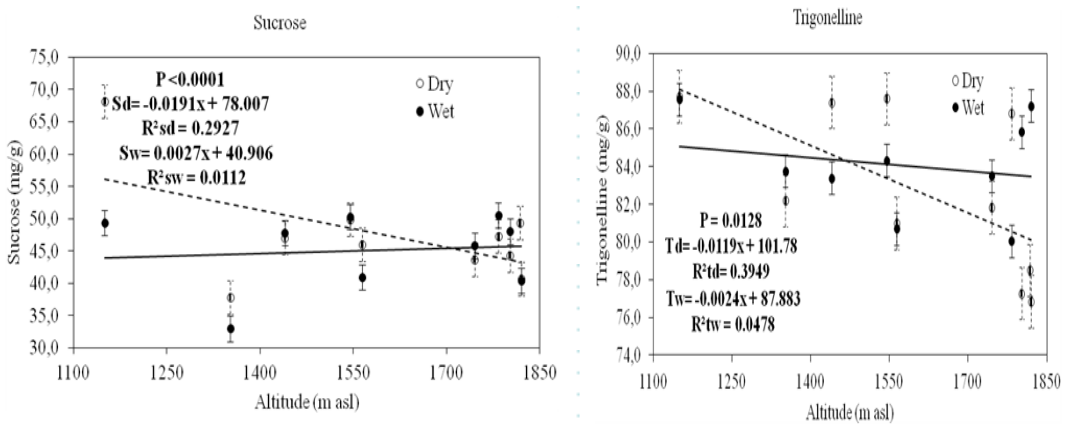

Fig. 2 Interaction effect of altitude and processing on sucrose and trigonelline compositions (mg/g DW): **solid line** (wet processing) and **broken line** (dry processing).

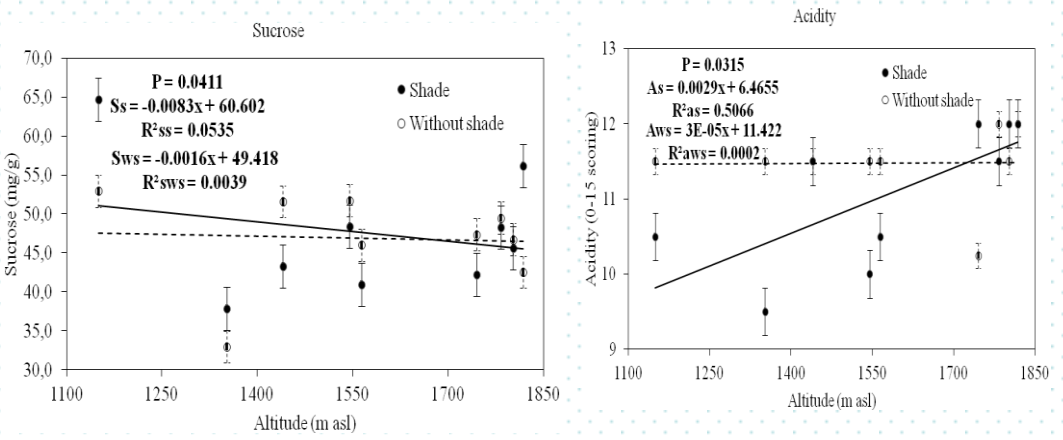

Fig. 3 Interaction effect of altitude and shade on sucrose composition (mg/g DW) and acidity (scoring): **solid line** (with shade) and **broken line** (without shade).

Caffeine and total chlorogenic acids significantly decreased with increasing altitude (Fig. 1), but favor showed the reverse. The result of caffeine contradicts with previous studies (Avelino et al., 2007; Vaast et al., 2004), but those of chlorogenic acids and flavor agree with past studies. Sucrose and trigonelline compositions of unwashed coffee considerably reduced with increasing altitude, but no effect of altitude on washed coffee was observed (Fig. 2). Sucrose composition of coffee grown under shade also decreased with increasing altitude, whereas acidity of the same coffee showed the opposite (Fig. 3). Bean physical quality of without shade grown and unwashed coffee was higher than that of the other samples.

Conclusion

Altitudinal effects on sucrose and acidity depended on shade, and sucrose and trigonelline on processing. Shade effect on physical quality of coffee beans also depended on processing. This confirms our initial hypothesis. But, its effects on caffeine, total chlorogenic acids and flavor were not associated with shade and processing. With increasing altitude, sucrose composition of coffee grown under shade, sucrose and trigonelline compositions of unwashed coffee, caffeine and chlorogenic acids decreased, but acidity of coffee grown under shade and favor increased. Dry processing produced higher bean physical quality for coffee grown without shade than all other samples.

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PA129

STUDY ON THE CHARACTERISTICS OF GRAFTING COFFEE NUTRIENT CONTENT AND THE INFLUENCE FACTORS OF ABSORBING NITROGEN, PHOSPHORUS AND POTASSIUM

SUN Yan*, YANG Jianfeng*, HUANG Lifang**, DONG Yunping**, YAN Lin**, LIN XingJun*, WANG Xiaoyang**, CHEN Peng*

*Spice and Beverage Research Institute, Chinese Academy of Tropical Agricultural Sciences, Wanning, Hainan, China. **Key Laboratory of Genetic Resources Utilization of Spice and Beverage Crops, Ministry of Agriculture, Wanning, Hainan, China

Abstact

The trial was carried out at Spice and Beverage Research Institute, Chinese Academy of Tropical Agricultural Sciences in China during 2013-2015 to study on the characteristics of grafted coffee nutrient content and the influence factors of absorbing N, P and K. To evaluate the ability of grafted coffee nutrient uptake and utilization, and find the influence factors of coffee nutrient high-efficient utilization.

Introduction

The pot trial was conducted by using grafted and own-root coffee. The coffee growth data was collected every 3 months. After 9 months, the chlorophyll SPAD, net photosynthesis rate, stomatal conductance, root volume, root area and root activity were determined. Also, the plants were separated into leaves, scion stems, rootstock stems and roots for determination of N, P and K content.

Table 1: Grafted and own-root coffee nutrient content

	grafted coffee（I）			own-root coffee（II）		
	root	stem	leaf	root	stem	leaf
N	23.68	20.45	41.79	13.79	18.94	36.87
P	1.18	0.94	1.63	0.68	0.57	1.43
K	18.88	10.11	20.89	22.48	11.08	20.77

Table 2: Grafted and own-root coffee root growth capacity

	volume (cm^3)	surface area (m^2)	lateral length (cm)	activity [μg/(g·h)]	soluble protein content (mg/g)
I	318.84	47.08	2521.05	142.32	0.14
II	294.17	49.42	3030.10	62.81	0.20

Main Text

Compared with own-root seeding, more N and P were absorbed by the grafted coffee. The grafted coffee showed a significant growth after six months of grafts take. In generally, the grafted coffee had a greater dry weight than the own-root seeding. The possible influential factors of coffee nutrient uptake and utilization were studied, the result indicated that there were significant difference in root volume and root activity between grafted and own-root coffee.

Conclusion

Based on the results of the present study, grafting was considered to be a good way to increase the nutrient content and improve the growth of coffee. The other important conclusion was that coffee nutrient uptake and utilization were positively correlated with root volume and root activity, and so promoting the root growth might be a future research direction for coffee nutrient high-efficient utilization.

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PA 130

Effects of selected *Saccharomyces cerevisiae* yeast inoculation on coffee fermentation and quality

Alter P.[1], Berthiot L.[2], Bertrand B.[3], Durand N.[1], Meile J.C.[1], Morel G.[1], Ortiz-Julien A.[2], N. Sieczkowski[2] , F.Vidal[2]
[1] UMR Qualisud (CIRAD, Université Montpellier, Supagro), 34095 Montpellier Cedex 5, France
[2] Lallemand SAS, 31700 Blagnac, France [3] UMR RPB (CIRAD, IRD, UM2), 34394 Montpellier Cedex 5, France.

Introduction

The Arabica coffee typically undergoes a wet preparation (Rolz et al., 1982) and is considered as the top level coffee, with its aroma, smoothness and softness. After harvesting cherries, the pulp is removed by a mechanical process, called depulping. A mucilaginous layer called mucilage adheres to the grain. Generally the mucilage is removed during a process step called "fermentation" were biochemical reactions occur, currently attributed to the presence of indigenous microorganisms and their enzymatic activities. Generally coffee brew produced from wet-process have superior aroma potential and higher acceptance (Subedi 2011, Velmourougane 2013). The aim of the study was to evaluate the impact of the inoculation of characterized selected *Saccharomyces cerevisiae* yeasts during the "fermentation" step on coffee processing and quality.

Methodology

- Four selected yeast strains, characterized and chosen among Lallemand collection were tested in duplicate in comparison with the current farm process (12h of fermentation under water) which was not inoculated (spontaneous fermentation).
- Inoculations with selected *Saccharomyces cerevisiae* strains (Active Dry Yeast) were performed on 50 kg of freshly harvested, depulped coffee (*Coffea Arabica*) batches (Fig. 1) with a ratio of 1g per kilo of depulped coffee. Each batch was treated equally in all the additional steps from demucilaginated coffee to roasted coffee beans (Fig. 2).
- Two fermentation durations were evaluated for each treatment : 12 and 36 hours.

Figure 1: Maceration of depulped coffee cherries after inoculation with selected yeast. Nicaragua 2015. Berthiot.

Figure 2: Drying of the different batches of green beans proceeding from the different yeast inoculations. Nicaragua 2015. Berthiot.

Results

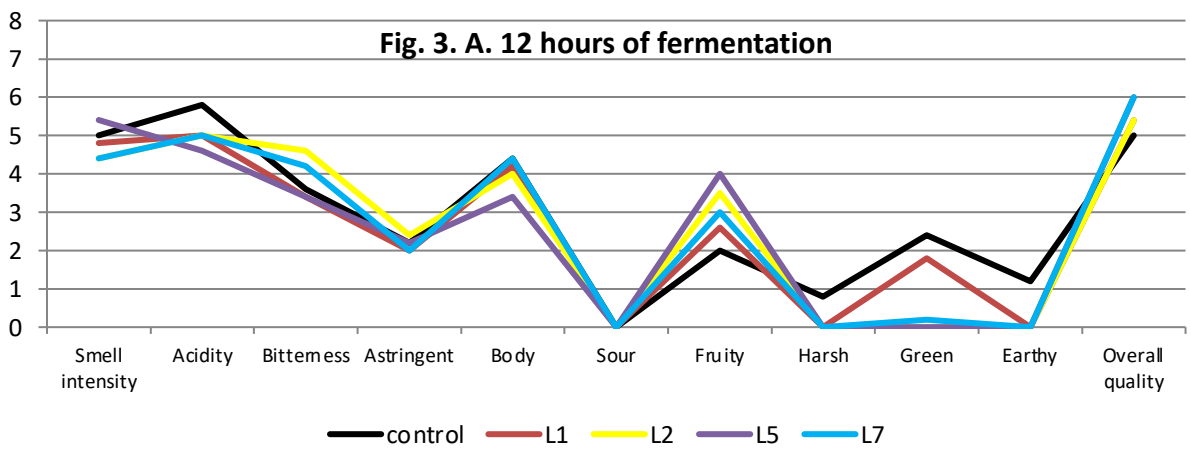
- ✓ All treatments led to the total demucilagination of the beans and no additional washing-step was required.
- ✓ Biochemical analysis (lipids, sugars, organic acid, caffeine, trigonellin and chlorogenic acid) : No statistically significant differences were evidenced between the treatments (Table 1).
- ✓ Sensory analysis : compared to the control, the inoculation with selected yeasts (L1, L2, L5 & L7) led to the decrease the perception of negative sensory attributes (green, earthy, harsh) and the improvement of the fruity character and the overall quality (Fig. 3) and the commercial value of the coffee (Table 2).
- ✓ Selected yeast L2 (and L5&L7 to a lower extant) showed a very positive impact already for the shorter fermentation duration (12h).
- ✓ A longer fermentation duration (36h) led to a higher sensory quality for L1 and to higher fruity notes for L5 & L7.

Sample	Citric acid	Lactic acid	Malic acid	Oxalic acid	Organic acid	Chlorogenic acid	Saccharide	Fats	Trigonelline	Caffeine
L1 12H	0,09	0,003	0,038	0,005	0,903	10,47	8,74	12,72	0,82	1,33
L1 36H	0,09	0,003	0,042	0,004	0,871	10,02	8,91	12,64	0,75	1,24
L2 12H	0,144	0,003	0,026	0,005	1,097	9,88	8,88	12,6	0,82	1,35
L 2 36H	0,127	0,002	0,036	0,004	0,742	9,55	7,96	12,82	0,75	1,33
L5 12H	0,083	0,001	0,038	0,004	0,534	9,22	8,15	12,37	0,75	1,3
L5 36H	0,119	0,003	0,048	0,004	0,916	9,73	8,54	12,79	0,75	1,3
L7 12H	0,117	0,004	0,044	0,008	1,145	9,33	8,03	13,05	0,72	1,25
L7 36H	0,105	0,003	0,045	0,005	1,019	8,74	7,97	12,32	0,67	1,24
Control	0,112	0,005	0,044	0,004	1,15	9,66	8,37	12,76	0,71	1,2

Table 1 : Biochemical analysis : results of the main compounds (%w/w) analyzed on green coffee beans.

Sample	Cup	Points SCAA
Farm process	B	78
L1 12H	B	80
L1 36H	B+	81
L2 12H	B+	82
L2 36H	B+	81
L5 12H	B+	81
L5 36H	B+	81
L7 12H	B+	81
L7 36H	B+	82

Table 2 : Sensory analysis : Results of the cup tasting run by a coffee buyer



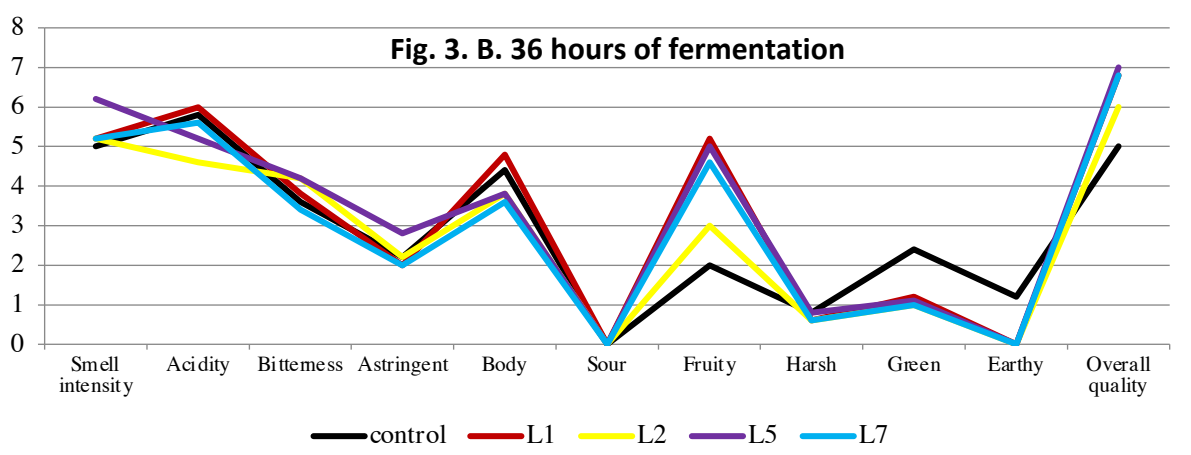


Figure 3: Sensory analysis : cup profiles of the different roasted coffees proceeding from the different treatments after 12 (Fig. 3. A.) and 36 hours of fermentation (Fig. 3. B.)

Conclusion

- ✓ This study shows how these selected yeast inoculations can improve the quality of green beans (wet-process).
- ✓ All the studied selected yeasts led to a better cup quality than the control. For 12h fermentation duration, L2, L5 and L7 perform the best at increasing cup quality and commercial value of coffee.
- ✓ A longer fermentation duration (36h) for one of these selected yeasts (L1) show very promising interest for the enhancement of fruity character and body of coffee and cup quality.
- ✓ The yeast didn't modify coffee quality but they limit the development of native microflora and their potential bad flavor. Thanks to their specific metabolism they allow to reveal the aromatic potential of coffees.

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Importance and Current Status of Coffee Thread Blight (*Corticium koleroga*) in Ethiopian Coffee Production

Kifle Belachew*, Demelash Teferi and Legese Hagos
Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Centre, Plant Pathology Research Section, P.O.Box 192; Email:-*kiflekef@gmail.com*

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Abstract
Besides its importance coffee production constraints with number biotic factors of which diseases are major. Thread blight of coffee caused by *Corticium koleroga* sporadically occurs and currently become significantly important disease in coffee growing areas of Ethiopia. Therefore, investigation including diagnostic survey on disease severity and incidence accompanied by infected coffee specimen collections were conducted along with isolation and identification of the causal pathogen. The results of study showed that disease invariably attacks coffee leaves, branches, twigs and berries with characteristic blight symptoms. White fungal threads were seen on the young stems and succulents tender tissues of coffee trees. These threads eventually become dark brown in color grow and spread to cover underside of leaves while coffee berries on infected braches are also completely destroyed leading to total crop failure. The isolation and identification of the causal pathogen from samples of leaves, berries, branches and shoots consistently produced fungal species which may be *Corticium koleroga* that further proved by pathogenicity tests. The disease mean incidence and severity at Limmu coffee plantation farm of "*Gummer"* in 2008 was 49.02 and 9.8 percent, respectively. In 2014 around southwestern Ethiopia mean incidence and severity of 58.44 and 32.59 percent, respectively, resulting in considerable damages. Among others, climatic factors prolonged rainfall with long period of wet favored the thread blight disease on Arabica coffee production in Ethiopia. In the pathogenicity test, the thread blight isolates inoculated after sporulation on detached leaves, twigs and berries of different coffee varieties showed similar symptom characteristically related to those observed on infected mature coffee trees. Thus, further in-depth research on the disease epidemiology and control practices are required along with exploring and developing resistant coffee varieties against the disease.

Introduction

Coffee is major cash crop in Ethiopian and sources of 25-30% export earnings of the country (Abu T., 2015). However it is prone to a number of diseases that attack fruits, leaves, stems and roots which in turn reduce yield and marketability (Kifle *et. al.,* 2014). Major coffee diseases in Ethiopia are coffee berry diseases caused by *Colletotrichum kahawae,* coffee wilt disease of *Gibberella xylarioides* and coffee leaf rust, however other diseases considered to be minor (Eshetu *et. al.,* 2000). Coffee thread blight diseases in Ethiopia was observed for first time at Gera and Metu agricultural research sub-stations in 1978 (Demelash *et.al.,* 2008). However, this disease sporadically occurs between June and September, but increasingly becoming important at highland coffee growing areas of Ethiopia. Therefore, investigation including diagnostic survey on disease severity and incidence accompanied by infected coffee specimens collections were conducted along with isolation and identification of the causal pathogen

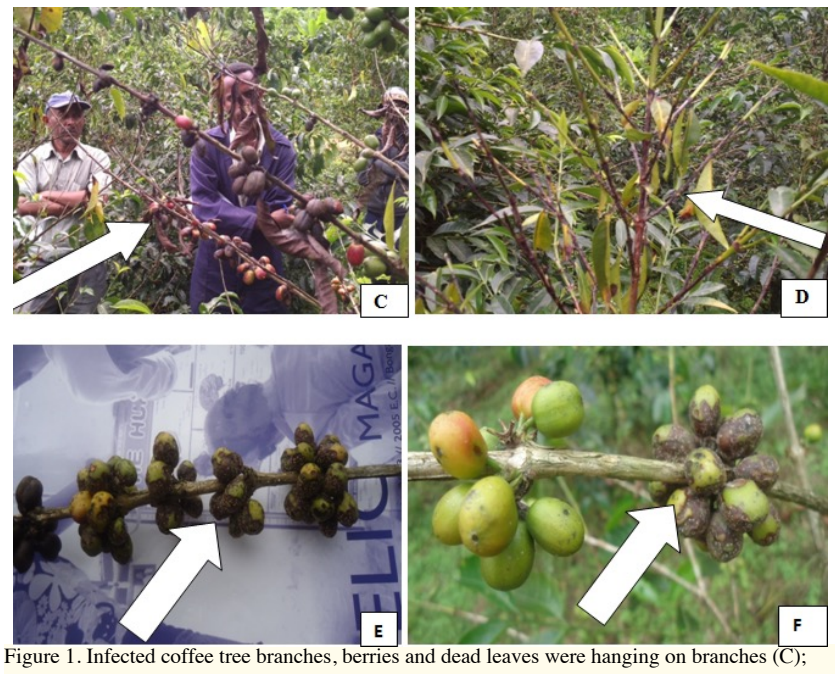

Figure 1. Infected coffee tree branches, berries and dead leaves were hanging on branches (C); dieback of primary branches (D), black decay and rot of mature berries (E &F). (August, 2014; Photograph by Kifle B.)

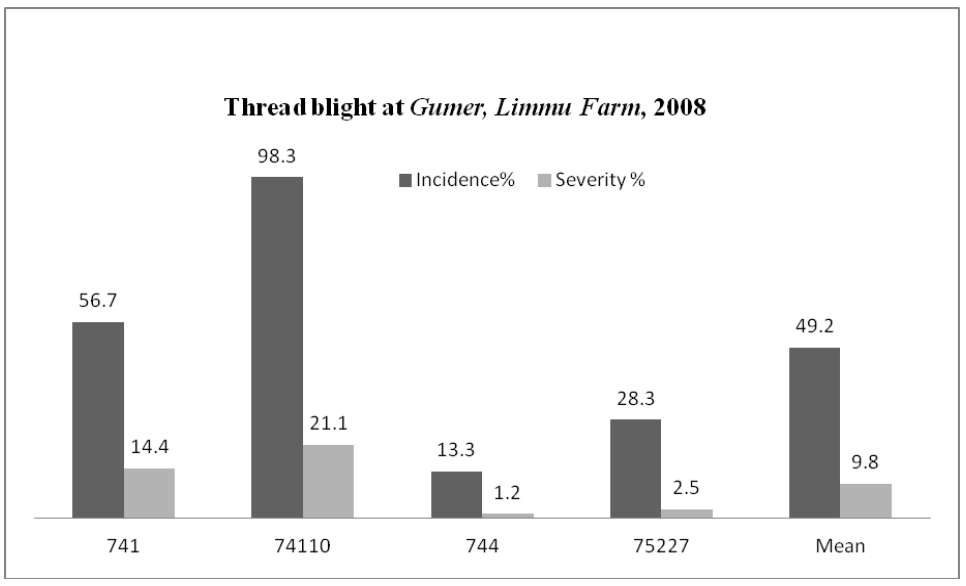

Figure 2. Thread blight percent incidence and severity with in different coffee varieties at Limu Coffee Plantation *of Gumer farm*, Horizen PLC, in 2008

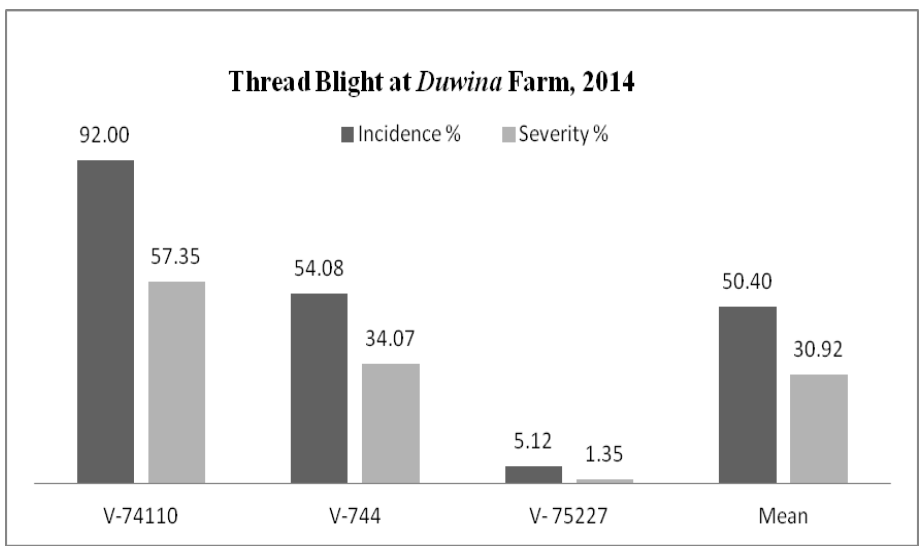

Figure 3. Thread blight percent incidence and severity at *Duwina* Coffee Plantation, Agriceft Ethiopia, 2014 (V-stands for variety)t

Result

In Ethiopia thread blight of coffee outbreak was seen for first time in 2008 at Limu coffee plantation farm of "Gumer" with mean diseases incidence and severity of 49.2 and 9.8, respectively. During the first disease outbreak assessment on four commercial varieties of coffee (741, 74110, 75227 and 744) diseases severity percent was found to be 14.42, 21.08, 2.48 and 1.17, respectively (Figure 2). The second reported outbreak of the diseases was from Bebeka coffee estate of "*Disadis*" farm on which 34 hectares of coffee damaged in 2012 (JARC, 2014). In 2014 number of coffee farms such as *Duwina* coffee farm of Agri Ceft PLC, Limmu coffee Plantation of Horizen PLC and coffee research sub centers such as *Gera, Haru, Mugi* and *Awada* reported similar coffee disease symptoms in the same season. At AgriCeft coffee plantation of *Duwina* farm disease incidence ranged from 5.12 to 92.0 percent per sample plot with average incidence of 50.4 percent and average disease severity of 30.92 percent (Figure 3). In the same year, the disease outbreak was further noted in coffee growing farm such as *Limmu Sintu and Gumer* with mean incidence and severity of 66.48 and 32.25%, respectively. According to Girma *et. al.,* (2009) thread blight diseases of coffee was one of locally important coffee diseases in Ethiopia. However, current scenario indicates wider distribution of the disease across geographical areas of coffee growing regions of Ethiopia. . In the pathogenicity test, the thread blight isolates inoculated after sporulation on detached leaves, twigs and berries of different coffee varieties showed similar symptom characteristically related to those observed on infected mature coffee trees.

Conclusion

Thread blight diseases on Ethiopian coffee was known for long time and considered as minor coffee disease. However, it sporadically occurs every five to six years between June and September, but increasingly becoming important and observed in wide coffee growing regions as an epidemic in 2014. The disease epidemics is found to be favored by prolonged rainfall and high relative humidity and prevalence of wet and humid conditions, that perhaps reflects one of the climate change scenarios. The disease has been causing severe damage to Arabica coffee since 2008 and it may be potential threat to coffee production in future. Thus, further in-depth research on the disease epidemiology and control practices are required along with exploring and developing resistant varieties against the emerging thread blight. Moreover, detailed characterization of the thread blight causing organisms is essential to clear out the present controversies in the pathogen population structure in relation to the disease symptoms.

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LOSS CAUSED BY *Meloidogyne exigua* UNDER CONTROLLED CONDITIONS IN COSTA RICA

ROJAS, Mainor[1] mrojas@icafe.cr, *RAMÍREZ, Daniel*[1], *SALAZAR, Luis*[2]

[1]Centro de Investigaciones en Café, Instituto del Café de Costa Rica; [2]NEMAFITO

Introduction

Meloidogyne exigua is the root-knot nematode most commonly found in the Costa Rican coffee plantations, although its effect on the coffee yield is not clearly determined yet. This study aims at determining the loss caused by *Meloidogyne exigua* on adult coffee plants of Caturra variety under semi-controlled field conditions.

Materials and Methods

The trial was conducted in a volcanic soil in Heredia, at 1180 m.a.s.l., with a mean annual temperature of 21.5° C and a total annual rainfall of 2650 mm. Coffee plants formed in two axis, with a 2.0 x 1.0 m. planting distance, were planted in 2009 in 100 L pots (Figure 1). A randomized complete block design with 10 replicates was used. The treatments were defined by the initial inoculum (Pi) of 0, 125, 250, 500, 1000 and 2000 eggs +J_2 *M. exigua*/100 cm^3 soil, applied two months after planting. Adequate fertilization has been provided. The plant development was evaluated in 2010 and 2011. Foliar samples were collected twice a year for chemical analysis of each plant. Root samples were collected in 2011, 2013 and 2015, for the analysis of the *M. exigua* density. Has been evaluated five year production.

Figure 1. Planting in pots.

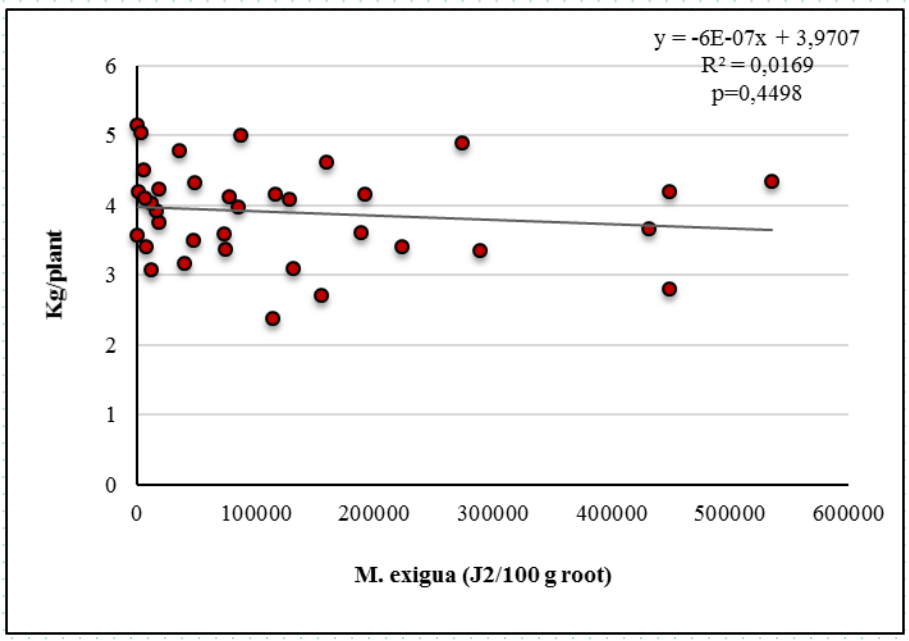

Figure 2. Production average years 2013 to 2016, depending on the density of *M. exigua*.

Results

The evaluations of development showed no response to the initial inoculum of nematodes. The foliar analysis indicated that the levels of elements were within the normal ranges and showed no response to initial inoculum. In 2011 the density of *M. exigua* in root reported a strong relationship with the initial inoculum and regression clearly separated the highest Pi (R^2 = 0.9766). Analysis of density in 2013 and 2015 showed no significant response to initial inoculum. The average production of the five harvests varied between 4.11 and 4.88 kg per plant, with no significant difference between treatments. Regression analysis of the average of the five harvests based on the initial inoculum showed no significant trend indicating the effect of the pathogen on production. The average crop yields 2012/2013 to 2015/2016 based on the average density of *M. exigua* from samples taken in 2013 and 2015, has high dispersion of data and the trend is not significant (Figure 2).

Conclusion

With production data can be estimated losses that ranged between +1.6% and -12.0%, with nematode densities of around 150.000 J_2/100 g root. Although this is not statistically significant, if that relationship were linear, the loss would be around 1% per 20000 J_2/100 g root.

CHARACTERIZATION OF FERTILITY IN SOILS DEVOTED TO COFFEE CULTIVATION IN COSTA RICA

PA 140

CHAVES-ARIAS, V.[1]; DELGADO-CHAVARRÍA, C.[2] ; ROMERO-MARTÍNEZ, G.[3]
[1] Instituto del Café de Costa Rica, Centro de Investigaciones en Café. vchaves@icafe.cr
[2] Instituto del Café de Costa Rica, Centro de Investigaciones en Café. cdelgado@icafe.cr
[3] Instituto del Café de Costa Rica, Centro de Investigaciones en Café. gromero@icafe.cr

INTRODUCTION

In order to update information on the fertility of coffee soils, and use it as input to guide farmers in their fertilization programs; between 2007 and 2012, ICAFE conducted a systematic soil sampling which covered most of the national coffee area. This paper summarizes the results obtained in different coffee regions.

Materials and Methods

Aerial photographs were used for selecting the collection sites. A grid with a density of one site every ten hectares was superimposed on the aerial photographs; every site was appropriated geo-referenced. A total of 9.607 samples (equivalent to 96.070 ha planted with coffee) were collected. Each sample was formed from six subsamples taken on the band of fertilization, at a depth of 0-20 cm, in a radius of approximately 10 m within the geo-referenced site. The water pH, interchangeable soil acidity (IA), Ca and Mg extracted with 1N KCl, and K, P, Cu, Zn, Mn and Fe with Modified Olsen were determined for each sample.
In addition, this paper provides the results for each variable classified by district, fertility maps using the Arc-Gis 9.2 program and recommendations for fertilization programs.

Results and Discussion

Variables related to soil acidity (Table 1) reached its highest expression in areas with predominance of Ultisols, among them Perez Zeledón, Los Santos and Los Santos Norte had the highest percentages of samples with low pH values (89% , 72% and 91% respectively); and high exchangeable acidity (69%, 70% and 82% respectively). Besides the two first-mentioned regions (Perez Zeledon and Los Santos) also they had the highest percentages of samples with high acid saturation (62% in both cases). Nationally, just over half of the samples showed low levels of calcium (56%) and magnesium (53%), while potassium they were about one-fifth (19%).
Regionally, the lower contents of calcium and magnesium in the soil were located in Coto Brus and Perez Zeledón, where both elements were presented at low levels in just over 70% of the samples.

As for potassium, the highest percentages of low samples corresponded to Coto Brus (38%) and Perez Zeledón (38%).

Samples with low concentrations of phosphorus accounted for 44% of the national total and not less than 70% of the samples collected in the regions of Coto Brus (89%), Perez Zeledon (70%).

Table 1: Percentage of samples with acidity problems

Coffee Region	n	% Samples		
		pH Low	IA (H+AI)	% IAS
		< 5,0	> 1,50 cmol(+)/L	> 40%
Los Santos	1268	90,9	82,2	62,0
L.S. Norte	1285	72,2	69,6	39,3
Coto Brus	972	28,5	19,7	26,9
Pérez Zeledón	1364	89,1	68,6	62,2
Valle Occidental	2120	64,5	55,8	41,6
Valle Central	1549	46,4	38,0	31,2
Turrialba-Orosi	831	70,4	51,6	37,1
National Total	9607	66,2	55,7	42,8

Table 2: Percentage of samples with nutrients problems

Coffee Region	n	% Low Samples			
		K	Ca	Mg	P
		< 0,20 cmol (+)/L	< 3,00 cmol (+)/L	< 0,80 cmol(+)/L	< 10 mg/kg
Los Santos	1268	10,9	64,2	44,6	39,7
L.S. Norte	1285	2,7	32,2	21,6	21,3
Coto Brus	972	38,3	67,6	72,0	88,8
Pérez Zeledón	1364	37,8	72,7	72,3	70,0
Valle Occidental	2120	12,1	53,7	46,1	34,7
Valle Central	1549	8,7	58,8	64,7	22,2
Turrialba-Orosi	831	35,7	50,9	60,4	48,1
National Total	9607	18,8	56,3	52,8	43,6

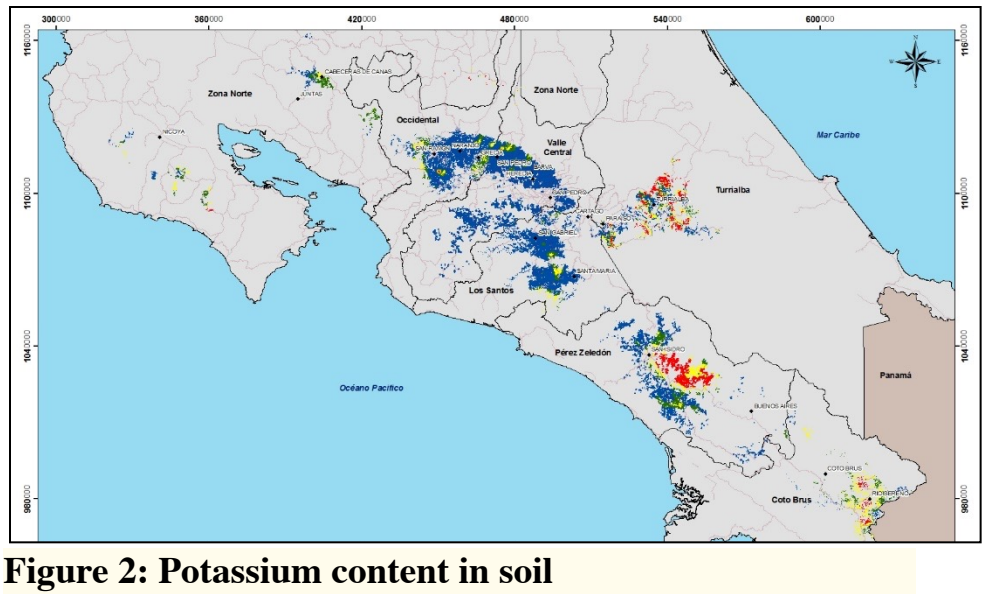

Figure 2: Potassium content in soil

Conclusion

Soil mapping allows a differential management of fertilizer recommendations according to the characteristics of each region

BEHAVIOUR AND DAMAGE OF PINK HIBISCUS MEALYBUG (*Maconellicoccus hirsutus*) IN COFFEE

PA 141

ROJAS, Mainor mrojas@icafe.cr; RAMÍREZ, Daniel
Centro de Investigaciones en Café, Instituto del Café de Costa Rica

Introduction

The pink hibiscus mealybug (Hemiptera: Pseudococcidae) is a quarantine pest of great economic importance worldwide. Damages are caused by all biological stages by sucking the sap and inject toxins into the plant, causing tissue malformations and drastically reduction of production. In Costa Rica, the pest was detected in 2012 affecting some coffee areas located in the Central Valley.

Materials and Methods

It followed monthly pest behavior in a lot of 2 ha, quantifying the number of individuals per branch in marked plants. Estimating crop loss was performed according to the degree of attack. Biological and chemical control was assessed. Monitoring was maintained for three years, until the problem was controlled.

Results

The plague caused fruit drop and rosetting in orthotropic and plagiotropic shoots, mainly in the upper third of adult plants, but also attacked shoots of pruning and young plants (Figure 1). The damage was complemented by the presence of sooty mold and caused losses were around 70% of harvest and the reduction of production potential for next year. The pest population remained its highest level during the driest months of the year and was very affected by rain. The monitoring in an affected area showed that the insect attacked between 60% and 80% of the plants. The sum of individuals of *M. hirsutus* remained around 13 per plagiotropic branch and 2 ovisacs with more than 200 eggs each (Figure 2).

From the year 2012 the parasitoid *Anagyrus kamali* was released at the sites most affected, reaching up to 50% parasitism with low pest populations. In 2013 the predator *Cryptolaemus montrouzieri* was released and the population of *M. hirsutus* practically disappeared from the affected area.

Figure 1. Symptoms and damage.

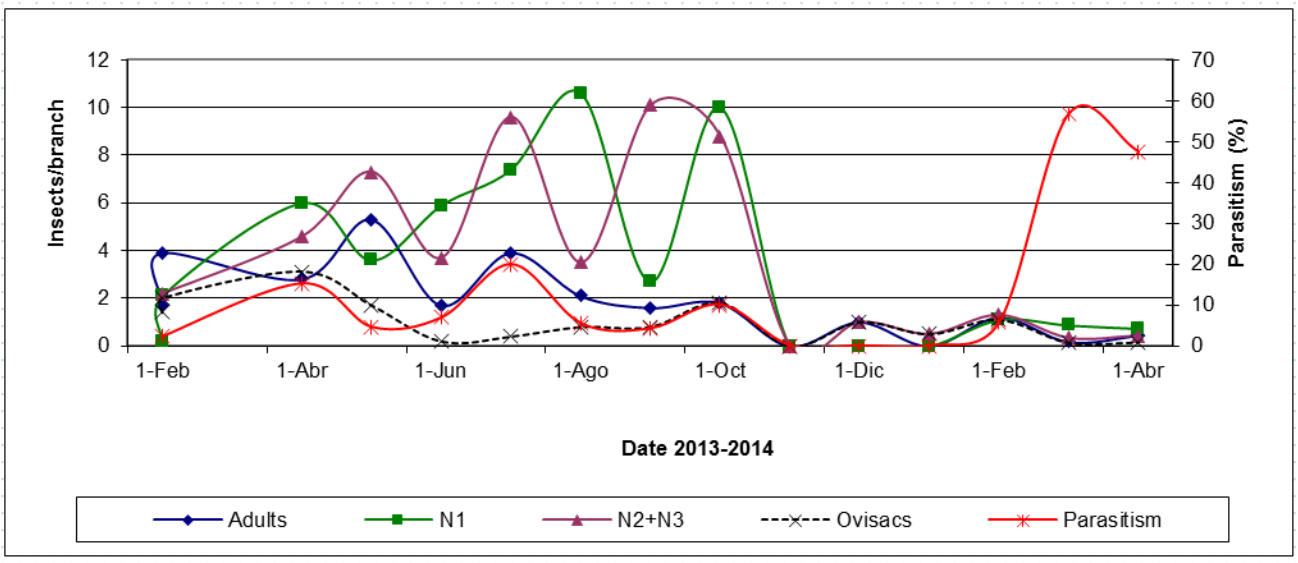

Figure 2. Insects per branch and parasitism of *A. kamali*.

Conclusion

The pink hibiscus mealybug has the potential to cause major damage to coffee. Its population is higher during the dry season and biological control can be effective.

F1 HIBRIDS RESPONSE TO FERTILIZER APLICCATION

PA 142

CHAVES-ARIAS, V.[1]; DELGADO-CHAVARRÍA, C.[2]
[1] Instituto del Café de Costa Rica, Centro de Investigaciones en Café. vchaves@icafe.cr
[2] Instituto del Café de Costa Rica, Centro de Investigaciones en Café. cdelgado@icafe.cr

INTRODUCTION

In order to evaluate the response to fertilization of the F1 hybrids (T-5296 x Rume Sudan) produced by somatic embryogenesis, a trial was established at the experimental station ICAFE (CICAFE) at 1180 masl, in a soil Andisol.

Materials and Methods

The coffee trees were planted in 2008, in full sunlight exposure, one plant per hole and at a distance of 2,10 x 1,10 m. The treatments were implemented since 2010, and evaluation consisting of 5 levels of fertilization, corresponding to 500, 750, 1000, 1250 and 1500 kg / ha of Complete Formula (C.F.) 18-5-15-6-0,2 (N, P_2O_5 , K_2O, MgO, B), respectively supplemented with extra Nitrogen of 45, 68, 90, 113 and 135 kg/N ha of nitrogen, based on ammonium nitrate (A.N.). Complete Formula partitioned into two applications (May, July), while ammonium nitrate was applied in October. Treatments were placed under a randomized complete block design, with 6 replicates.

Table 1 : Nutritional supply of fertilizers

Nº		kg/ha				
	C.F. + A.N.	N	P_2O_5	K_2O	MgO	B
1	500 + 135	135	25	75	30	1,0
2	750 + 204	204	38	112	45	1,5
3	1000 + 270	270	50	150	60	2,0
4	1250 + 339	339	63	188	75	2,5
5	1500 + 405	405	75	225	90	3,0

C.F. = 18-5-15-6-0,2 (N-P_2O_5-K_2O-MgO-B)
A.N.= Ammonium Nitrate (35,5 % N)

Results and Discussion

Coffee trees did not show a clear response to treatment in the first (2010) and fourth harvest (2014), but they showed a positive effect of fertilizer application in 2011, 2012, 2013 and 2015 (Table 2).
The effect was linear in 2013 and quadratic in 2011, 2012 and 2015.
With the exception of 2014, where the harvest was very small; in the other 5, treatments with lower amounts of fertilizers (500 and 750 C.F.) were those with lower harvest.

When considering the average of the 6 harvests, a quadratic effect was found (figure 2); that according to the response curve, the maximum harvest (21,8 t/ha) is obtained with 1.568 kg C.F. + 421 kg A.N./ha; and the economic optimum (21,7 t/ha) with 1418 kg C.F. + 381 kg A.N./ha.

Figure 1: Pictures of experimental plot

Table 2 : Harvests 2010-2015 and average

Treatments C.F. + A.N.	Kg coffee cherries/ha						
	2010	2011	2012	2013	2014	2015	X
500 + 134	19.200	21.274	15.438	15.423	7.383	17.428	16.024
750 + 203	19.426	25.259	18.397	19.840	6.126	22.755	18.634
1000 + 269	19.742	26.674	21.021	21.509	5.589	25.245	19.963
1250 + 337	20.958	28.581	21.870	23.698	4.348	27.403	21.143
1500 + 403	28.864	28.661	22.398	27.697	4.717	26.375	21.619
Effect	ns	Q	Q	L	ns	Q	Q

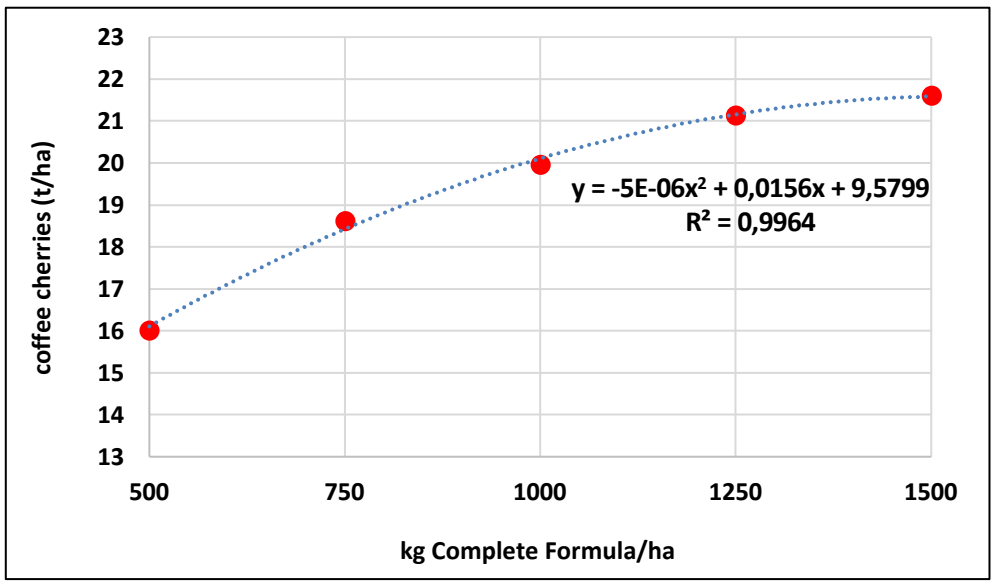

Figure 2: Yield response to fertilization. Average 6 crops (2010-2015)

Conclusion

The high productivity of F1 hybrids, must be accompanied by proper fertilization that allows them maximize their genetic potential.

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EFFECT OF *Meloidogyne exigua* AND SOIL TYPES ON THE DEVELOPMENT OF COFFEE PLANTS

PA 143

ROJAS, Mainor mrojas@icafe.cr
Centro de Investigaciones en Café, Instituto del Café de Costa Rica

Introduction

The nematode *Meloidogyne exigua* is distributed in 96% of the area of coffee grown in Costa Rica, where a recent study indicates that this species can cause losses around 1% per 20000 J_2/100 g root. The plant has a major influence on the dynamics of plant parasitic nematodes, but physical and chemical soil factors also affect the behavior of the pest. The aim of the study was to evaluate the effect of *M. exigua* on the development of coffee plants grown in pots with soil from different coffee regions of Costa Rica.

Materials and Methods

The trial was conducted in nursery, using shade 50% and under conditions of 1000 m. a. s. l., and annual average temperature of 23 °C. Polyethylene bags with pasteurized soil was used and two seedlings of red Catuaí variety were planted. Design randomized complete block was used, with five replications in factorial 6x2 (two Andisols, two Inceptisols and two Ultisols, with and without inoculation of *M. exigua*). The plants were inoculated a month after transplanting, applying 3.4 eggs+J_2 / cm^3 soil. The evaluation considered height, stem diameter, aerial fresh weight, galls index and density of *M. exigua* in the roots.

Table 1. Characteristics of the soils used in the assay.

Soil	pH	Acidity	K	Ca	Mg	P	Cu	Zn	Mn	Fe	Acidity saturation	O. M.	Sand	Clay	Silt
	H2O	cmol(+)/L				mg/L									
Heredia	5.5	0.43	1.94	6.08	0.95	17.8	20	5.4	77	97	4.57	11	46.70	31.48	21.83
Naranjo	6.1	0.23	1.38	7.89	1.37	13.2	19	8.1	73	62	2.12	12	41.48	39.05	19.48
Turrialba	5.9	0.29	0.68	7.51	0.85	25.7	17	2.6	51	95	3.11	12	33.83	54.05	12.13
León Cortés	5.0	1.35	1.04	2.03	0.99	39.4	6	4.9	56	491	24.95	10	21.25	66.55	12.20
Pérez Zeledón	5.1	0.86	0.13	0.50	0.16	3.2	4	0.7	9	150	52.12	13	29.13	58.98	11.90
Coto Brus	5.9	0.20	0.63	5.80	1.36	11.7	12	6.5	36	66	2.50	15	68.83	14.05	17.13

Results

Effect of soil type, nematode and interaction between them was obtained. Soils with unfavorable chemical characteristics hinder the development of plants. The highest rate of reproduction of the nematode was obtained in sand soil and the lowest in one of the clay soils, but with good chemical features (Figure 1). Aerial weight on the best soils fell more than half in infested plants, while the others was reduced 15% on average (Figure 2).

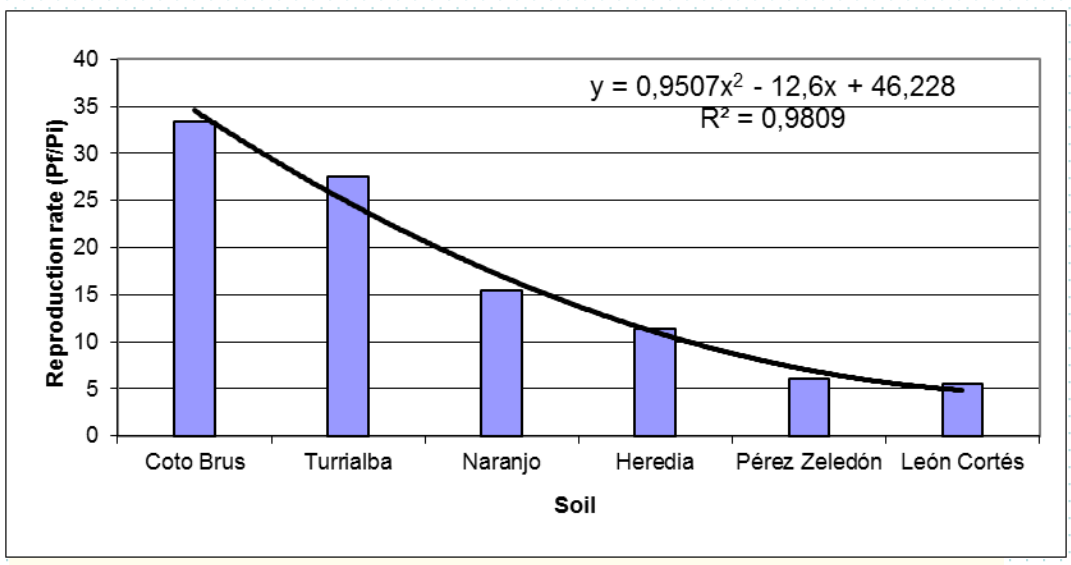

Figure 1. Reproduction rate according to soil type.

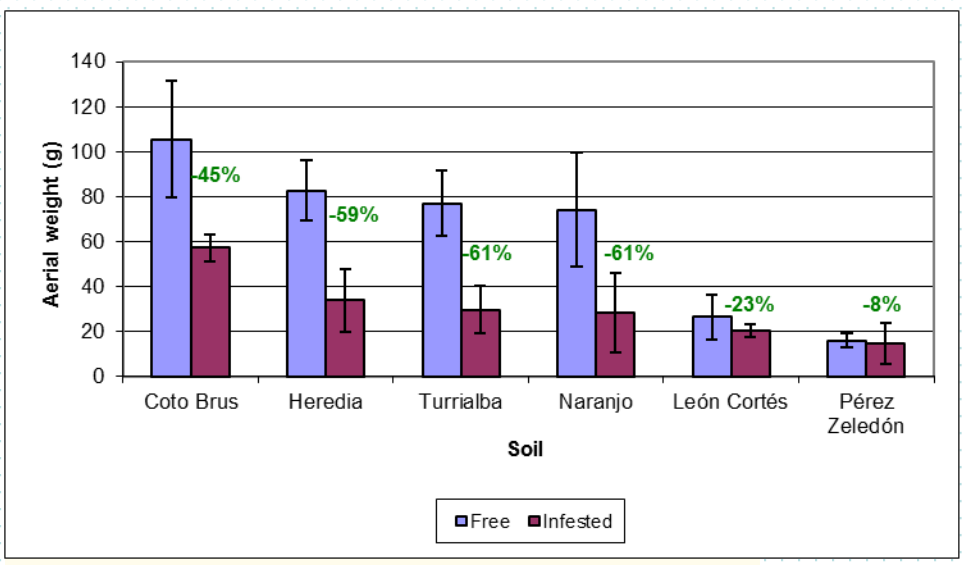

Figure 2. Effect of soil and *M. exigua*.

Conclusion

The nematode strongly affected the development of plants grown in soils with good chemical characteristics. The reproduction rate was also higher in the most fertile soils. The plantation management should be different according to the coffee region.

Assessing the Livelihood Outcomes of Value Chain Interventions in the Indonesian Specialty Coffee Sector: The Case of Kintamani, Bali

D.F.S. HARTATRI[1], M. VICOL[2], J. NEILSON[2]
[1] The Indonesian Coffee and Cocoa Research Institute, Jember, Indonesia
[2] The University of Sydney, Australia

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Abstract
Small coffee producers in Indonesia face myriad difficulties in accessing new market opportunities. These difficulties are mainly attributed to farmers' limited access to knowledge and information. Furthermore, the low capability of Indonesian farmers to produce high quality coffee also limits their access to the global specialty coffee market. Given that more than 90% of coffee production in Indonesia is by small farmers, coffee industry stakeholders have implemented value chain interventions (VCIs) with the dual aims to upgrade the capabilities of farmers to supply specialty coffee value chains and reduce rural poverty. In 2001, the Government of Indonesia (GoI) commenced collaborative work with the Indonesian Coffee and Cocoa Research Institute (ICCRI) to design and initiate VCIs through a farm-level industrialization program. Through this program, the GoI and ICCRI encouraged farmer groups (UPHs) to produce higher quality coffee by supplying local mechanized processing units and facilitating closer relationships between farmers and coffee buying/roasting firms.
This paper presents a case study of a VCI in the specialty coffee value chain in Kintamani, Bali. The results of the case study show that the implementation of the VCI faced several challenges, including the low institutional capabilities of the UPHs; the failure of the VCI to develop appropriate risk minimization tools for UPHs, resulting in exposure to unsustainably high risks associated with fluctuations in the global coffee price and production risks; and the changing role of coffee production in household livelihood strategies. Further, the paper argues that because of the way the VCI 'coupled' with local institutional structures, it is highly likely that the benefits of farm-level industrialization will only accrue to leaders of the UPHs, and this has exacerbated social concerns amongst members. These outcomes suggest that without attention to locally specific institutional and livelihood contexts, VCIs in the Indonesian specialty coffee sector may lead to sub-optimal outcomes for rural development and the long-term security of specialty coffee supply.

Introduction

Since 1989, the coffee industry has been globalized. This has increased smallholder farmers' opportunity to access to the global market and develop their livelihood and income. However, in developing countries, including Indonesia there have also been several challenges for smallholder farmers to obtain to access new market opportunities. These difficulties are mainly attributed to farmers' limited access to knowledge and information. In order to address these challenges, several actors have initiated various value chain interventions (VCIs).
The main aims of the VCIs conducted by stakeholders are to upgrade the capabilities of farmers to supply specialty coffee value chains and reduce rural poverty. In 2001, the Government of Indonesia (GoI) commenced collaborative work with the Indonesian Coffee and Cocoa Research Institute (ICCRI) to design and initiate VCIs through a farm-level industrialization program. Through this program, the GoI and ICCRI encouraged farmer groups (*Unit Pengolahan Hasil*, UPHs) to produce higher quality and quantity of coffee by supplying local mechanized processing units and facilitating closer relationships between farmers and coffee buying/roasting firms. Thus, farmers can obtain higher farm-gate price. The aim of this study is to assess a case study of a VCI in the specialty coffee value chain in Kintamani, Bali.

Methodology

This study applied a case study approach by incorporated both quantitative and qualitative research methods to explore and analyze the data, including a household impact assessment survey. Other methods included surveys of coffee farmer organisations, in-depth semi-structured interviews with key informants, such as farmers, ICCRI, the government, traders, exporters, exporters associations, and participant observation in coffee-growing communities.
The survey was conducted in Belantih and Ulian villages, Kintamani in 2013. Fifty coffee farmers who are involved in the VCIs from each village were selected as target group. Meanwhile, the control group of farmers consisted of households that not involved in the VCIs. Fifty coffee farmers from each village were identified as this control group for comparative purposes. Thus, the number of household surveys undertaken totaled 200 household farmers.

Table 1. Farm-gate price of green bean coffee in Kintamani, 2012

Indicators	Farmers who not involved in VCIs (IDR/kg green bean equivalent)	Farmers who involved in VCIs (IDR/kg green bean equivalent)	Statistical significance
Lowest price received	16,740	21,600	-
Highest price received	47,058	52,941	-
Median price received	31,176	32,400	-
Mean price received	30,755	35,283	Significant

The Challenges of Coffee Farmer Organizations

There are several challenges faced by the UPHs in conducting the VCIs. Many of the UPHs were essentially business units managed by the UPHs' leaders and the UPHs leaders' families. The system applied by most of the UPHs, where UPHs purchase red cherries from farmers has forced the leaders to act as coffee collectors/traders. In addition, none of the UPHs shared the coffee processing activities' profits with the members of UPHs. Furthermore, the big amount of money needed by UPHs to conduct upgrading activities. However, most of the UPHs had limited working capital and limited access to credit,. This has led the leader of the UPHs had to utilize their own assets as collateral when accessing bank loans, which had relatively high bank interest rates. Therefore, the leaders of the UPHs were in effect conducting a high risk business, which these risks were not shared by other UPHs' members. On the other hand, when the UPHs earned high income, it is highly likely that only the UPHs' leaders and their families enjoyed the income of the VCIs. Therefore, the VCIs has encouraged farmers to become involved in small scale business that have relatively high risk. Because of this high risk business, during the implementation of VCIs in Kintamani, several UPHs collapsed. There are six key reasons of the UPHS' failures, they are:

No.	Six reasons the failures of UPHs
1.	The leaders of the UPHS had low managerial, entrepreneurial, and marketing skills. These skills played a crucial role in managing the businesses.
2.	The farmer organizations had limited access to financial services, particularly credit, a situation exacerbated by the slow turnover because of the long time required by UPHs for producing fully washed coffee.
3.	The high level of competition between UPHs and the private sector forced farmer organizations into difficulties. For instance, in 2012, several UPHs offered a higher price in order to purchase larger volumes of red coffee cherries in an environment of high competition with other UPHs and local traders.
4.	UPHs' high coffee processing costs and low efficiency level, fluctuations coffee price in the global market and deliberate return resulted in financial losses.
5.	It seems that the advantages of the VCIs will only accrue to UPHs' leaders, and this has exacerbated social concerns amongst members.
6.	(6) the UPHs did not expand proper risk minimization systems and were exposed to high risks in related with quality, price certainty and price fluctuations.

Conclusion

Farm-level industrialization program was established in answer to numerous challenges in the international environment. As business units, UPHs are hoped to bring several benefits to farmers. The VCIs provide several benefits for farmers; e.g., better opportunity for accessing knowledge and credit, buyer certainty, and higher farm-gate prices. A significant aspect in VCIs involved the development of direct market links with specialty coffee buyers. This study offers insights on the potential of smallholders to improve their livelihood through links to the VCIs and higher value coffee markets. However, during the implementation of the VCIs, farmer organizations continue to face myriads challenges. In order to address these challenges, it emerges that it is at the present timely to reconsider this approach, and it's underpinning assumptions about rural development, at least as it is presently being promoted by the GoI and other related actors. Supporting the private sector through the implementation of the VCIs program may increase the impact of farm-level industrialization, given the high competences of the private sector to manage both quality control and business. Also, focusing on both on-farm and off-farm aspects at the same time might help address the livelihood of Indonesian coffee farmers.

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QUALITY IN VARIETIES OF ORGANIC COFFEE IN THREE REGIONS OF MEXICO

PA 149

ESCAMILLA, Esteban*, RUIZ, Octavio, ZAMARRIPA, Alfredo***, GONZÁLEZ Víctor A.****
Universidad Autónoma Chapingo, CRUO, Huatusco, Veracruz, México, *
Colegio de Postgraduados, México, ***INIFAP, México.

Abstract

Amongst the important options to help coffee growers face the constant crisis are both the organic production system and quality improvement. In this work, sensorial and physical qualities of 17 coffee varieties grown under organic conditions since 1998 were evaluated in experimental areas in the states of Veracruz, Oaxaca and Puebla. From August 2004 to March 2005, agroecological variables were collected, and ripe fruits were harvested and treated through a wet process. As per physical evaluation, a specialist board evaluated green coffee and carried out sensorial analysis of the beverage. It is concluded that varietal factors influence the physical and sensorial characteristics of the coffee grown under organic management.

Introduction

More than 200 years after its introduction and integration as an agroindustrial crop in Mexico (Pérez y Díaz, 2000), coffee is one of the products of major economic, social, cultural and environmental importance in the national agricultural field (UACh, 2005). Coffee quality is determined by several factors: environmental, genetic, agronomic and agroindustrial (Wintgens, 2004), but it is also a controversial issue (Anzueto et al., 1998). According to Santoyo et al. (1996), genetic factors determine the size, grain shape and color, chemical composition and organoleptic properties of the infusion. Although not formally proven, many coffee buyers indicate that the original varieties such as Typical and Bourbon outperform other Arabica both cup quality, and physical appearance of the grain (Bertrand et al., 1999). Studies on coffee quality have relied on conventional cupping, since research incorporating sensory analysis by tasting panels are still new (Perez et al., 2005). Therefore, the objective of this study was to determine the physical and sensory quality of 17 varieties of coffee grown under organic management, established at the states of Veracruz, Oaxaca and Puebla.

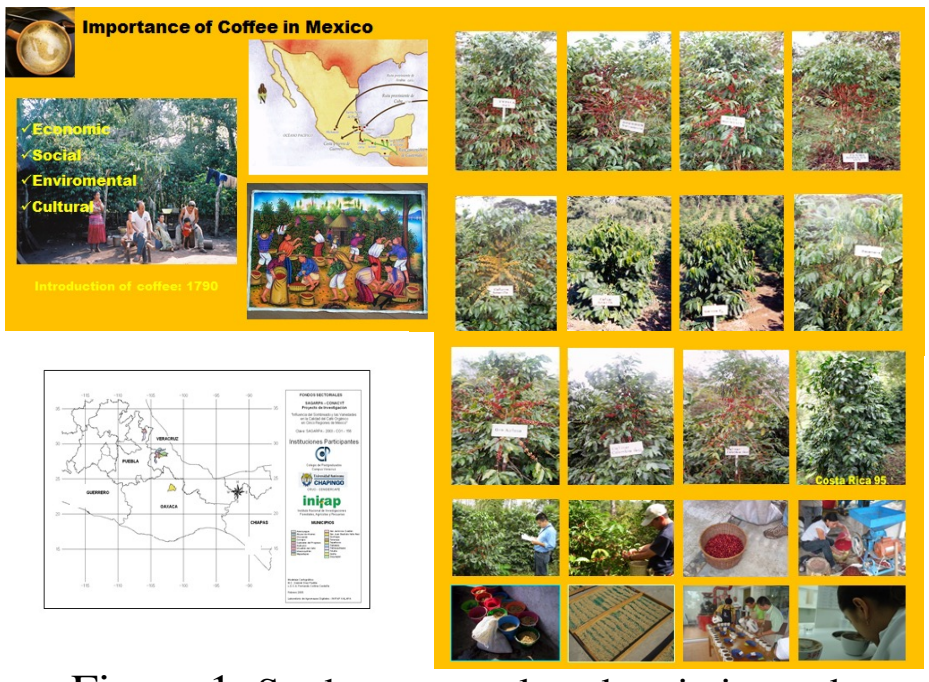

Figure 1. Study area, analyzed varieties and methodology

Variety	Weight Fruit (g)	Performance Cherry Parchment (kg)§	Performance Parchment Green Coffee (kg)#	Bean Borer Fruit (%)	Empty Fruit (%)	Normal Grain Planchuela (%)	Snail Grain (%)	Triangle Grain (%)	Abnormal Grain (%)	Grain Z19¥	Grain > Z16¥	Grain Color¶
Pluma Hidalgo	1.79 bcd†	250.2 a	55.12 a	1.7 a	3.3 a	83.5 a	10.6 a	3.4 abc	16.5 a	22.7 cdef	89.3 ab	F-VF
Típica Xhantocarpa	1.68 cde	242.3 a	55.12 a	2.2 a	2.5 a	84.0 a	10.7 a	3.2 abc	15.9 a	17.6 cdef	90.4 ab	F-VF
Colombia BC	1.84 abc	273.5 a	55.39 a	0.3 a	3.6 a	81.4 a	10.0 a	5.8 abc	18.5 a	36.3 abcd	91.9 ab	VF
Colombia BV	2.0 ab	271.2 a	55.66 a	0.0 a	3.8 a	81.7 a	12.7 a	2.3 ab	18.2 a	30.3 abcde	92.4 ab	F-VF
Blue Mountain	1.63 cde	238.2 a	55.67 a	0.9 a	5.3 a	88.2 a	8.3 a	1.7 a	11.8 a	19.4 cdef	90.9 ab	F-VF
Oro Azteca	1.71 cde	263.7 a	55.82 a	0.4 a	6.4 a	78.3 a	13.8 a	3.9 abc	21.7 a	20.6 cdef	87.4 ab	F-VF
Batie	1.55 de	258.4 a	55.84 a	0.4 a	20.7 a	84.6 a	12.5 a	1.7 a	15.4 a	41.0 abc	92.7 ab	F-VF
Dessie	1.90 abc	267.5 a	55.88 a	0.6 a	2.3 a	81.7 a	14.6 a	1.3 a	18.2 a	46.6 ab	93.6 ab	F-VF
Catuai Amarillo	1.76 bcd	256.5 a	55.99 a	0.2 a	2.6 a	86.7 a	6.5 a	4.3 abc	13.2 a	13.9 def	91.4 ab	F-VF
Costa Rica 95	1.79 bcd	287.5 a	56.25 a	0.0 a	2.9 a	78.5 a	12.5 a	3.4 abc	21.5 a	24.6 bcdef	88.2 ab	F-VF
Caturra Rojo	1.63 cde	253.3 a	56.33 a	0.4 a	2.3 a	85.8 a	7.4 a	4.6 abc	14.2 a	12.2 ef	89.3 ab	F-VF
Típica 947	1.62 cde	252.5 a	56.48 a	1.0 a	4.3 a	78.5 a	10.0 a	6.6 bc	21.5 a	16.3 def	86.8 ab	F-VF
Borbón Salvadoreño	1.46 e	268.9 a	56.51 a	1.0 a	2.5 a	81.0 a	9.9 a	6.8 bc	18.9 a	3.9 f	77.4 c	F-VF
Garnica Iquimite	1.67 cde	266.9 a	56.53 a	0.2 a	2.3 a	80.1 a	8.3 a	7.2 c	19.8 a	10.8 ef	85.2 bc	VF
Caturra Amarillo	1.68 cde	266.4 a	56.58 a	0.2 a	3.0 a	85.7 a	6.4 a	5.5 abc	14.2 a	10.3 ef	89.1 ab	F-VF
Garnica F5	1.75 bcd	251.0 a	56.58 a	0.4 a	2.4 a	81.0 a	10.2 a	4.6 abc	18.9 a	14.0 def	88.2 ab	F-VF
Pacamara	2.1 a	255.3 a	56.63 a	0.3 a	5.7 a	81.2 a	6.7 a	5.7 abc	18.7 a	52.8 a	95.7 a	F-VF

† Values with the same letter in a column are not statistically different (Tukey, 0.05).
§ Number of coffee cherries(kg) required to obtain 57.5 kg of parchment coffee. The comercial standard is 250 kg of coffee cherries.
# Number of parchment coffee (kg) required to obtain 46 kg of green coffee. The comercial standard is 57.5 kg of parchment coffee.
¥ Grain size measured in screens with holes diameter: z 19 (1.18 mm) y z > 16 (> 1.0 mm), based on the NMX-F-551-1996 (Secretaría de Comercio y Fomento Industrial, 1996).
¶ Grain color (Green coffee), con base en la NMX-F-551-1996 (Secretaría de Comercio y Fomento Industrial, 1996): F = Fine; VF = Very Fine.

Table 1 :Physic quality of the organic coffee bean in 17 varieties.

Variety	Aroma intensity	Acidity intensity	Body intensity	Astringency cups	Wine cups	Loose cups	Herbal cups	Defective cups
Pluma Hidalgo	3.46 a†	3.43 a	3.00 a	0.0 a	0.0 a	0.0 a	1.0 a	2.6 ab
Típica Xhantocarpa	3.50 a	4.15 a	3.00 a	0.0 a	0.0 a	1.5 b	0.0 a	3.0 ab
Colombia BC	3.56 a	3.63 a	3.03 a	4.0 a	1.0 a	0.0 a	0.0 a	6.0 ab
Colombia BV	3.50 a	3.13 a	3.13 a	2.6 a	0.0 a	0.0 a	1.0 a	5.3 ab
Blue Mountain	3.53 a	3.56 a	3.06 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
Oro Azteca	3.43 a	3.43 a	3.03 a	0.6 a	0.0 a	0.0 a	0.0 a	1.0 ab
Batie	3.36 a	3.50 a	3.06 a	1.0 a	0.0 a	0.0 a	2.6 a	3.6 ab
Dessie	3.36 a	3.23 a	3.03 a	1.6 a	0.0 a	0.0 a	0.0 a	3.3 ab
Catuaí Amarillo	3.43 a	3.40 a	3.13 a	0.6 a	0.0 a	0.0 a	1.0 a	3.0 ab
Costa Rica 95	3.50 a	3.25 a	3.15 a	2.5 a	0.0 a	0.0 a	1.0 a	6.5 b
Caturra Rojo	3.40 a	3.50 a	3.06 a	2.0 a	0.0 a	0.0 a	0.6 a	3.3 ab
Típica 947	3.46 a	3.43 a	3.03 a	0.0 a	0.0 a	0.0 a	0.0 a	1.0 ab
Borbón Salvadoreño	3.46 a	3.73 a	3.03 a	0.0 a	1.0 a	0.0 a	0.0 a	1.0 ab
Garnica Iquimite	3.50 a	3.66 a	3.13 a	0.0 a	0.0 a	0.0 a	0.0 a	0.6 ab
Caturra Amarillo	3.46 a	3.26 a	3.10 a	0.0 a	0.0 a	0.0 a	0.0 a	1.3 ab
Garnica F5	3.40 a	3.53 a	3.03 a	0.6 a	0.0 a	0.0 a	0.6 a	2.3 ab
Pacamara	3.5 a	3.30 a	3.16 a	0.6 a	1.6 a	0.0 a	0.0 a	3.0 ab

† Values with the same letter in a column are not statistically different(Tukey, 0.05)

Table 2 :Sensorial quality of the organic coffee bean in 17 varieties.

Main Text

Sensory and physical quality were evaluated to 17 coffee varieties grown under organic conditions since 1998 in experimental areas of the states of Veracruz, Oaxaca and Puebla. From august 2004 to march 2005, agroecological variables were evaluated, harvesting ripe fruits and processing by the humid path. Physical evaluation of green coffee and the sensorial analysis of the beverage was done by a specialist board. Blue Mountain variety had the better agroindustrial yields, better grains shape and size and less unwanted cups. Resistant varieties to *Hemileia vastatrix* such as Costa Rica and Colombia yielded more unwanted cups, specially astringents. Sensorial attributes, such as: fragance, aroma, nose and after taste are given for all varieties in this research. It is concluded that varietals' factor influences the physical and sensorial characteristics of coffee grown under organic management.

Conclusion

There are differences in the physical and sensorial quality of the 17 varieties tested, all grown with the organic system. Blue Mountain variety, selection derived from Typical, stands out for its agroindustrial yields, the shape and size of the grains and the absence of defects in cup tests. The Costa Rica 95 variety, recommended for its resistance to rust and high productivity, the highest number of cups with defects, especially astringency. For each variety preliminary characterization of its fragrance, aroma, nose, aftertaste and sensory attributes is provided.

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PA152

MULTIVARIATE ANALYSIS FROM ECOPHYSIOLOGICAL RESPONSES OF COFFEE PLANTS INTERCROPPED WITH WOODY SPECIES AND UNDER WATER DEFICIT

Silva, Vânia A. *, Santos, Meline O.*, Silveira, Helbert R. O*, Dantas, Mayara F. *, Volpato, Margarete M. L. *, Venturin, Regis P.*, Ferreira, Ester A.*

* EPAMIG SUL, Lavras, MG, Brasil.

ABSTRACT

The intercropping of coffee plants with woody species can change its ecophysiological interactions, causing impacts on water relations. These impacts may cause positive or negative physiological changes depending, among other factors, on climatic conditions. This study aimed to use multivariate analysis as a technique to characterize ecophysiological responses of coffee intercropped with woody species under water deficit in south region of Minas Gerais. The 'Catuaí Vermelho 99' was planted in monoculture and intercropped with *Khaya ivorensis*, *Tectona grandis* e *Acrocarpus fraxinifolius* in two plant spacings (9 x 13.6 m and 18 x 13.6 m) in the line of trees. The coffee assessments were performed in the second and third year after planting. Evaluations of spectral index, gas exchange, fluorescence and water potential were performed in August of 2014 and 2015. The physiological variables were analyzed by canonical variables analysis by R program. In August 2014 and 2015 higher water potential values (Ψpd) were found in coffee trees in monocultures and intercropped with *K. ivorensis*. In August 2014, the variables with highest positive score in first canonical were carotenoid reflectance index (CRI1) and structure-insensitive pigment index (SIPI) and negative score were Ψpd, plant senescence reflectance index (PRSI), electron transport rate (ETR) and effective quantum yield of photosystem II (PSII). Coffee plants intercropped with *T. grandis* in closer spacing showed high values of CRI1 and SIPI indexes with low Ψpd values, PRSI, ETR and PSII, while opposing responses were found in coffee plants intercropped with *K.* ivorensis. The variables most positive score in the second canonical were Ψpd, water band index (WBI) and anthocyanin reflectance index (ARI1) and negative scores the non-photochemical quenching (qN). Monoculture were discriminated for presenting positive scores with high Ψpd , WBI and ARI1 values with low qN values, while intercropping coffee with *A. fraxinifolius* had negative scores, with high qN values and low values of WBI, Ψpd and ARI1. In August 2015, trees reached Ψpd lower than in previous year. Based on CAN1, it was observed that Ψpd showed high positive score, while it was negatively correlated with PSII, ETR, flavonol reflectance index (FRI) and PRSI. It was observed that coffee plants under monoculture, intercropped with *K. ivorensis* and intercropped with *T. grandis* presented higher values for Ψpd, with lower values of PSII, ETR, FRI and PRSI. Moreover, coffee trees intercropped with the *T. grandis* in close spacing and *A. fraxinifolius* showed higher PSII, ETR, FRI and PRSI and lower Ψpd. Tree species influences the water potential of coffee plants in water deficit stage. The canonical variables allowed discriminating coffee monoculture, intercropped with *K. ivorensis* and *T. grandis* in wide spacing of coffee intercropped with *T. grandis* in close spacing and *A. fraxinifolius*, indicating a negative effect of intercropping with *T. grandis* in close spacing and *A. fraxinifolius* on water potential and also coffee photochemical efficiency.

INTRODUCTION

The intercropping of coffee plants with woody species can change its ecophysiological interactions, causing impacts on water relations. These impacts may cause positive or negative physiological changes depending, among other factors, on climatic conditions. This study aimed to use multivariate analysis as a technique to characterize ecophysiological responses of coffee intercropped with woody species under water deficit in south region of Minas Gerais.

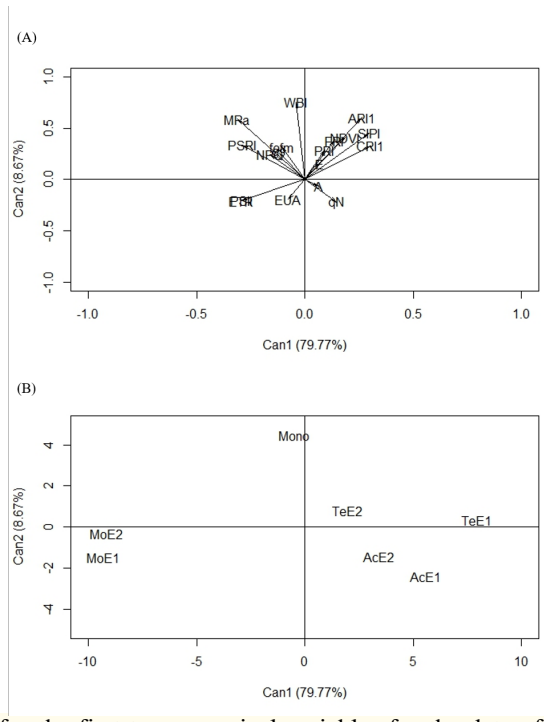

Figure 1: Biplot for the first two canonical variables for the data of the seven treatments on the basis of physiological parameters evaluated in August 2014 (A) The correlation coefficients for all parameters were represented by vectors. (B) Segregation of seven treatments studied based on physiological parameters evaluated. MoE1= *K. ivorensis* (9 X 13.6 m); MoE2= *K. ivorensis* (18 X 13.6 m); TeE1= *T. grandis* (9 X 13.6 m); TeE2= *T. grandis* (18 X 13.6 m); AcE1= *A. fraxinifolius* (9 X 13.6 m); AcE2= *A. fraxinifolius* (18 X 13.6 m), Mono= monocultivo.

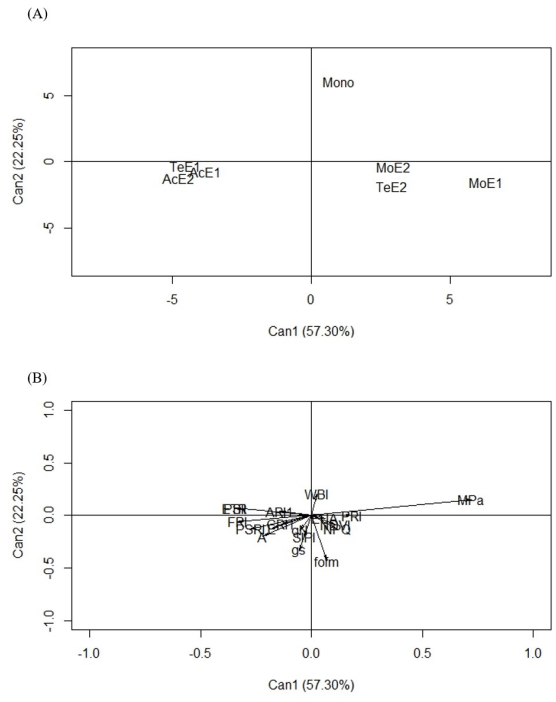

Figure 2: Biplot for the first two canonical variables for the data of the seven treatments on the basis of physiological parameters evaluated in August 2015 (A) The correlation coefficients for all parameters were represented by vectors. (B) Segregation of seven treatments studied based on physiological parameters evaluated. MoE1= *K. ivorensis* (9 X 13.6 m); MoE2= *K. ivorensis* (18 X 13.6 m); TeE1= *T. grandis* (9 X 13.6 m); TeE2= *T. grandis* (18 X 13.6 m); AcE1= *A. fraxinifolius* (9 X 13.6 m); AcE2= *A. fraxinifolius* (18 X 13.6 m), Mono= monocultivo.

MAIN TEXT

In August 2014, the variables with highest positive score in first canonical were CRI1, SIPI and negative score were Ψpd, PRSI, ETR and PSII. Coffee plants intercropped with *T. grandis* in closer spacing showed high values of CRI1 and SIPI indexes with low Ψpd values, PRSI, ETR and PSII, while opposing responses were found in coffee plants intercropped with *K.* ivorensis. In August 2015, based on CAN1, it was observed that Ψpd showed high positive score, while it was negatively correlated with PSII, ETR, FRI and PRSI. It was observed that coffee plants under monoculture, intercropped with *K. ivorensis* and intercropped with *T. grandis* presented higher values for Ψpd, with lower values of PSII, ETR, FRI and PRSI. Moreover, coffee trees intercropped with the *T. grandis* in close spacing and *A. fraxinifolius* showed higher PSII, ETR, FRI and PRSI and lower Ψpd.

CONCLUSION

The canonical variables allowed discriminating coffee monoculture, intercropped with *K. ivorensis* and *T. grandis* in wide spacing of coffee intercropped with *T. grandis* in close spacing and *A. fraxinifolius*, indicating a negative effect of intercropping with *T. grandis* in close spacing and *A. fraxinifolius* on water potential and also coffee photochemical efficiency.

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Sharing Advance in Coffee Science & Technology

MANAGEMENT INDICATORS OF BRAZILIAN COFFEE BUSINESS: A MULTIVARIATE ANALYSIS

PA 153

BLISKA, Flavia*; BLISKA JR., Antonio**; FIRETTI, Ricardo***; TURCO, Patricia***; LEAL, Paulo**.
* Agronomic Institute of Campinas, **University of Campinas, ***APTA Regional, Campinas. Brazil.

Abstract

The aim of this study was to gather information addressed to the rural business manager, to help him improving business, turning it into an organized and profitable organization.

We use the **Identification Method of Management Degree** (MIGG Coffee):

- It uses a quick and easy to apply questionnaire in order to classify management activities into different organizational levels.
- allows comparisons among companies, production processes, technological levels and regions.
- assists in evaluating competitiveness of local arrangements for sustainable regional development.
- allows pointing out strengths and weaknesses and indicates corrective actions.

We evaluate de management level of 920 coffee farms, from 2013 to 2016.

The results of this study highlight the main strengths and weaknesses of the management of coffee farms in the different Brazilian regions.

Furthermore, they can assist in the preparation of sectoral public policies to sectoral sustainability.

Introduction

- The contribution of the coffee farming is historically relevant to the Brazilian economy, due to the generation of product and income, and absorption of the labor force.
- Recently, the use of new technologies has contributed to the significant increase in productivity.
- Also to coffee growing consolidation, in regions with different soil and weather conditions, that resulted in a heterogeneous coffee economy, adapted to regional characteristics.
- However the producer or rural entrepreneur still dispenses much of his time and energy on technical issues and routine tasks, relegating to the background the administrative aspects of the business.
- Therefore, in coffee companies the creation of internal management mechanisms is very important, as much as in other economic sectors.

Methodology

Identification Method of Management Degree - MIGG Coffee

- MIGG Coffee is based on the identification of the level of management of a coffee farm, according to scores obtained by applying a questionnaire.
- This questionnaire evaluates 64 indicators relating to eight criteria: Planning, Leadership, Customers, Society, Information and Knowledge, People, Processes, Results.
- Each test provides the assessment with a sum of points, which ranges from zero to 1000.
- This score ranks the degree of management levels from one (the lowest) to nine (the highest) (Bliska jr. 2010; Bliska Jr. et al 2014).
- **Multivariate analysis by two-way joining method.**

Number of States, municipalities, micro and meso Brazilian geographic regions analyzed

Brazilian States	Municipalities	Microregions	Mesoregions	Organizations (farms)
07	215	74	32	920

Conclusion

- It was observed that in organizations with lower levels of management, administrative decisions are not based on methods that allow systematic reproduction of production processes.
- In general, technical, administrative or financial data are not registered systematically. When they are used only to meet legal requirements and can not extract such data information for planning and future decision making in order to improve production processes.
- In organizations where were identified high levels of management, management practices were integrated into modern farming practices, regardless of company size, number of employees or Brazilian region.
- The results support the view that, despite the technical expertise in farming, business management in Brazilian coffee production is still primitive and intuitive.

Different regions: diversity of management systems

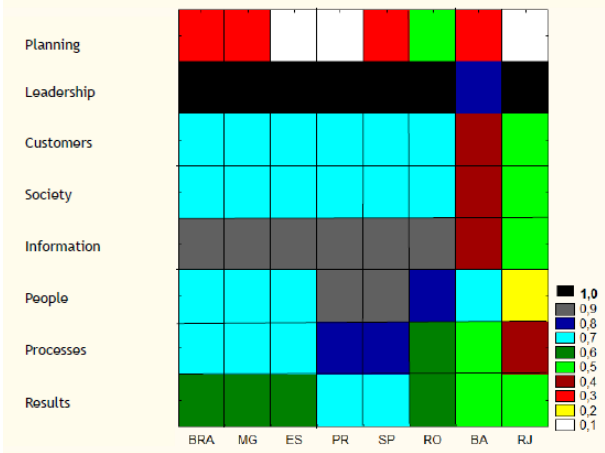
Results

Descriptive statistics: average management level in Brazilian coffee production

Dispersion of the management level, in coffee production	Brazil	Major Brazilian coffee growers states				
		Minas Gerais	Espirito Santo	São Paulo	Bahia	Parana
Average management	6,29	6,45	6,02	6,36	5,59	6,22
Median	6,00	7,00	6,00	6,00	5,00	6,00
Variance	3,18	2,95	3,36	3,63	3,69	3,23
Standard deviation	1,78	1,72	1,83	1,91	1,92	1,80
Coefficient of variation (%)	28,35	26,61	30,46	29,93	34,34	28,91
Number of companies	920	398	173	74	91	169
Best management performance: State of Minas Gerais			Worst management performance: State of Bahia			

Multivariate analysis by two-way joining method

% regarding the relative importance of each criterion in a particular region
We used the relativized average: the mean value of each criterion was divided by its maximum possible score, so all eight values assumed results between 0 and 1.



- Brazil as a whole (BRA)
- States: Minas Gerais (MG), Espirito Santo (ES), Paraná (PR), Sao Paulo (SP), Bahia (BA), Rio de Janeiro (RJ) and Rondonia (RO)

- **States of MG, ES, PR, SP, RO: relative importance of management criteria have similar behavior.**
- **Leadership: criteria with the highest relative importance in all regions.**
- **Planning and strategies: criteria with the lowest relative importance in all regions.**

Percentage of management indicators attended positively 920 coffee farms, Brazil, 2013-2016

	Management criterion _ Indicator	%
Main strengths	[39] Processes _Employees /responsible for production have knowledge to carry out the harvest at the right time.	95,5
	[5] Leadership_Authority is exercised with fairness and respect, without embarrassment and bullying.	95,3
	[40] Processes_The harvest procedure is done in order to avoid contact with the ground grain.	92,4
	[9] Leadership_The administration takes lead of main actions/delegate duties and responsibilities, including OSH.	89,8
	[44] Processes _The coffee storage in or benefit is made in an appropriate place.	89,4
	[17] Society_The organization properly dispose of water, processing of waste and empty agrochemical packaging.	88,3
	[22] Information_The organization seeks information from public/private services to improve production processes.	87,9
	[37] Processes - In the nutritional control of crops the organization makes regular use of chemical analysis and apply the recommendations of laboratories and/or professionals.	87,0
	[64] Results_Corrective actions made on time by suppliers results in better relationships among farms and them.	85,9
	[24] Information_The organization regularly attend trade fairs, congresses, field days, visits to other productions.	85,8
Main weaknesses	[35] Processes_The organization uses/tests varieties looking for resistance to pests/diseases and new market s.	47,0
	[60] Results_The organization assesses the continuous improvement of productivity per person periodically.	44,1
	[13] Customers_The organization maintains registers or database with the history of relationship with clients.	42,2
	[27] Information_The organization has registers and protocol procedures of technologies, production methods and processes and seeks to identify, develop and incorporate innovations to their products and services.	41,9
	[21] Society_The organization has Code of Conduct and promote its implementation throughout the chain.	39,0
	[62] Results_The operational efficiency of the production system is evaluated in terms of fuel consumption, energy, water or fertilizers per unit produced.	37,4
	[38] Processes_The organization uses electrical conductivity meters, pH and soil moisture.	33,0
	[2] Planning_The organization has documented clear definition of their reason for existence (Mission), plan to defined future (Vision) and on the organizational principles to guide the actions of employees on a daily basis.	29,1
	[1] Planning_The organization has a business plan.	13,7
	[29] People_The organization offers health insurance to its employees.	12,9

Brazilian coffee regions

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OCCUPATIONAL SAFETY AND HEALTH OF RURAL WORKERS IN BRAZILIAN COFFEE PRODUCTION

PA 154

BLISKA, Flavia*; BLISKA JR., Antonio**; BARROS, Thaís***; FIRETTI, Ricardo****; TURCO, Patricia****; LEAL, Paulo**.
* Agronomic Institute of Campinas, **University of Campinas, ***Fundacentro, Campinas, ****APTA Regional, Campinas. Brazil.

Abstract

The social and economic impacts of Occupational Safety and Health (OSH) problems throughout the coffee production process are significant, mainly because the coffee sector employs significant amount of hand labor.

As a result, this work identify the weaknesses of coffee production regard Brazilin OSH standards, through 24 management indicators related to OSH.

We used the Identification Method of Management Degree – MIGG Coffee, through questionnaires applied to 920 farms in Brazilian coffee regions, from 2014 to 2016.

To analyze those indicators we used descriptive statistics and factor analysis.

We observed that there is still much to be improved in Brazil with respect the OSH, regardless of the type of coffee, state assessed, farm size or number of employees.

Introduction

Agriculture: one of the most dangerous sectors to workers

There are many physical, chemical, biological, ergonomic and accidents hazards

- Agriculture employs more than a third of the global workforce.
- In many countries, agriculture is the most important sector for female employment
- Agriculture also employs 70% of child labor worldwide.
- Brazil: there is great underreporting of information on occupational accidents.
- Brazilian Health System serves large number of accidents and diseases from informal market.
- 23440 work accidents in Brazilian agricultural: typical and path accidents, occupational diseases.
- 1.055 of them in coffee production (MPS, 2013).

Agricultural work accidents: very high costs for the country - US$ 22 billion per year (Pastore, 2011)

- **Direct and indirect costs of companies**: first aid, equipment destruction and materials, disruption of production, fines, workers' compensation, damage to company image
- **Costs of Brazilian Social Security** regarding payments of benefits and special retirement.
- **Cost of families** due to the injured and sick relatives (underestimated due to the informal labor market).

Methodology

- We analyzed the management indicators related to OSH in the coffee segment by the **Identification Method of Management Degree – MIGG Coffee (**Bliska Jr., 2010; Bliska et al, 2014).
- The MIGG Coffee is based on the identification of the level of management of a coffee farm (company), according to scores obtained by applying a questionnaire.
- This questionnaire evaluates 64 indicators relating to eight criteria: Planning, Leadership, Customers, Society, Information and Knowledge, People, Processes, Results.
- Each test provides the assessment with a sum of points, which ranges from zero to 1000.
- This score ranks the degree of management levels from one (the lowest) to nine (the highest)
- 24 are directly or indirectly related to OSH.

Conclusion

- Some indicators show entrepreneurial capacity and high commitment to production process.
- May have been largely weaken by poor capability for planning as well as for low index registers and protocols of technologies, methods and processes.
- Despite many positive points, there is still much to be improved in Brazil about the OSH, regardless type of coffee, state assessed, farm size or number of employees.
- Some important concepts for the development of rural entrepreneurs have not yet been fully incorporated into the coffee activity.

Strengths: water treatment

Results

Percentage of management indicators attended positively
920 coffee farms, Brazil, 2013-2016

	Indicator	%
Main strengths	2 - Authority is exercised with fairness and respect, without embarrassment and bullying.	95,3
	18 - The harvest procedure is done in order to avoid contact with the ground grain, respecting the OSH.	92,4
	4 - The administration takes lead of the main actions and delegate duties and responsibilities, including OSH.	89,8
	6 - The organization properly dispose of water, processing of waste and empty agrochemical packaging.	88,3
	9 - The organization seeks information to improve their production processes, including OSH.	87,9
	22 - Environmental regulations, legislation and environmental commitments are undertaken.	84,4
	7 - The organization always hires employees aged greater than that established in the legislation.	84,1
	3 - Objectives /results, including preservation of workers' physical integrity are frequently /clarity reported.	79,4
	5 - The organization uses biological control and/or rationally use agrochemicals, with prescription and monitoring of professional application by a legally qualified.	76,7
	19 - The newly harvested products are transported quickly and properly, respecting the OSH.	76,4
	21 - There is a routine procedure to keep clean and organized work environment.	76,2
	12 - Workers regularly use PPE correct, recommended by qualified professional, provided by the organization.	74,8
	16 - The working system allows better performance of employees/staff, including on the OSH.	73,8
	15 - The organization seeks to identify and develop leadership characteristics, including respect to OSH.	70,0
Main weaknesses	17 - The working system has contributed to improving the performance of employees and identify those with the ability to pursue and achieve new knowledge including respect to OSH.	65,7
	23 - The organization regularly evaluates well-being, satisfaction and motivation of employees , work activities and adequacy of living area, according Brazilian rules.	64,1
	20 - There are preventive maintenance for machinery and equipment with moving parts protection.	63,0
	14 - The organization provides opportunities and encourages the participation of employees in educational training programs and professional training, including OSH.	61,3
	10 - The organization uses internet for communication, dissemination and search information, including OSH.	57,1
	24 - The organization periodically evaluates the improvement of productivity respecting OSH.	44,1
	11 - There are records /protocol procedures of technologies, production methods, processes, including OSH.	41,9
	8 - The organization has Code of Conduct and encourages its use throughout the chain, including OSH.	39,0
	1 - There are steps, planning, defined goals, risk analysis, improvements in environment /working conditions.	33,0
	13 - The organization offers health insurance to its employees.	8,0

Weaknesses: absence of protection gloves, boots, leggings

Management indicators that must be respected in order to meet the Brazilian standards of Occupational Safety and Health
Principal components analysis (PCA)

- 6 factors: explain 57.2% of the sample variance
- Number of questionnaires evaluated – Brazil: 920
- Variable number initially analyzed: 24
- Number of variables excluded during the review process: 06
- First factor: explain 19,7% of sample variance
- Variables included have minimum correlation of 0.573 with a Factor
- Orthogonal Rotation: Varimax Raw
- Factors extraction method: eigenvalues (eigenvalues) above 1.0

Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
15 - Identification and development of leadership characteristics	11 - Systematic records of information and protocols of technologies, methods and processes	2 - The authority is fair and respect without embarrassment and bullying	7 - Employee recruitment according to age set out established by law	12 - Correct use of PPE 6 - Right treatment of processing water, waste and agrochemical packaging	23 - Regular assessment of well-being and employee satisfaction and respect for the law
16 - The working system allows better performance of employees/staff	24 - Continuous assessment of productivity	4 - Delegation assignments and responsibilities	22 - Commitment to environmental regulations	14 - Encouragement of employee participation in educational training	21 - Continuous process of cleaning and organization
17 - The working system improves the performance of employees, identifies abilities and proactivity	1 - Steps and planning setting goals, risk analysis, environment and working conditions improvements 08 - Code of Conduct applied throughout the chain	3 - Objectives and results, including workers' physical integrity, are clear and frequently reported	5 - Rational use of agrochemicals and/or biological control	 Strengths: waste disposal	
Respect for people	**Planning and organization**	**Respect for law and community**	**Leadership**	**Respect for persons and community**	**Working environment appropriate to the worker welfare**

Factors such as "OSH and human resources", “Management commitment to OSH", "Organizational Commitment to OSH", “Search for high-performance processes " and “Labor legislation" were observed in different Brazilian region.

Strengths: PPE in the application of agrochemicals and machinery operations

Weaknesses: electrical hazards (shock and fire)

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BLISKA JR., A. Identification Method of Management Degree (MIGG) in the cut flower production activities. Campinas, SP. 2010 (PhD Thesis).
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QUALITY OF BRAZILIAN COFFEE: CRITICAL ISSUES IN THE PROCESS AND PRODUCT MANAGEMENT AIMED AT SUSTAINABILITY

PA 155

BLISKA, Flavia*; BLISKA JR., Antonio; FIRETTI, Ricardo***; TURCO, Patricia***; LEAL, Paulo**.**

*** Agronomic Institute of Campinas, **University of Campinas, ***APTA Regional, Campinas. Brazil.**

Abstract

In this study we use the Identification Method of Management Degree (MIGG Coffee) to evaluate 30 quality management indicators, 2013-2016, through 920 questionnaires, based on descriptive statistics and principal component analysis.

Purpose

Identify weaknesses in the management of quality processes and products in the coffee agricultural sector, in the main Brazilian producing regions

Provide subsidies for corrective actions to:

- increase production efficiency
- increase the quality of coffee
- increase agricultural yields
- aggregate value
- **Consequently: increase the competitiveness and sustainability of Brazilian coffee sector**

Introduction

Worldwide demand for technical high quality coffee has grown continuously

International market has recognized, promoted and encouraged the quality of coffee

The competitiveness of the agricultural sector has taken place mainly through

- differentiation processes and products
- increase in the production efficiency
- cost reduction
- adoption of technologies for pre and post-harvest specific to regional conditions

Creation of internal management mechanisms is essential to increase the quality of coffee and the segment's competitiveness, particularly for micro and small farms, the basis of world production, from process improvement agricultural placing the product on the target market.

Despite the importance of coffee to the national economy and its significant technological development, administrative aspects of the business are in the background.

Methodology

Identification Method of Management Degree - MIGG Coffee

- MIGG Coffee is based on the identification of the level of management of a coffee farm, according to scores obtained by applying a questionnaire.
- This questionnaire evaluates 64 indicators relating to eight criteria: Planning, Leadership, Customers, Society, Information and Knowledge, People, Processes, Results.
- Each test provides the assessment with a sum of points, which ranges from zero to 1000.
- This score ranks the degree of management levels from one (the lowest) to nine (the highest) (Bliska Jr., 2010; Bliska Jr. et al 2014).
- 30 indicators are directly or indirectly related to coffee quality.
- 920 questionnaires (farms)
- Regions analyzed: Minas Gerais, Espirito Santo, Sao Paulo, Bahia, Parana, Rio de Janeiro, Rondonia

Conclusion

- There are many critical issues to be overcome in order to achieve the quality throughout the coffee beans production process.
- The low index registers denotes not only administrative deficiency, but probably also low capacity usage of systematic information for decision making.
- Despite the success of many competitive strategies, most of Brazilian coffee company has no information to support rational decisions in the quality process.
- Public policies must be adapted to regional needs, due to profile and the degree of management of farms and due to increase of competitiveness strategies
- Adding value and sustainability of the activity, training of human resources, training new leaders and family succession should be the agenda items of government entities, cooperatives and rural entrepreneurs associations.
- If the weakness are not clearly identified as such by the coffee industry, therefore persist difficulty in strike them appropriately.

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BLISKA JR., A. Identification Method of Management Degree (MIGG) in the cut flower production activities. Campinas, SP. 2010 (PhD Thesis).

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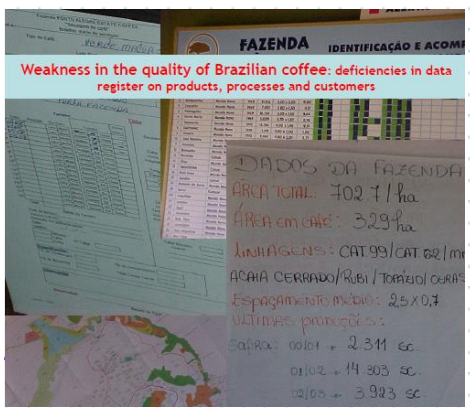
Strengths in the quality of Brazilian coffee: production process, harvest, post-harvest

Results

	Management indicators attended positively, 920 coffee farms, Brazil, 2014-2015	%
Main strengths	19 - Employees and responsible for the production have knowledge to carry out the harvest at the right time.	**95,5**
	20 - The harvest procedure is done in order to avoid contact with the ground grain.	92,4
	24 - The coffee storage in or benefit is made in an appropriate place.	89,4
	9 - The organization seeks information from the public and private services to improve their production processes.	87,9
	17 - In the nutritional control of crops the organization makes regular use of chemical analysis and apply the recommendations of laboratories and/or professionals.	87,0
	10 - The organization regularly attend trade fairs, congresses, "field days" and visits to other areas of production.	85,9
	2 - The administration seeks to inform all factors that influence organization's productive and commercial aspects.	83,0
	22- The organization has adequate post-harvest unit.	80,9
	11 - The organization maintains a close relationship with customers through regular contacts and visits.	78,9
	8 - The organization uses biocontrol and/or rational use of agrochemicals in their production processes.	76,7
Main weaknesses	26 - Are regularly made quality control and standard inspections in their products.	54,6
	29 - In the relationship with the market, solving problems and implementing corrective action is recorded internally by the organization and reported to client.	53,0
	30 - The percentage of grain harvested within the higher standard classes is evaluated regularly.	51,1
	27 - There is planning aimed at quality control and reduction of conferences and inspections.	47,7
	28 - Satisfying of your direct customers is monitored or measured somehow.	47,2
	15 - The organization uses and tests varieties looking for resistance to pests and diseases and new market trends.	47,0
	5 - The organization maintains registers or database with the history of relationship with clients.	42,1
	13 - The organization has registers and protocol procedures of technologies, production methods and processes and seeks to identify, develop and incorporate innovations to their products and services.	41,9
	18 - The organization uses electrical conductivity meters, pH and soil moisture.	29,2
	1 - The organization has document with clear definition of their reason for existence (Mission), plan to defined future (Vision) and on the organizational principles that guide how employees should act in their day-to-day (values).	13,8

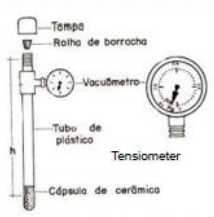
Strengths: harvest and post-harvest

Strengths: assessment of storage parameters, storage in an appropriate place



Weakness in the quality of Brazilian coffee: deficiencies in data register on products, processes and customers

Weakness in the quality of Brazilian coffee: low use of precision instruments - tensiometer, conductivimeter, ph meter



Brazilian coffee quality management indicators: Principal components analysis (PCA)

- 7 factors explain 53.1% of the sample variance
- Number of questionnaires evaluated - Brazil: 920
- Variable number initially analyzed: 30
- Number of variables excluded during the review process: 07
- First factor: explain 20.0% of sample variance
- Variables included have minimum correlation of 0.373 with a Factor
- Orthogonal Rotation: Varimax Raw
- Factors extraction method: eigenvalues (eigenvalues) above 1.0

Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
25-Assessment of storage parameters	6-Communication of complaints to management	9-Search for information	3-Definition of sales values of products	29-Relationship with the market	18-Use of precision instruments	20-Harvest without contact of grain with the ground
23-Control parameters in the drying process	7-Communication deliveries to customers failures	17-Regular use of chemical analysis in the nutritional control	13-Existence of registers and protocols and search for innovations	28-Monitoring of customer satisfaction	27-Planejamento do controle da qualidade	19-Adequate guidance for harvest
22-Existence of adequate post-harvest unit						
24- Appropriate storage	5 - Registration of customer relationship	14-Search for information about new varieties	12 Search for new markets			
21-Proper transport of newly harvested products						
26-Regular quality inspections		10-participation in technical events				
Process control in post-harvest and storage	**Relationship record with customers**	**Search and use of information**	**Innovation and search for new markets**	**Monitoring of customer satisfaction**	**Planning and monitoring of production**	**Care with the harvest**

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PA 161

The Maragogype Bean Appearance of Indonesian Robusta

SUMIRAT Ucu[1]* & YUSIANTO[1]

[1]*Indonesian Coffee and Cocoa Research Institute (ICCRI), *Corresponding author:* ucu_sumirat@yahoo.com

Introduction

Indonesian Robusta has been known having large size of green bean. This situation is also supported by strong farmer preference to have planting material with large bean. Therefore, large bean is one of the most important considerations in coffee breeding of Robusta coffee in Indonesia. This research was aimed to communicate our finding of two promising Robusta clones having giant bean size which similar to Maragogype of Arabica, and its prospect to be developed as specialty product as Maragogype[1].

Materials and Methods

The two promising clones (IRD1 & IRD2) were identified during the National Competition of Superior Coffee, held by the Indonesian Coffee and Cocoa Research Institute (ICCRI). Agronomic performances was on-site assessed in Lampung province. Coffee processing was done by the farmer, whereas assessment of green bean and cup quality were observed in ICCRI.

Result and Discussion

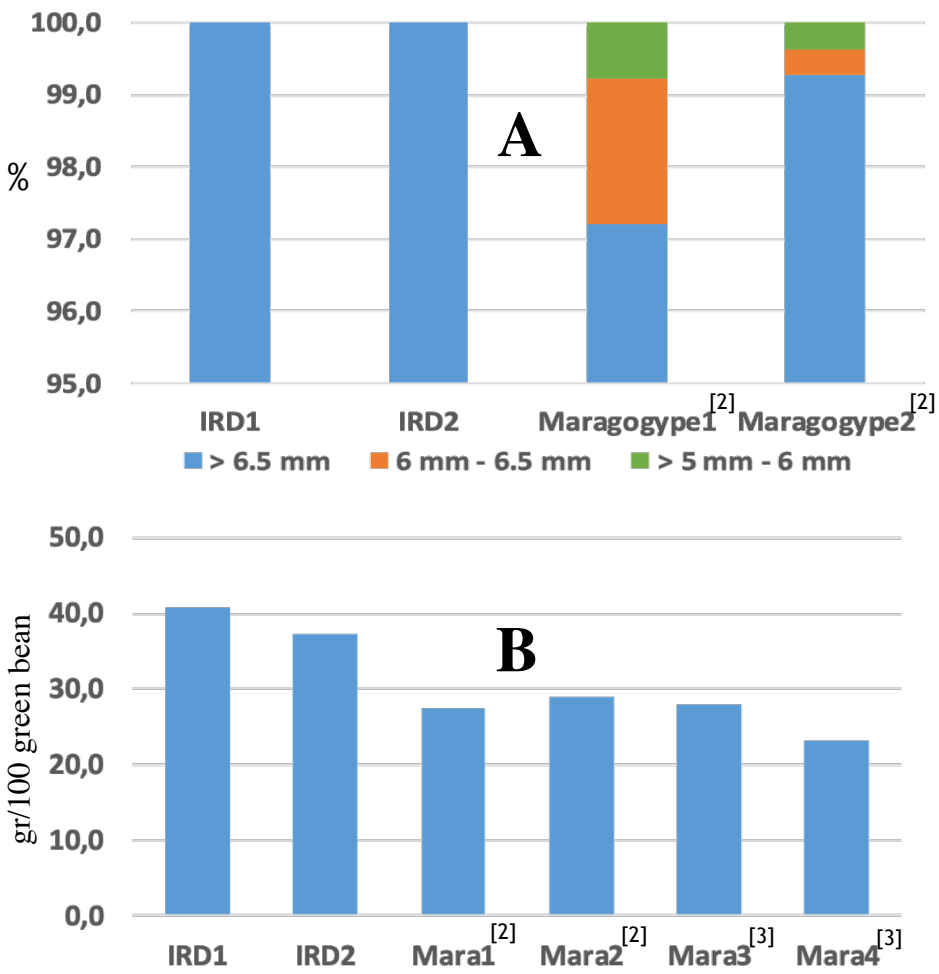

Figure 1. Bean appearance between two promising Robusta clones and Maragogypes based on Indonesian Arabica sieving system (A) & weight of 100 green bean (B).

Figure 2. Bean size between IRD1 (A) and Maragogype (B).

Frag./Arm. = Fragrance/Aroma
Flav. = Flavor
Aft.= Aftertaste
Sal./Aci. = Salt/Acid
Bit./Swe. = Bitter/Sweet
Mfl. = Mouthfeel
Bal. = Balance
Ove. = Overall

Total Score IRD1 = 77,0
Total Score IRD2 = 81,8

Comments of cup taste:
IRD1 = Greenish, Astringent
IRD2 = Caramelly, Chocolaty

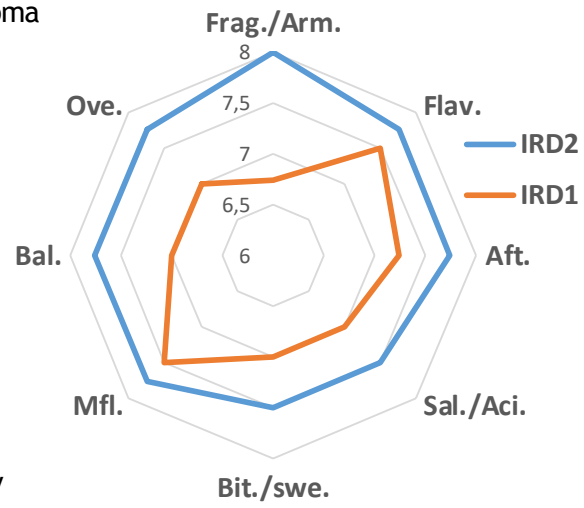

Figure 3. Cup quality[4] of two promising Robusta clones

According to Figure 1, sieving on the two selected Robusta clones (IRD1 & IRD2) showing 100 % of their green bean were classified as large bean, compared to two reported selected Maragogypes[2]. On the other hand, weight per 100 green bean of those two Robustas were found far heavier rather than four reported selected Maragogypes[2,3]. However, observation on cup quality of this two promising clones were showed differently, whereas IRD2 showed excellent-class compared to very good-class of IRD1. This result is confirm the high opportunity of clone IRD2 to be developed as specialty product as like as Maragogype in Arabica coffee. Furthermore, this clone was also showed good yield capacity of 2,5 ton/ha under Indonesian planting system.

Conclusion

Among two selected Robusta clones, IRD2 is showed higher opportunity to be developed as specialty product considering it outstanding size of green bean, excellent cup quality & supported by high productivity.

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ICCRI

PA 162

EFFECT OF THE WEED CONTROL METHODS IN COFFEE INTER ROWS, DURING EIGHT YEARS ON COFFEE YIELD*.

Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café *
ALCÂNTARA, E.N., FERREIRA, M.M.***, MENICUCCI, N, P.****, * * Researcher of the Minas Gerais state agricultural research institution (EPAMIG), Brazil, FAPEMIG's scholarship holder;*** Professor of the UFLA Federal University of Lavras, MG, Brazil; *** *UFLA, under graduated student.**

ABSTRACT

The coffee crop is very sensitive to competition from weeds for water, light, and nutrients. Weed control represents 30% of production cost. Because of that, several weed control methods were tested to find the best one for use in the inter-rows of the coffee crop. For that purpose, in 2006, an experiment was set up in a randomized block design with seven treatments to control weed at inter-rows: a mower, disk harrow, rotary tiller, post-emergence herbicide (glyphosate) at 720g ai / ha, pre-emergence herbicide (oxyfluorfen) at 720 g ai / ha, hand weeding, and inter-rows without weed control as a check, using a randomized block design, in three replications. The experimental area is a red Oxisol with an 8% slope and 3364 coffee plants of the Paraíso (MGH 419) cultivar, planted with 4.0 m inter-rows and 0.7 m plant spacing at the EPAMIG Experimental Station of São Sebastião do Paraíso, MG, Brazil. Yields from 2008, 2009, 2010, 2011, 2012, 2013 and 2015 were analyzed. After eight years, results show that the pre-emergence herbicide led to the highest yield. The inter-row without control had the lowest production. The use of the disk harrow, rotary hoe, manual weeding, and post-emergence herbicide showed intermediate yields because they are methods that depend on the timing and availability of weed control. Furthermore, use of the disk harrow contributed to infestation by bermudagrass [*Cynodon dactylon* (L.) Pers] due to the shearing of stolons into diverse single segments giving rise to many plants, and the rotary tiller led to the predominance of purple nutsedge (*Cyperus rotundus* L.) due to the release of several tubers, increasing and distributing their propagules throughout the agricultural area.

Keywords: Weed control methods, coffee, production.

INTRODUCTION

The coffee plant is very sensitive to weed competition by water, light and nutrients. According to GARCIA -BLANCO et al 1982, yield losses due to competition, may reach to 77%.

At inter rows coffee, weeds have been controlled by several ways, including intercropped plantings, due to, the weed control cost.

Some studies have shown that although some weed control methods increased the soil organic matter, they have not increased the coffee yield.

MATERIALS AND METHODS

To study the effects of weed control methods at inter-rows on yield coffee a experiment was installed in 2006, in randomized block design with seven treatments on coffee inter rows: mower, disk harrows, rotary tiller, post-emergency herbicide (glyphosate) at 720g a.i./ha pre-emergency herbicide (oxyfluorfen) at 720 g a.i./ ha, manual weeding and no weeding, using three replications, in a coffee planted in Oxisol soil area with 3366 plants from Paradise (MGH 419) coffee cultivar spaced 4 by 0.7 m at EPAMIG at São Sebastião do Paraíso, MG. Yields from 2008, through 2015 for each treatment, were analyzed.

Figure 1 **- No weed control at inter row**

Figure 2 **– Post emergency herbicide at inter row.**

Figure 3 **– Pre emergency herbicide at inter row.**

RESULTS

Table 1 - Nº of processed bags 60 kg coffee/ha. São Sebastião do Paraíso, MG. Brazil.

Inter-row	Years								
Treatments	2008	2009	2010	2011	2012	2013	2014	2015	Average
Mower	8.0 bc	26.0ab	41.7ab	27.0 a	45.0 ab	36.6 ab	43,00 ab	29,80 a	32,18 ab
Disk harrow	11.7 bc	20.7 b	55.3ab	20.0 a	30.0 b	45.4 a	29,81 b	27,99 a	28,96 b
Rotary hoe	17.0 bc	25.7ab	48.3ab	28.0 a	46.0 ab	20.1 b	43,7 ab	21,90 a	32,36 ab
Post E.Herb.	14.4 bc	25.0ab	49.0ab	28.0 a	42.2 ab	24.1 ab	46,7 a	26,37 a	31,57 ab
PRE- herb.	31.3 a	33.0a	61.0a	31.7 a	48.3 a	16.6 b	56,67 a	22,55 a	38,66 a
Hand hoeing	17.7 b	24.0ab	48.3ab	36.7 a	42.3 ab	19.3 b	50,33 a	21,52 a	32,36 ab
No control	4.0 c	18.7 b	37.3 b	25.3 a	30.0 b	37.0 ab	43,00 ab	29,67 a	28,17 b
C.V. (%)	16.4	9.34	8.55	14.25	7.93	15.64	3,62	15,98	12,33

*Mean values with the same letters are not significantly different by the Tukey test at 0.5% level.

Pre-emergency herbicide treatment presented the best weed control and so the highest production, and no wedding treatment, presented the lowest yield. Mechanical methods yielded 15% to 20% less than the best treatment. Results shown that, the effectiveness of these methods depend on the operational availability of weed control. Disk harrow method did spread the bermuda grass [*Cynodon dactylon* (L.) Pers], at the area, and the rotary tiller spread purple nutsedge (*Cyperus rotundus* L.).

CONCLUSIONS

The pre-emergency herbicide treatment showed the greater yield. No weed control presented the lowest yield. Mechanical weed control methods, presented 15 to 20% less yield than pre-emergency herbicide. Mechanical weed control methods contributed to spread bermuda grass and nutsedge at coffee area.

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EFFECT OF WEED CONTROL METHODS ON COFFEE INTER ROWS ON DISPONIBILITY OF MICRONUTRIENTS AT TWO SOIL LAYER.

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Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café *
VENTORIN, R, ALCÂNTARA, E.N.**, PEREIRA, A.B..***, ** Researchers of the Minas Gerais state agricultural research institution (EPAMIG), Brazil, FAPEMIG's scholarship holder;*** UFLA Federal University of Lavras, MG, Brazil, under graduated student.**

ABSTRACT
The coffee plant is very sensitive to weed competition by water, light and nutrients. Because of this, the needs of weed control on coffee is imperative. According to GALLO et al (1958), absorption of water and nutrients by weeds are factors that provoke disturbs on coffee nutrition, as the Zn deficiency that may be appointed as one of these effect. So according to GARCIA -BLANCO et al 1982, yield losses due to competition, may reach to 77%. This study was made to evaluate the effect of weed control methods at coffee inter rows on the soil micronutrient availability. To study the effects of weed control at inter rows on soil chemical quality an experiment, in randomized block design with seven treatments on coffee inter rows: disk harrows, rotary tiller, post-emergency herbicide (glyphosate) at 720g a.i./ha pre-emergency herbicide (oxyfluorfen) at 720 g a.i./ ha, manual weeding and no weeding, using three replications, in a coffee crop in Oxisol soil area with 3366 plants with Paraíso (MGH 419) coffee cultivar spaced 4 by 0.7 m at Experimental Station at EPAMIG in São Sebastião do Paraíso, MG, was installed in 2006. Soil samples from each treatment, were analyzed. All treatments affected the availabities of Zn, Mn, Boron and Sulfur at layer of 0 to 15cm. The pre-emergency herbicide reduced Zn, Mn and Boron and increased sulfur content. At the 15 to 30 cm soil layer the pre emergency treatment reduced the Mn content. The soils without cover treatments shown the tendency to reduce Zn content, probably due to lixiviate this mineral. The treatments affected micronutrients content in soil, specially on the first layer. The treatments without soil cover reduced the micronutrient content on the soil surface.
Key words: weed control methods, soil micronutrients, coffee crop.

INTRODUCTION

The coffee plant is very sensitive to weed competition by water, light and nutrients. Because of this, the needs of weed control on coffee is imperative. According to GALLO et al (1958) weeds compete for all nutrients but they present low interest on MN, Cu and boron competition, but accumulate great amounts of zinc and iron. So according to GARCIA - BLANCO et al 1982, yield losses due to competition, may reach to 77%. This study was made to evaluate the effect of weed control methods at coffee inter rows on the soil micronutrient availability.

METHODS

To study the effects of weed control at inter rows on soil chemical quality an experiment was installed in 2006, in randomized block design with seven treatments on coffee inter rows: disk harrows, rotary tiller, post-emergency herbicide (glyphosate) at 720g a.i./ha pre-emergency herbicide (oxyfluorfen) at 720 g a.i./ ha, manual weeding and no weeding, using three replications, in a coffee crop in Oxisol soil area with 3366 plants with Paradise (MGH 419) coffee cultivar spaced 4 by 0.7 m at EPAMIG at São Sebastião do Paraíso, MG. Soil samples from each treatment, were collected and analyzed.

RESULTS

***Table 1*- Micronutrients contents in mg/dm3 at 0 to 15cm soil layer on weed control methods. S.Seb.Paraiso, 2013.**

Treatments	Zn	Fe	Mn	Cu	Boron	Sulfur
Mower	13,11a	26,78a	69,89ab	18,91a	0,137ab	17,08 b
Disk harrow	11,87bc	23,81a	67,10ab	18,90a	0,213 ab	22,21 b
Rotary tyller	9,55bc	31,94a	49,81ab	19,12a	0,187 ab	20,27 b
Post E. herb.	15,24a	26,71a	49,77ab	21,88a	0,203 ab	21,45 b
P.E. herb.	5,24 c	30,31a	24,90 b	13,55ab	0,100 b	59,18 a
Hand hoeing	15,35a	27,17a	76,39a	19,73a	0,237 a	23,90 b
No hoeing	15,32a	24,01a	64,72ab	13,31a	0,233 a	21,45 b
V. Coef.	12,18	8,28	13,06	9,52	3,86	8,46

***Table 2*- Micronutrients contents in mg/dm3 at 15 to 30cm soil layer on weed control methods. S.Seb.Paraiso, 2013.**

Treatments	Zn	Fe	Mn	Cu	Boron	Sulfur
Mower	13,70a	35,33a	77,68ab	20,10a	0,137ab	17,08b
Disk harrow	16,20a	31,00a	87,10a	22,48a	0,213ab	22,21b
Rotary tyller	12,60a	45,67a	60,31ab	19,71a	0,187ab	20,27b
Post E. herb.	12,37a	28,33a	59,76ab	20,45a	0,203ab	21,45b
P.E. herb.	10,47a	37,67a	41,49b	18,58a	0,100b	59,18a
Hand hoeing	7,48a	37,37a	59,13ab	14,17a	0,237a	23,90b
No hoeing	5,41a	37,33a	62,01ab	13,18a	0,233a	21,45b
V. Coef.	22,49	8,48	10,26	18,36	3,86	8,46

All treatments affected the availabities of Zn, Mn, Boron and Sulfur at layer of 0 to 15cm. The pre-emergency herbicide reduced Zn, Mn and boron and increased sulfur content. At the 15 to 30 cm soil layer the pre emergency treatment reduced the Mn content. The soils without cover treatments showed the tendency to reduce Zn content, probably due to lixiviate this mineral.

***Figure 1* - No weed control at inter row**

***Figure 2* – Post emergency herbicide at inter row.**

***Figure 3* – Pre emergency herbicide at inter row.**

CONCLUSIONS

The treatments affected micronutrients content in soil, specially on the first layer.
The treatments without soil cover reduced Zn content on the surface soil, mailing due to leaching.

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PRODUCTION PLANTS COFFEE (*Coffea arabica* L.) IN HYDROPONIC GREENHOUSES MESH RED SHADOW

PA 166

ROBLEDO, José*; CONTRERAS Francisco**. Universidad Autónoma Chapingo, GANAECO A.C. Mexico.

Abstract

the Mexican government implemented in 2014 under the PROCAFE program, production plants in conventional nurseries and hydroponic greenhouses. It was considered the protocol designed hydroponic greenhouse; greenhouse construction; training of technicians and nurserymen for the operation of the project; seed selection in seed farms; substrate preparation, planting seed, nutrition management, pest and disease control; in the phenological evaluation parameters germination, plant height, stem diameter and pairs of leaves per plant were considered. 36 greenhouses were established rachel red shadow mesh type 50% in seven states coffee growers for the production of 16.9 million coffee plants with a capacity of 500,000 plants per greenhouse.

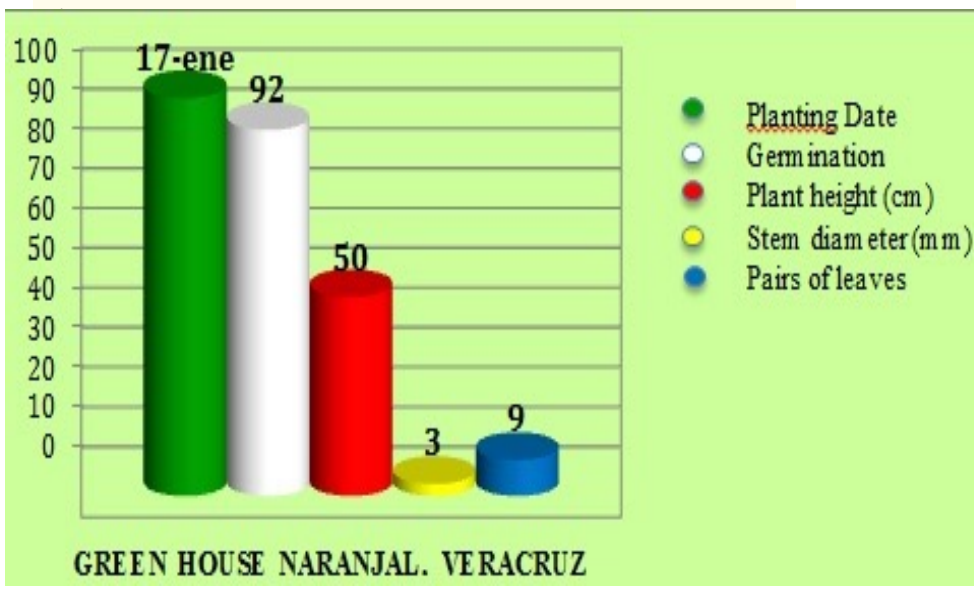
Introduction

Mexico as a producer globally ranked ninth after Honduras, situation created by the presence of coffee rust (*Hemileia vastatrix*), which affected production decline in the 2015-2016 cycle to 2.34 million bags of 60 kg. The lowest reported in úñtimos years. In response, the Mexican government implemented in 2014 under the PROCAFE, production plants in conventional nurseries and hydroponic greenhouses to produce 200 million plants needed to revive the national coffee production.

Figure 1: Production Process plant coffee

Figure 2: Greenhouses rachel mesh red shadow and Modules hydroponic greenhouses six mesh sahadow

Graphic 1 : Phenologic development of



Main Text

36 Greenhouses were established rachel red shadow mesh type 50% in seven states coffee growers for the production of 16.9 million coffee plants with a capacity of 500,000 plants per greenhouse. Propagated varieties were: Costa Rica 95, Oro Azteca, Colombia, Geisha, Catuai, bourbon and mundo novo. the best substrate as a mixture of paet moss, agrolita, lombricompost, adding to the mixture mycorrhizae and PSD was determined. the best nutrition formulas applied to soil drench and foliar type considering chemical and organic nutrition were determined; germination rates of 85-95% were determined; phenological evaluation reported in six months due to the mesh red shade height 45-50 cm plants, 8-10 pairs of leaves and stem diameter of 3-4 mm. the evaluation of six modules hydroponic greenhouses have underway to evaluate mesh 6 colors, 2 substrates and 3 sizes of trays.

Conclusion

Production of coffee plants in hydroponic greenhouses mesh red shadow is a viable strategy as it allows two cycles of plants per year, optimizing space producing more plants per unit area, plants safe quality, free soil pests and excellent root development, ensuring his arrest in the field. This strategy has been validated by organizations and producers.

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REGISTRATION VARIETY OF COFFEE (*Coffea arabica L.*) IN THE NATIONAL CATALOGUE OF PLANT VARIETY (CNVV) OF MEXICO

PA167

ROBLEDO, José*, ESCAMILLA, Esteban**, RODRIGUEZ, Dulce***
*Universidad Autónoma Chapingo, CRUO, Huatusco, Veracruz, México

Abstract

In Mexico, only record a variety of coffee, Oro Azteca, entered in the National Catalogue of Plant Varieties (CNVV), given that this is an essential requirement in the process of approval rating systems seed, you had was vital important documentary record characteristics of coffee varieties commonly used in our country, coming to his aid in some controversy of biopiracy.

Introduction

In 2015, twenty varieties of coffee commonly used, established in the Germplasm Bank Coffee CRUO, which represents the most important collection of coffee from Mexico, with more than 250 accessions were selected. To make descriptors guidelines distinctness, uniformity and stability of the International Union for the Protection of New Varieties of Plants (UPOV) they were used.

Figure 1: Characteristics of plant

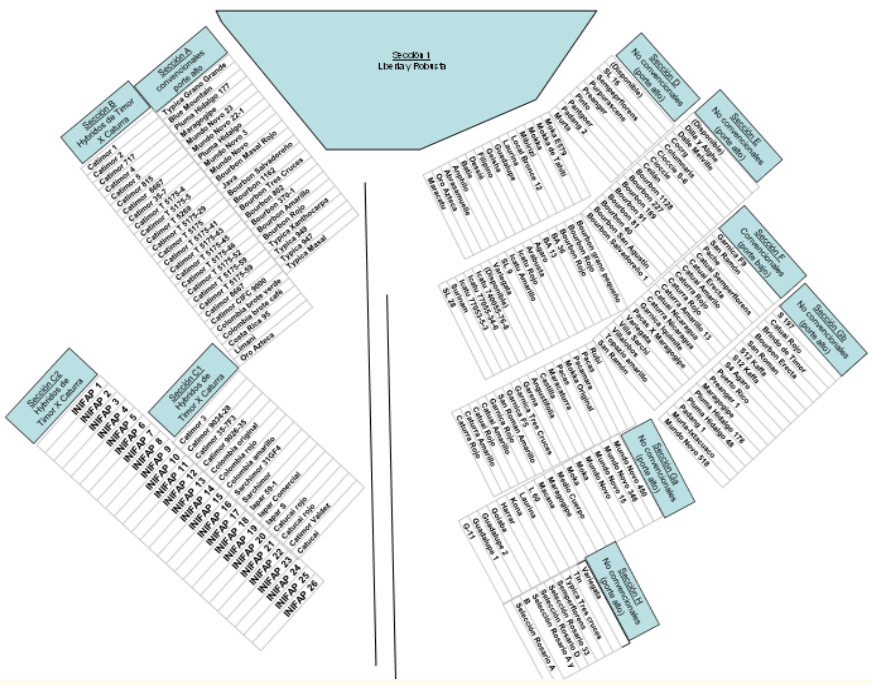

Figure 2: Germplasm Bank Coffee CRUO

Figure 3: Twenty coffee varieties registered in the CNVV

Main Text

Twenty varieties of coffee in the CNVV of Mexico were enrolled: Blue Mountain, Costa Rica 95, Bourbon Amarillo, Geisha, red Bourbon, Iapar 59, Bourbon Salvadoreño Maracatú, yellow Catuaí, Maragogipe, red Catuaí, New World, Catucaí, Pacamara, red Caturra, Pluma Hidalgo, Colombia and red Garnica, through the National Service of Seed Inspection and Certification (SNICS). In addition to verification farms and seed certification

Conclusion

The registration of these varieties of coffee in the CNVV, is an important contribution to the Mexican coffee, essential to enter certification programs coffee seed requirement.

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分享咖啡科技进步

PRODUCTIVITY AND VEGETATIVE GROWTH OF CONILON COFFEE UNDER DIFFERENT RATES OF BIOREGULATOR

PA172

GILES, João Antonio D.*, RODRIGUES, Joice P.*, VALANI, Gustavo P.*, BENTO, Fernanda Bruna**, PARTELLI, Fábio Luiz*#,

* Universidade Federal do Espírito Santo, São Mateus, Espírito Santo, Brazil. # Corresponding author: partelli@yahoo.com.br
**Stoller do Brasil Ltda, Campinas, SP, Brazil.

INTRODUCTION

Within several chemical products which regulate plant growth, Stimulate® stands out due to its composition, where gibberellic acid, indole-3-butyric acid and kinetin are found.

OBJECTIVE

This study aimed to evaluate the effect of different application rates of Stimulate® under growth and production parameters on conilon coffee, contributing to adjust its rates for the crop.

MATERIAL AND METHODS

The experiment was conduced in a field of *Coffea canephora* in the municipality of São Mateus – state of Espírito Santo, Brazil, during the crop years of 2012/13, 2013/14 and 2014/15. A randomized block experimental design was used, with four repetitions and five treatments (0, 200, 400, 800 and 1600 mL ha-1 of Stimulate®). Three foliar applications were made, the first in the pre-flowering stage, the second when the petals were falling and the third in the stage of small green grains.

The number of nodes was determined annually by the difference between its number at the beginning and at the end of the experiment, the first was assessed just before the first application and the last close to grain harvest.

RESULTS, DISCUSSION AND CONCLUSION

The data about the nodes number on orthotropic branches were adjusted to a quadratic regression model (ŷ = 14.651 + 0.0015x - 9E-07x2; R2 = 0.82**), with a maximum value reached at the rate of 833 mL ha-1 of Stimulate®, resulting in an average of 15.3 nodes, corresponding to 4.6% more in comparison to the control.

Analyzing the effect of Stimulate® on productivity (ŷ = 103.87 + 0.0117x - 7E-06x2; R2 = 0.57*), there was a trend of increase in production, with a maximum value at the rate of 835 mL ha-1 of Stimulate®, resulting in a productivity of 108.8 bags ha-1, representing an increase of 6.7 bags compared to the control.

During the third year of harvesting, a difference of more than 15 bags ha-1 was noticed between the rates of 0 and 800 mL ha-1 of Stimulate®. This fact is primarily associated with the cumulative effect on growth, provided by Stimulate® (greater vegetative growth and number of nodes than previous year). This did not occur in the first year of harvesting, even with foliar application, decreasing the average difference in productivity.

The use of Stimulate® (800 mL ha-1) contributed to boost productivity and vegetative growth on conilon coffee, especially after two years of application. This work suggests that higher rates do not bring benefits to coffee production.

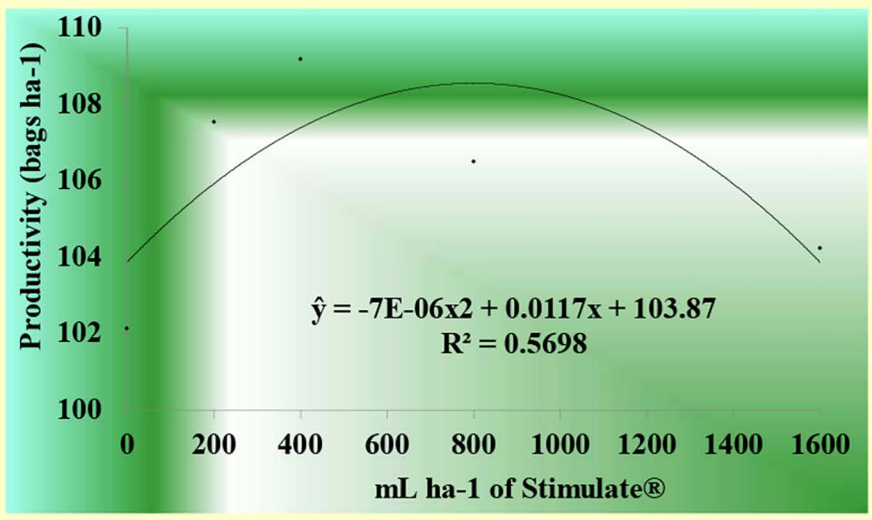

Fig.: Productivity and 0, 200, 400, 800 and 1600 mL ha-1 of Stimulate®

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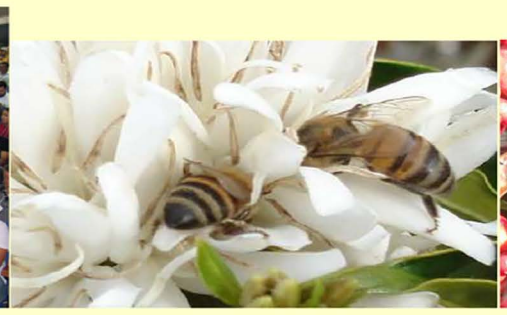
Support



PRODUCTIVITY AND VEGETATIVE GROWTH OF CONILON COFFEE UNDER DIFFERENT RATES OF BIOREGULATOR

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*Universidade Federal do Espírito Santo, UFES, São Mateus, ES, Brazil. **Stoller do Brasil Ltda, Campinas, SP, Brazil. *Contact author: partelli@yahoo.com.br

RATIONALE

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RESULTS

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CONCLUSIONS & PERSPECTIVES

The use of Stimulate® (800 mL ha-1) contributed to boost productivity and vegetative growth on conilon coffee, especially after two years of application. This work suggests that higher rates do not bring benefits to coffee production.

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VARIABILITY FOR LEAF NUTRIENT LEVELS IN *Coffea canephora* GENOTYPES DURING TWO PHENOLOGICAL STAGES

PA173

GOMES, Wander Ramos*, RODRIGUES, Weverton Pereira**, OLIVEIRA, Marcos Goes*, VIEIRA, Henrique Duarte**, RAMALHO, José Cochicho***,****, PARTELLI, Fábio Luiz*#,

* Universidade Federal do Espírito Santo, São Mateus, Espírito Santo, Brazil. # Corresponding author: partelli@yahoo.com.br
**Universidade Estadual do Norte Fluminense, UENF, Campos dos Goytacazes, RJ, Brazil.
***PlantStress&Biodiversity Group, DRAT, LEAF, ISA, Universidade de Lisboa, Portugal. ****GeoBioTec, FCT, UNL, Portugal.

INTRODUCTION

Understanding the dynamics of nutrients and their relationship with climate conditions is an important tool for crop management, as regards fertilizers application. For that was analysed the mineral accumulation in leaf over two years in *C. canephora* cv. Conilon plants, under irrigated and non-irrigated conditions. The interpretation of the nutritional status of Conilon coffee leaves has mainly been based on a method known as the "sufficiency range", that allows a quite simple and direct analysis [1]. The standard values from the "sufficiency range" method are usually regional, and use a mean value of the leaf nutrient content as a reference, according to the time of year [2]. However, standard values for specific genotypes have not been established.

OBJECTIVE

Consequently, the objective of this work was to establish leaf nutrient contents and leaf nutrient content relationships for leaves from seven Conilon coffee genotypes, during two phenological stages (pre-flowering and bean filling).

MATERIAL AND METHODS

Twenty crops scattered around several cities along the northern region of Espírito Santo state, Brazil, with crop yield either equal to or greater than 100 bags per hectare (during two harvests) were assessed.

A total of 140 samples were collected during each evaluation period for quantification of leaf nutrient contents (N, P, K, Ca, Mg, S, Fe, Zn, Cu, Mn and B). To examine differences between the leaf nutrient contents and leaf nutrient content relationships, F- and Scott-Knott tests were used.

RESULTS, DISCUSSION AND CONCLUSION

There is genetic diversity in leaf nutrient contents and leaf nutrient content relationships among Conilon coffee genotypes, during both pre-flowering and bean-filling stages. The 8V, 9V, and 12V genotypes exhibited the highest values for most of the nutrients, especially N, P and Cu.

Leaf diagnosis can be genotype-specific and may differ between phenological stages, which can help farmers to reduce the cost of fertilizer and improve their income. Therefore, genetic diversity exists in standard leaf nutrient levels such that leaf diagnosis can be specific to each genotype and phenological stage.

Table 1. Mean values, coefficient of variation (CV), and F- and Scott-Knott test results for nutrient contents measured in seven conilon genotypes of the clonal variety Vitória Incaper 8142 with crop yield either equal to or greater than 100 bags per hectare (average from 2012 and 2013 harvests) during the pre-flowering stage.

	Genotypes								
Nutrients	5V	6V	8V	9V	10V	12V	13V	CV	F test
N (g/kg)	24.2b	25.5b	26.6a	26.9a	25.1b	28.1a	24.8b	9.70	**
P (g/kg)	1.9b	1.2b	1.3a	1.3a	1.2b	1.2a	1.3a	16.8	**
K (g/kg)	11.7a	12.4a	13.7a	12.2a	12.7a	12.3a	12.0a	20.2	NS
Ca (g/kg)	20.7b	16.9c	21.6b	21.3b	26.1a	21.2b	18.2c	24.1	**
Mg (g/kg)	3.3a	3.3a	3.3a	3.8a	3.9a	3.9a	3.6a	28.5	NS
S (g/kg)	1.0d	1.3c	1.6a	1.4b	1.2c	1.7a	1.2c	20.0	**
B (mg/kg)	76.8a	60.9a	75.2a	79.6a	77.2a	83.5a	71.4a	31.7	NS
Cu (mg/kg)	7.9b	8.1b	11.4a	9.4a	7.1b	11.0a	10.9a	52.1	*
Fe (mg/kg)	149.1a	118.4a	129.9a	130.6a	131.3a	145.1a	113.5a	49.1	NS
Mn (mg/kg)	139.9a	150.9a	144.7a	136.3a	153.4a	135.1a	147.8a	57.7	NS
Zn (mg/kg)	5.4c	6.4c	7.0b	5.9c	6.2c	8.2a	5.8c	21.2	**

Table 2. Mean values, CV, and F- and Scott-Knott test results for nutrient contents measured in seven conilon genotypes belonging to the clonal variety Vitória Incaper 8142 with crop yield either equal to or greater than 100 bags per hectare (average from 2012 and 2013 harvests) during the bean-filling stage.

	Genotypes								
Nutrients	5V	6V	8V	9V	10V	12V	13V	CV	F test
N (g/kg)	24.2b	25.5b	26.6a	26.9a	25.1b	28.1a	24.8b	9.70	**
P (g/kg)	1.9b	1.2b	1.3a	1.3a	1.2b	1.2a	1.3a	16.8	**
K (g/kg)	11.7a	12.4a	13.7a	12.2a	12.7a	12.3a	12.0a	20.2	NS
Ca (g/kg)	20.7b	16.9c	21.6b	21.3b	26.1a	21.2b	18.2c	24.1	**
Mg (g/kg)	3.3a	3.3a	3.3a	3.8a	3.9a	3.9a	3.6a	28.5	NS
S (g/kg)	1.0d	1.3c	1.6a	1.4b	1.2c	1.7a	1.2c	20.0	**
B (mg/kg)	76.8a	60.9a	75.2a	79.6a	77.2a	83.5a	71.4a	31.7	NS
Cu (mg/kg)	7.9b	8.1b	11.4a	9.4a	7.1b	11.0a	10.9a	52.1	*
Fe (mg/kg)	149.1a	118.4a	129.9a	130.6a	131.3a	145.1a	113.5a	49.1	NS
Mn (mg/kg)	139.9a	150.9a	144.7a	136.3a	153.4a	135.1a	147.8a	57.7	NS
Zn (mg/kg)	5.4c	6.4c	7.0b	5.9c	6.2c	8.2a	5.8c	21.2	**

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VARIABILITY FOR LEAF NUTRIENT LEVELS IN *Coffea canephora* GENOTYPES DURING TWO PHENOLOGICAL STAGES

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*Universidade Federal do Espírito Santo, UFES, São Mateus, ES, Brazil. **Universidade Estadual do Norte Fluminense, UENF, Campos dos Goytacazes, RJ, Brazil. ***PlantStress&Biodiversity Group, DRAT, LEAF, ISA, Universidade de Lisboa, Portugal. ****GeoBioTec, FCT, UNL, Portugal. *Contact author: partelli@yahoo.com.br

RATIONALE
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The standard values from the "sufficiency range" method are usually regional, and use a mean value of the leaf nutrient content as a reference, according to the time of year [2]. However, standard values for specific genotypes have not been established. Consequently, the objective of this work was to establish leaf nutrient contents and leaf nutrient content relationships for leaves from seven Conilon coffee genotypes, during two phenological stages (pre-flowering and bean filling).

METHODS
Twenty crops scattered around several cities along the northern region of Espírito Santo state, Brazil, with crop yield either equal to or greater than 100 bags per hectare (during two harvests) were assessed. A total of 140 samples were collected during each evaluation period for quantification of leaf nutrient contents (N, P, K, Ca, Mg, S, Fe, Zn, Cu, Mn and B). To examine differences between the leaf nutrient contents and leaf nutrient content relationships, F- and Scott-Knott tests were used.

RESULTS, CONCLUSIONS & PERSPECTIVES
There is genetic diversity in leaf nutrient contents and leaf nutrient content relationships among Conilon coffee genotypes, during both pre-flowering and bean-filling stages. The 8V, 9V, and 12V genotypes exhibited the highest values for most of the nutrients, especially N, P and Cu. Leaf diagnosis can be genotype-specific and may differ between phenological stages, which can help farmers to reduce the cost of fertilizer and improve their income.
Therefore, genetic diversity exists in standard leaf nutrient levels such that leaf diagnosis can be specific to each genotype and phenological stage.

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EVALUATION AND INTRODUCTION OF *Coffea canephora* GENOTYPES IN BRAZIL: INITIAL ASSESSMENT

PA175

OLIOSI, Gleison*, GILES, João A. D.*, COVRE, André M.*, GOMES, Wander. R. G.*, FERREIRA, Adésio*, SILVA, Marcelo B.*, GONTIJO, Ivoney*, VIEIRA, Henrique D.**, RAMALHO, José C.***,****, PARTELLI, Fábio Luiz*#,

*Federal University of Espírito Santo, UFES, São Mateus, ES, Brazil. # Corresponding author: partelli@yahoo.com.br, **State University of Northern Rio de Janeiro, UENF, Campos dos Goytacazes, RJ, Brazil. ***Plant Stress & Biodiversity Group, DRAT, LEAF, ISA, Universidade de Lisboa, Portugal. **** GeoBioTec, FCT, UNL, Portugal.

INTRODUCTION

New promising genotypes of *Coffea canephora* Pierre ex A. Froehner have been selected by coffee growers, although without a statistical evaluation in field trials.

OBJECTIVE

Here, the development and productivity of 43 potential genotypes of *C. Canephora* grown at Espírito Santo and Bahia states, Brazil, will be evaluated, aiming at identify high potential genotypes for farmers.

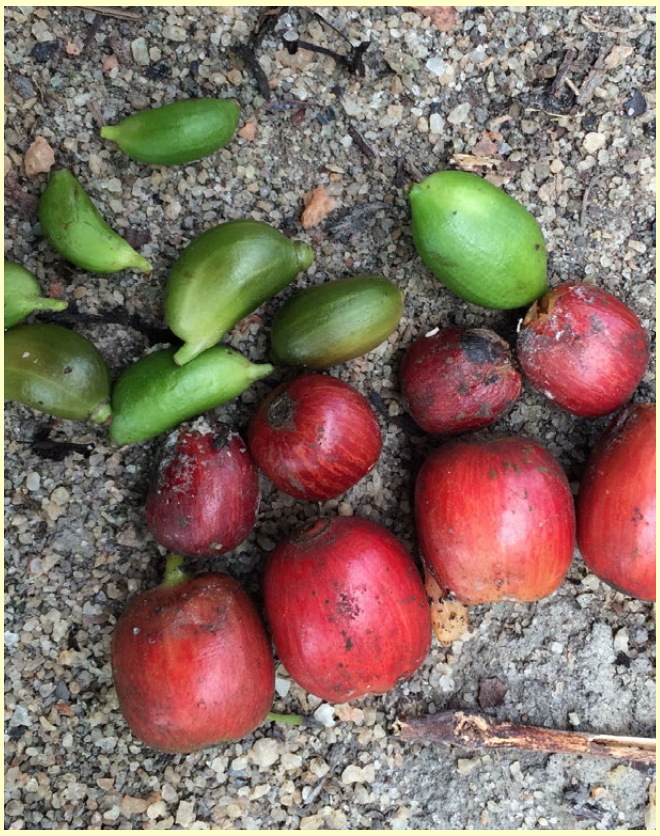
MATERIAL AND METHODS

Plantations of *C. canephora* were set on May and June 2014 at the municipalities of Nova Venécia (State of Espírito Santo) and Itabela (Bahia state). Both fields are currently under development and the first harvest is expected on June 2016. Within the genotypes tested, 42 were propagated via cuttings and one from seeds. The randomized block design was used with three blocks and seven plants on each experimental plot. Plant height and crown diameter were evaluated in order to characterize the initial development. The data were submitted to variance analysis, with averages pooled by the Scott-Knott test at a significance level of 5%.

RESULTS, DISCUSSION AND CONCLUSION

Plant height led to three plant groups: 5 genotypes classified as taller, 13 as intermediates and 25 as smaller plants, with average height of 1.68, 1.55 and 1.43 m, respectively. Considering the crown diameter, the plants were also pooled into three groups: 2 genotypes with greater diameter, 12 classified as intermediate and 29 with smaller diameter, with average diameter of 2.04, 1.75 and 1.54 m. Variability among these genotypes has already been found on seedlings [1] and it is very common to be found on a wide diversity of *C. canephora* [2, 3, 4]. This initial evaluation evidenced important variability among the genotypes, which could enable the selection of promising ones at the end of the experiment.

In a second phase, selected genotypes may be registered as varieties and indicated to planting. For that, the following steps of this applied research aims at to assess the genotype productivities and bean quality characteristics along five years, generating scientific knowledge, building capacities (technicians) and advanced training (at graduation, MSc and PhD levels).



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[3] Partelli FL, et al. (2013) JAS, 5:108-116. DOI: 10.5539/jas.v5n8p108.
[4] Partelli FL, et al. (2014) RBCS, 38:214-222. DOI: 10.1590/S0100-06832014000100021.

Support

•Farmer:
•T. Pianissoli

EVALUATION AND INTRODUCTION OF *Coffea canephora* GENOTYPES IN BRAZIL: INITIAL ASSESSMENT

OLIOSI, Gleison*, GILES, João A. D.*, COVRE, André M.*, GOMES, Wander. R. G.*, FERREIRA, Adésio*, SILVA, Marcelo B.*, GONTIJO, Ivoney*, VIEIRA, Henrique D.**, RAMALHO, José C.***,****, PARTELLI, Fábio L.*

*Federal University of Espírito Santo, UFES, São Mateus, ES, Brazil. **State University of Northern Rio de Janeiro, UENF, Campos dos Goytacazes, RJ, Brazil. ***Plant Stress & Biodiversity Group, DRAT, LEAF, ISA, Universidade de Lisboa, Portugal. **** GeoBioTec, FCT, UNL, Portugal. *Contact author: partelli@yahoo.com.br

RATIONALE
New promising genotypes of *Coffea canephora* Pierre ex A. Froehner have been selected by coffee growers, although without a statistical evaluation in field trials. Here, the development and productivity of 43 potential genotypes of *C. Canephora* grown at Espirito Santo and Bahia states, Brazil, will be evaluated, aiming at identify high potential genotypes for farmers.

METHODS
Plantations of *C. canephora* were set on May and June 2014 at the municipalities of Nova Venécia (state of Espírito Santo) and Itabela (Bahia state). Both fields are currently under development and the first harvest is expected on June 2016. Within the genotypes tested, 42 were propagated via cuttings and one from seeds.
The randomized block design was used with three blocks and seven plants on each experimental plot. Plant height and crown diameter were evaluated in order to characterize the initial development. The data were submitted to variance analysis, with averages pooled by the Scott-Knott test at a significance level of 5%. This work was supported by several coffee growers, CNPq and FAPES.

RESULTS, CONCLUSIONS & PERSPECTIVES
Plant height led to three plant groups: 5 genotypes classified as taller, 13 as intermediates and 25 as smaller plants, with average height of 1.68, 1.55 and 1.43 m, respectively. Considering the crown diameter, the plants were also pooled into three groups: 2 genotypes with greater diameter, 12 classified as intermediate and 29 with smaller diameter, with average diameter of 2.04, 1.75 and 1.54 m. Variability among these genotypes has already been found on seedlings [1] and it is very common to be found on a wide diversity of *C. canephora* [2, 3, 4].
This initial evaluation evidenced important variability among the genotypes, which could enable the selection of promising ones at the end of the experiment. In a second phase, selected genotypes may be registered as varieties and indicated to planting. For that, the following steps of this applied research aims at to assess the genotype productivities and bean quality characteristics along five years, generating scientific knowledge, building capacities (technicians) and advanced training (at graduation, MSc and PhD levels).

REFERENCES
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[4] Partelli FL, et al. (2014) RBCS, 38:214-222. DOI: 10.1590/S0100-06832014000100021.

PA177

Agrobacterium tumefaciens-MEDIATED TRANSFORMATION: AN EFFICIENT TOOL FOR PATHOGENICITY STUDIES ON *Colletotrichum kahawae*

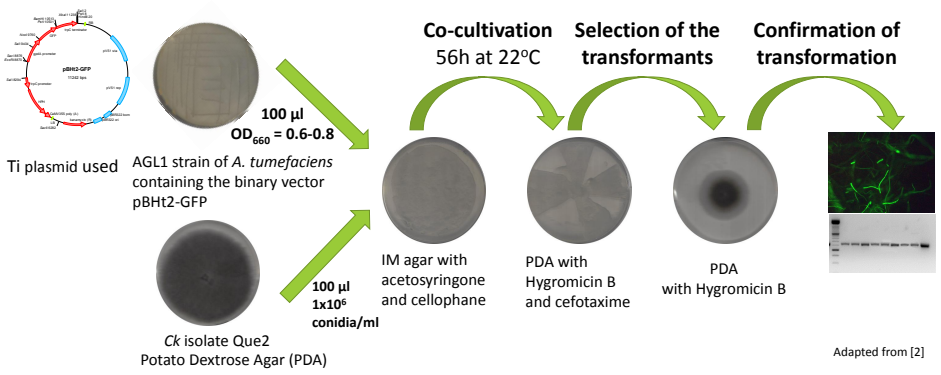
CABRAL, Ana[1,2], TALHINHAS, Pedro[1,2], NASCIMENTO, Teresa[2], VÁRZEA Vitor[1,2], SILVA, Maria do Céu[1,2] AZINHEIRA, Helena Gil[1,2], OLIVEIRA, Helena[2]

[1]CIFC- Centro de Investigação das Ferrugens do Cafeeiro, Instituto Superior de Agronomia (ISA), Universidade de Lisboa (ULisboa). Quinta do Marquês, 2784-505 Oeiras, Portugal
[2]LEAF- Linking Landscape, Environment, Agriculture and Food, ISA,ULisboa. Tapada da Ajuda, 1349-017 Lisboa, Portugal

Introduction

- Coffee Berry Disease, caused by the hemibiotrophic fungus *Colletotrichum kahawae* (*Ck*), is the major limiting factor to the *Coffea arabica* production in Africa, especially at high altitudes, where it can cause losses up to 80% if no control measures are applied. The possible introduction of this pathogen to other continents like America and Asia is a threat [1].
- A deep knowledge of pathogenicity mechanisms involved in the infection process may contribute to more rational and effective breeding programs.
- A powerful way to study pathogenicity mechanisms of fungi is to disrupt their genes.
- The heterologous integration of a DNA fragment causes a random process of gene disruption (insertional mutagenesis) and leads to the isolation of numerous fungal mutants exhibiting changes in pathogenicity.

Material and Methods



- Pathogenicity tests of the transformants were performed on detached green berries of susceptible *C. arabica* plants, according to [3] with slight modifications.
- Symptoms on the green berries were evaluated 5, 7, 10, 13 and 16 days post-inoculation (dpi) (Fig 1).

Fig. 1 – Scale of symptoms adopted to evaluate the progress of the infection (adapted from [3]).

- Conidial germination and appressoria formation were evaluated *in vitro* in a glass slide or *in vivo* with a nail polish replica technique [4].
- The most interesting transformants were selected for the identification of DNA segments flanking the T-DNA.

Results

- 75-150 transformants were generated with a concentration $1x10^5$ conidia per 90 mm diameter plate.
- The 173 transformants obtained were mitotically stable.
- All transformants were able to germinate and produce melanised appressoria, after 18-22h (Fig. 2).

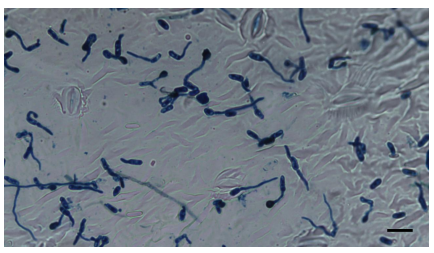


Fig. 2 – Conidial germination and appressoria formation. Bar = 20 µm

- Different aggressiveness patterns were observed among the transformants tested (Fig. 3).

Fig. 3 – Symptoms observed for three transformants after 13 days post-inoculation (dpi).

- 7% of the transformants tested exhibit reduced aggressiveness (<30% of the fruits presented symptoms). The transformant Que2-441 was non-pathogenic, as it was unable to produce symptoms on green berries, even on wounded fruits (Fig. 4)

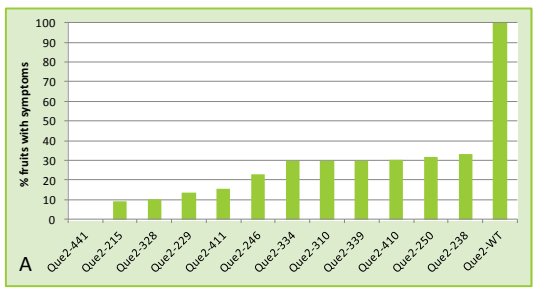


Fig. 4 – Pathogenicity tests of 12 transformants and control (Que2-WT). **A** - percentage of fruits observed with symptoms; **B** - Area Under the Disease Progression Curve, from 0 to 16 days post-inoculation.

- A "MFS_1 Major Facilitator Superfamily" was identified as a candidate disrupted gene in a transformant with reduced aggressiveness, suggesting its involvement in pathogenesis.

Gene Ontology	Description
Cellular component	integral component of membrane (GO:0016021)
Biological process	transmembrane transport (GO:0055085)

Conclusions

Ck was successfully transformed using *A. tumefaciens* thus facilitating the identification of Ck genes involved in pathogenesis. This will enable a better understanding of plant-pathogen interaction mechanisms, opening routes for the deployment of more informed plant protection strategies.

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UNVEILING THE COFFEE DEFENCE MECHANISMS AGAINST *Colletotrichum kahawae*

PA 178

SILVA Maria do Céu[1,2], DINIZ Inês[1,2], FIGUEIREDO Andreia[3], LOUREIRO, Andreia[1,2], GUERRA-GUIMARÃES Leonor[1,2], BATISTA Dora[1,2,4], AZINHEIRA Helena[1,2], PEREIRA, Ana Paula[1], TALHINHAS Pedro[1,2], OLIVEIRA Helena[2], PAIS Maria Salomé[3], GICHURU Elijah[5], VÁRZEA Vitor[1,2]

[1]CIFC - Centro de Investigação das Ferrugens do Cafeeiro, Instituto Superior de Agronomia (ISA), Universidade de Lisboa (UL). Oeiras, PT. [2]LEAF - Linking Landscape, Environment, Agriculture and Food, ISA/UL. Lisboa, PT, [3]BioISI - Instituto de Biossistemas e Ciências Integrativas, Faculdade de Ciências (FC), UL. Lisboa, PT, [4]Ce3c - Centre for Ecology, Evolution and Environmental Changes, FCUL, Lisboa, PT,[5] Coffee Research Institute, KALRO, Ruiru, KE

Introduction

Coffee Berry Disease (CBD) caused by the hemibiotrophic fungus *Colletotrichum kahawae* (Ck) is a major constraint for Arabica coffee production at high altitudes in Africa [1,2,3] representing a threat for its cultivation in Latin America and Asia. In green berries the disease symptoms are the formation of dark sunken lesions with sporulation (acervuli), followed by their premature dropping and mummification. 50-80% of crop losses are expected if no control measures are applied. Although plant breeding is an important strategy to produce coffee cultivars resistant to CBD, the basis of this resistance is still scarce.

Objectives

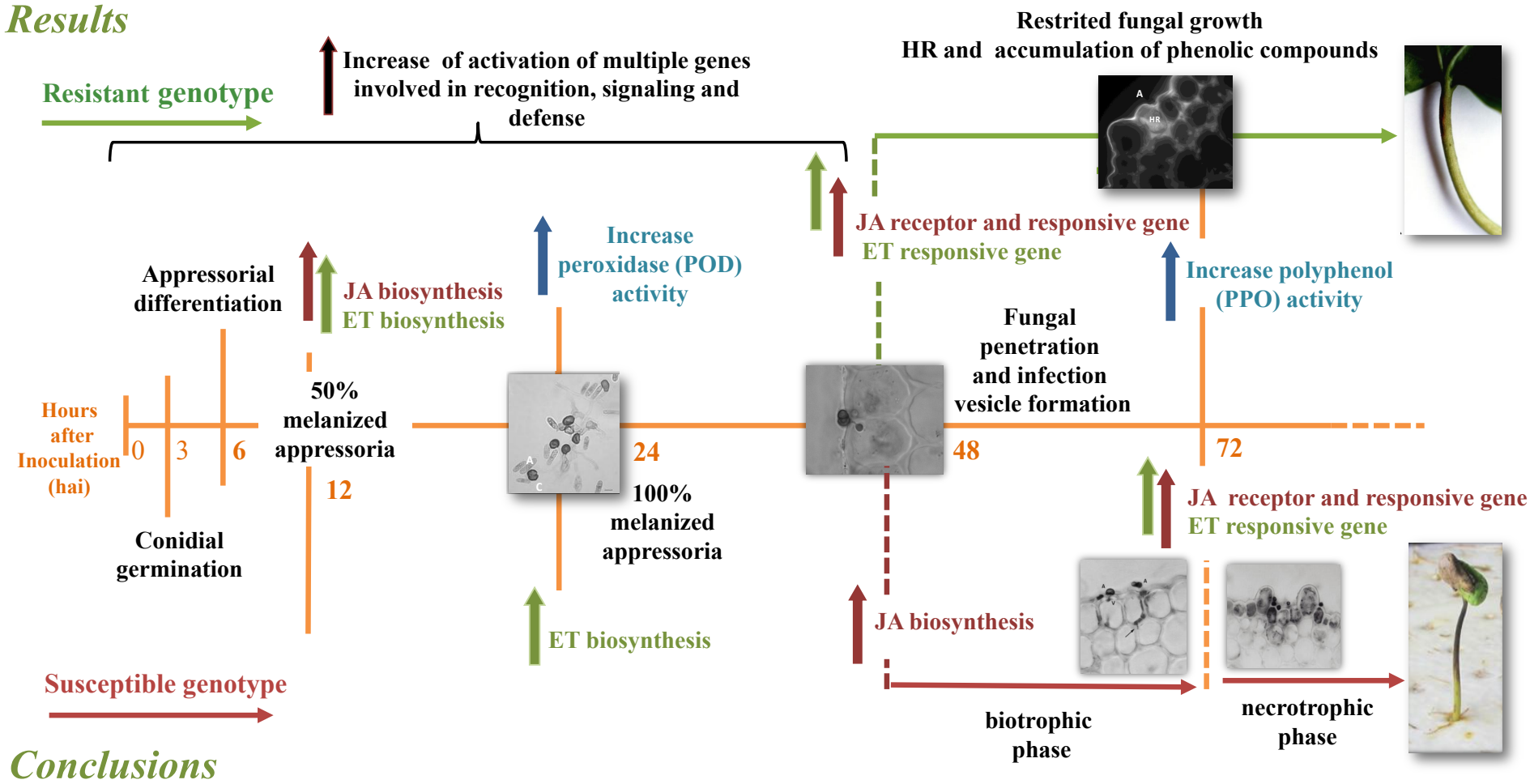
This programme intends to characterize the resistance mechanisms of the coffee genotype Catimor 88 used with success as sources of resistance to Ck in Kenyan breeding programmes comparatively with the susceptible variety Caturra. Thus, microscopic, biochemical and molecular analysis are being used to 1) quantify the fungal growth and early cellular host responses; 2) evaluate activities of oxidative enzymes; 3) study the expression profiles of genes putatively involved in coffee resistance mechanism.

Biological material
health and inoculated with Ck

Sample collection (6-72 hai) →

- **CITOLOGICAL STUDIES** — Light microscopy
- **BIOCHEMICAL STUDIES** — Spectrophotometry /IEF /PAGE
- **MOLECULAR STUDIES** — qPCR

Results



Conclusions

Coffee resistance to Ck is characterized by a restricted fungal growth associated with hypersensitive reaction (HR), accumulation of phenolic compounds an increase of peroxidase (POD) and polyphenol oxidase (PPO) activity. In the resistant genotype early activation of genes involved in recognition, signaling and defense was also observed. The monitorization of the jasmonic acid (JA) and ethylene (ET) pathways indicate that JA and ET signalling was activated earlier and before fungus penetration in the resistant genotype and rather later and coincident with the necrotrophic phase in the susceptible genotype. This integrative approach was able to shed some light on coffee defence mechanisms to Ck, although there is still much to learn about gene function, and ultimately to identify potential biomarkers for disease resistance.

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ACKNOWLEDGMENTS

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PA180

Why Coffee Farming has been on the decline in Zimbabwe

Gabriel Vusanimuzi Nkomo

Africa University. Zimbabwe

ABSTRACT

The lack of finance, expertise and machinery has hampered the growth of the industry in Zimbabwe. The Land Reform programme had a knock down effect too on the industry as some new farmers prefer to grow low cost crops such as maize instead of coffee that needs processing. New farm owners want instant profit while a coffee tree once planted takes three to five years to mature. Production has plummeted as the new land owners cannot secure bank loans to buy fertilisers or repair ageing infrastructure. Many of the farmers were new to the business, and lacked the expertise to keep quality high. Most of the country's coffee farmers lack cash reserves to support themselves when the crop fails or yields are low. There is need to train farmers and offer much-needed supplies such as fertilisers, irrigation systems and pesticides for the industry to grow in Zimbabwe.

RATIONALE

Production of coffee in Zimbabwe has declined over the years due to under capitalization and a lack of a formal body to represent the sector (Mhondera, 2013). In 1980 coffee production was 14 664 tonnes per season. However this has declined to 350 tonnes per seasons (Herald, 2010). The largest coffee processing plant in Zimbabwe had to close as deliveries dwindled for it to run profitably.

METHODS

A literature review of the coffee sector in Zimbabwe was done to assess the trends on why the coffee sector has been lagging behind especially in the smallholder farming sector especially after the Land Reform Programme in 2000.

RESULTS

Many of the farmers were new to the business, and lacked the expertise to keep quality high. Most of the country's coffee farmers lack cash reserves to support themselves when the crop fails or yields are low. In 1980 coffee production was 14 664 tonnes per season but this has declined to 350 tonnes per season (Zimbabwean Independent,2010). Many inexperienced farmers were given coffee farms despite the fact that the crop requires great skill. Apart from this, the government promoted maize and wheat production, leading to the destruction of coffee trees as farmers prepared to farm the targeted crops. Newly resettled farmers they did not have the expertise, so they did not continue with coffee farming as one has to be capacitated with coffee farming skills (The Zimbabwean,2015).

CONCLUSIONS & PERSPECTIVES

There is need to train farmers and offer much-needed supplies such as fertilisers, irrigation systems and pesticides for the industry to grow in Zimbabwe. However farmers are still unable to compete with better organised growers in countries such as Rwanda, Kenya and Malawi. Most of the profit in coffee farming in Zimbabwe goes to shippers, roasters and retailers. A review of property rights especially on land where large scale estates whose land was taken away needs to be done(The Zimbabwean,2015). Financing and incentives to the coffee industry need to be mooted and introduced. The government has to avail more land and help farmers access cheap loans so that many farmers in coffee producing region can revert to the crop. The involvement of other players in the coffee industry is set to benefit many smallholder farmers. Nongovernmental organizations such as World Vision and SNV have played a major role in the revival of coffee industry. The two obtained funds from USAID to revive the two value chains(Mudyazvivi,2011).

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PA184

Using local knowledge to link shade tree species composition to the provision of ecosystem services in coffee plantations in Yunnan Province, China

RIGAL, Clément[a,b], XU, Jianchu[a,b], VAAST, Philippe[b,c]

[a] Key Laboratory of Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Kunming, China
[b] World Agroforestry Centre
[c] CIRAD, UMR Eco&Sols, Montpellier, France

Background

Coffee farming in China's Yunnan Province only took off in the 1990's with the arrival of international buyers and the introduction of Catimor, a dwarf cultivar resistant to leaf rust. In the past 20 years, Arabica coffee production has been growing at a double digit rate to reach 1.8 million bags in 2013/2014. Coffee farming systems largely depend on intensive management practices under full sun conditions. Nonetheless, the local government has been promoting the use of shade trees in recent years, by distributing free tree seedlings to coffee farmers.

Method

Associated tree species in coffee estates were listed through on-farm tree species inventories. Local knowledge and key ecosystem services and disservices provided by these tree species were collected through interviews of farmers and ranking. Data were analyzed and ranked using the BradleyTerry2 package in R (Lamond et al 2016).

Key Result 1

160 tree species were identified in coffee farms amongst which 124 were indigenous. This high diversity is the result of both natural trees left uncut during recent episodes of forest land opening and natural regeneration during subsequent fallow land periods.

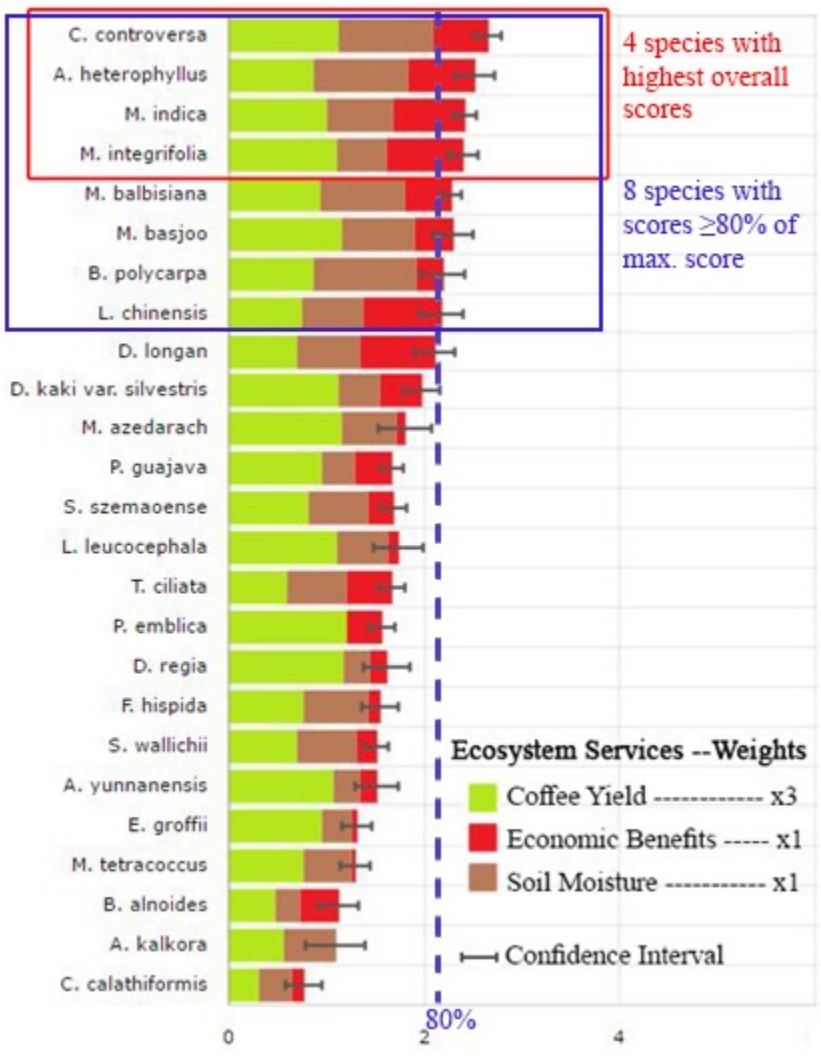

Figure 2: Scores and recommendations of shade tree species for Catimor, taking into account 3 services, with higher weight placed on coffee yield than on economic benefits and soil moisture enhancement provided by shade trees.

Detailed results available at www.shadetreeadvice.org

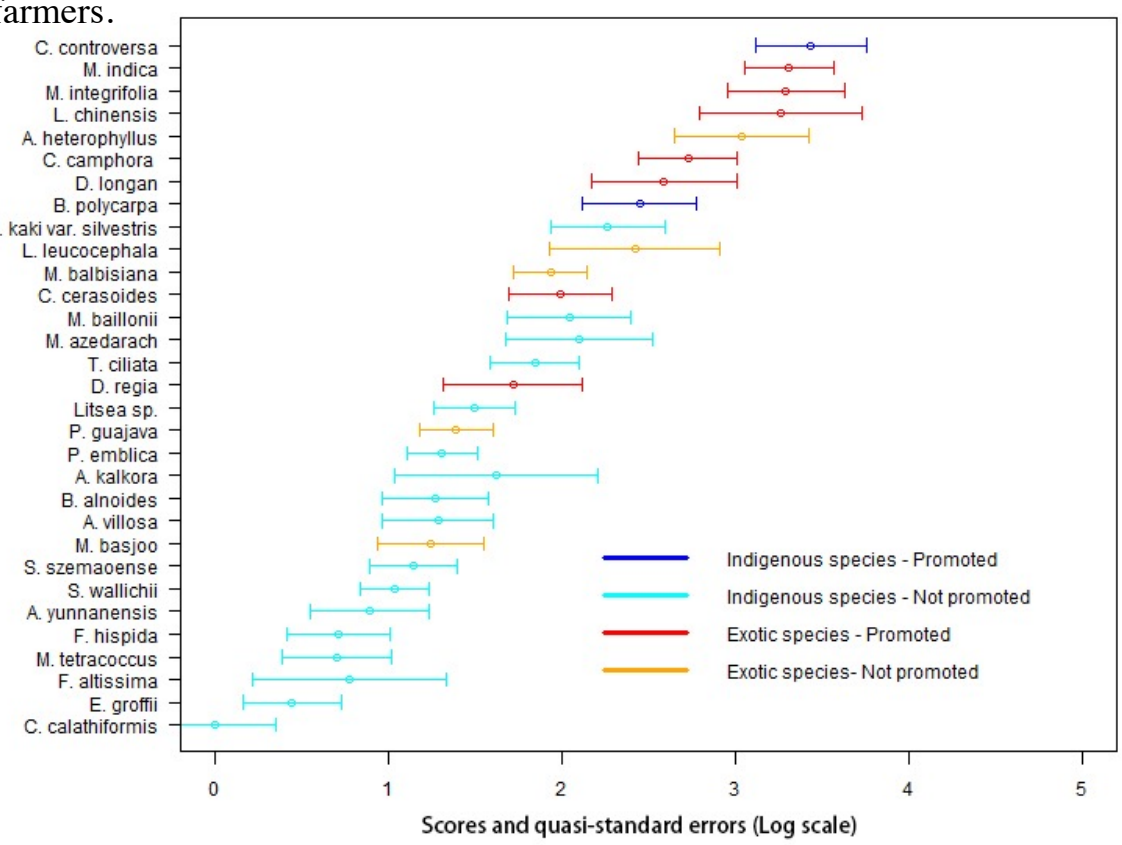

Figure 1: Scores and confidence intervals of shade tree species ranked according to overall preferences by coffee producers

Key Result 2

8 key local ecosystem services provided by shade trees according to rural communities: 1) protection against heat, 2) protection against cold, 3) soil erosion control, 4) soil moisture enhancement, 5) litter provision, 6) protection against white stem borer (*Xylotrechus quadripes*), 7) weed control and 8) economic benefits from shade trees. 2 key ecosystem disservices : 1) root competition with coffee trees, and 2) coffee yield reduction

Key Results 3

Promoted species are said to perform significantly better than non-promoted species for all studied ecosystem services ($p < 0.001\%$) except for root competition with coffee trees ($p > 35\%$).

Indication of a relevant selection of promoted species	Indication of a bias introduced by promotion activities
Coffee growers with higher diversity of shade tree species in their farms agree with the positive impacts of promoted species for protection from cold, erosion control and weed control ($p<1\%$)	Migrant coffee growers and those belonging to mountain ethnic minorities displayed a significant preference for promoted species compared to local farmers and those derived from valley minorities ($p<1\%$)

Box 1: Performances of promoted species

With increasing farm size, farmers perceived less positively the promoted trees in terms of protection from heat, soil moisture enhancement, root competition, weed control and coffee yield ($p<1\%$).
Gender, age and education are poor explanatory predictors of perceived performances of promoted trees.

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Practical method for non-destructive measurement of stem and leaf dry biomass in three diploid African *Coffea* species

PA186

Okoma P.1, Akaffou S.1, De Reffye P.2, Hamon P.3, Hamon S.3, Konan O.1, Kouassi K. H.1, Legnate H.4, Letort V.5, Sabatier S.1
1 Université Jean Lorougnon Guédé (UJLoG), B. P. 150, Daloa, Ivory Coast
2 CIRAD, UMR AMAP, F-34398 Montpellier, France
3 UMR DIADE, IRD, B.P. 64501, 34394 Montpellier Cedex 5, France
4 Centre National de Recherche en Agronomie, Divo, Ivory Coast
5 Ecole Centrale Paris, Department of Applied Mathematics, Chatenay-Malabry, France

Abstract:

Coffea **architecture is known to play a key role in production. Analysis have been undertaken in Côte d'Ivoire via GreenLab, a generic powerful function-structure plant model. It can compute hidden parameters like as organ source-sinks strength through direct measurements on plant. Thus architecture set (organogenesis) and biomass production could be perfectly assessed. Input data for growth analysis required dry biomass therefore plants were generally felled and the organs (internode, leaves and flowers) were cut and dry.**

We conducted analysis upon three diploids cultivated coffee species: ***Coffea canephora*** **(Robusta) designated as CAN,** ***C. liberica var. dewevrei*** **(DEW) and** ***C. liberica var. liberica*** **(LIB), to search a power allometric equation between fresh internode size and leaves area and their corresponding dry mass. Three compartments consisted of A1 (main stem), A2 (plagiotrop branches) and A3 (branchlets) axis were made. For each compartment fresh internode length and diameter and leaves size and area were measured.**

Two synthetic variables represented by internode volume and leaves area were calculated. Allometric relationships were performed between these synthetic variables and the corresponding dry mass. We found that internode volume was very highly related to it dry mass, as well in CAN as in DEW and LIB: R^2 > 98 %, 96 % and 91 % respectively for A1, A2 and A3 compartment. Also very high relationships were recorded between fresh leaf area and it dry mass whatever the species, R^2 ranging from 89% to 98% according to the compartments.

Hence this study provided evidence for a non-destructive measurement method to estimated dry mass.

Key words: Plant modeling, ***Coffea*****, Allometric relationship, Biomass, Architecture.**

INTRODUCTION

Coffea **architecture (Fig. 1) is known to play a key role in coffee trees production (Cilas** ***et al.*****, 2006). Thus the architecture analysis has been undertaken in Côte d'Ivoire via GreenLab, a generic powerful function-structure plant model (Yan** ***et al.*****, 2004). Input data required dry biomass therefore plants were generally felled and the organs (internode and leaves) were cut and dry. We searched a power allometric equation between fresh internodes and leaves size and their corresponding dry mass to avoid destructive measurements.**

Figure 1: Long branches and branchlets of CAN bearing numerous fruits

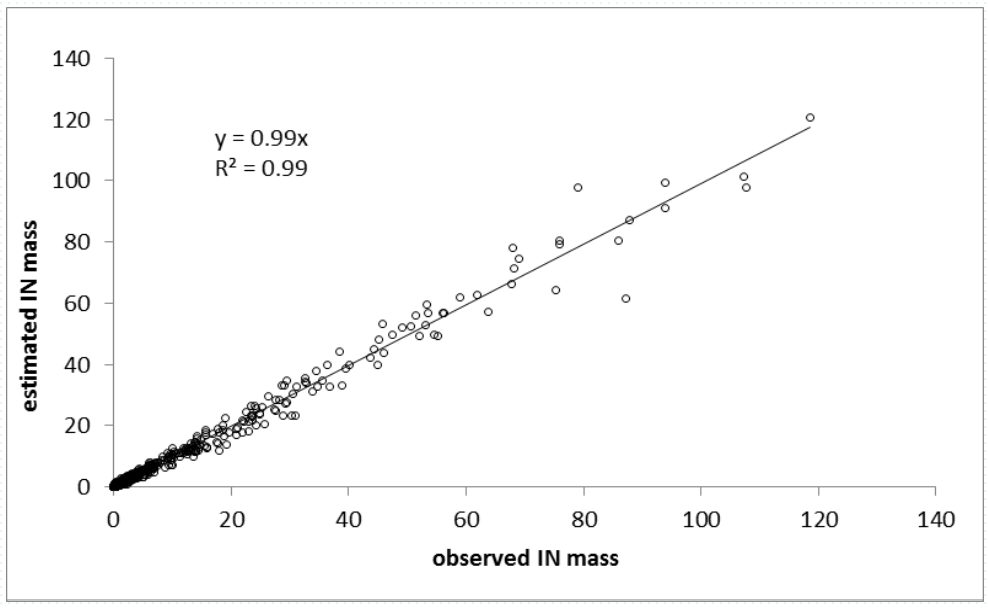

Figure 2: Relationship between observed and estimated internode dry mass

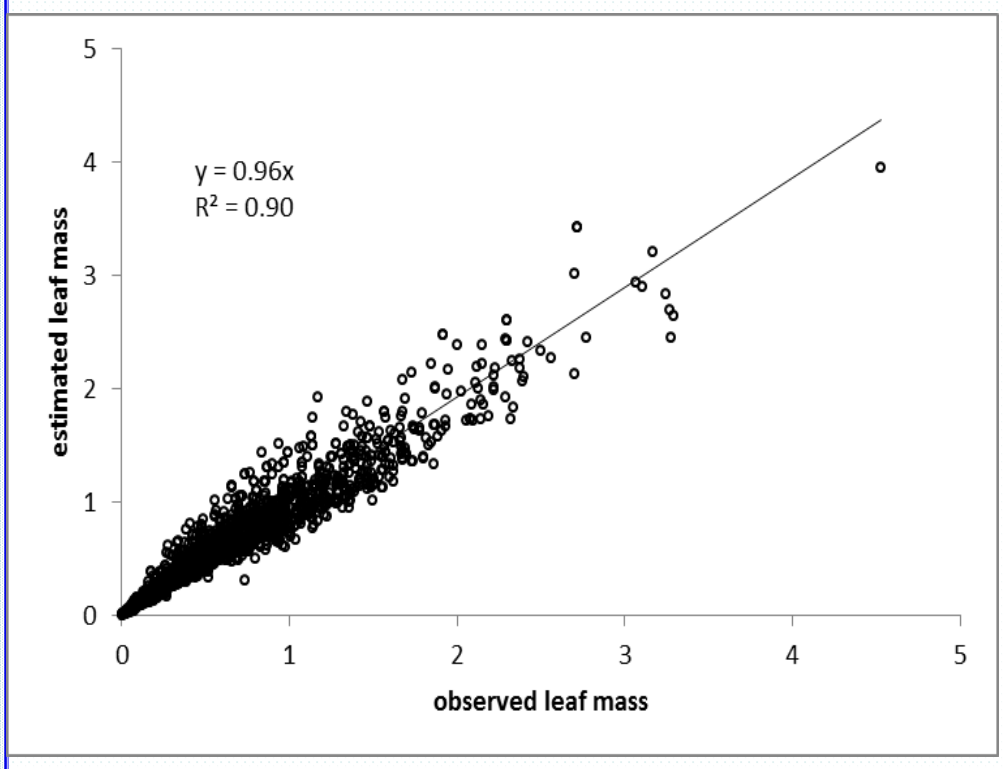

Figure 3: Relationship between observed and estimated leaf dry mass.

MATERIAL AND METHODS

Three genotypes of four years old for three diploids cultivated coffee species: ***Coffea canephora*** **(CAN),** ***C. liberica var. dewevrei*** **(DEW) and** ***C. liberica var. liberica*** **(LIB) were sampled. Three distinct compartments representing A1 (main stem), A2 (plagiotrop branches) and A3 (branchlets) axis were made. An average of 80 internodes and 16 leaves were observed in the A1 compartment per species while more than 160 internodes and 90 leaves were investigated for the A2 and A3 compartments. Allometric relationships were performed between the fresh internode volume V= 3.14 * IL * D^2/4 or the leaf area (L * l) and the respective corresponding dry mass. IL=internode length, D=internode diameter, L=leaf length and l=leaf wide.**

RESULTS

The internode dry mass was highly related to it volume, as well in CAN as in DEW and LIB whatever the compartment. The linear equation found was y=0.7x. The dry mass predicted was perfectly correlated with that measured (Fig. 2). For leaves, the length*width variable was a good estimator of leaf area. This latest variable showed also a high relationships with the dry mass throughout a power equation $y=0.007(L*l)^{1.02}$, whatever the compartment and the species, R^2 ranging from 89% to 98%. The estimated leaves dry mass from the power equation showed a good relationship with the measured leaves area as illustrated in the Figure 3.

CONCLUSIONS & PERSPECTIVES

This study provided evidence for a non-destructive measurement method to estimated dry mass. Validation should be done on young (two years) and old trees (five years). Contrasting species like as ***Coffea racesoma*** **should be investigated to gain further insights in allometrics relations.**

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THE PHYLLOCHRON UNDER ELEVATED AIR [CO_2] AND IRRIGATION IN ACTIVE GROWTH PERIOD OVER THE TREE HYERARCHY

ASIC 200

RAKOCEVIC, Miroslava*, ALVIM, Carolina A.**, CORREIA, Laís E.**, FILIZOLA, Heloisa F.**, MANZATTO, Celso V.**, BATISTA, Eunice R.**

*Embrapa Agriculture Informatics and Institute of Biology, University of Campinas, Campinas, SP, Brazil, mimarako@unicamp.br, **Embrapa Environment, Jaguariúna, SP, Brazil*

Abstract

Climate forecasts suggest that [CO_2] in atmosphere will continue to increase. Plant growth dynamics regarding elevated [CO_2] climate changing could be estimated by phyllochron, which is defined as a time interval between the appearances of two successive leaves. Leaf appearance rate is considered a major component of crop yield, since it contributes to crop leaf area index, which determines the interception of solar radiation used for CO_2 assimilation and biomass production. The hypothesis of this study was that the hierarchy over the same tree could have the impact on time intervals of leaf emission, while the elevated air [CO_2] and irrigation would promote the phyllochron in adult Arabica coffee plants.

In adult Arabica coffee plants, about 78% of vegetative growth occurs in the warm, rainy season, named active growth period. Coffee plants were grown, started from winter 2011, under actual (~390 µL CO_2 L^{-1}) and elevated [CO_2] (actual + 200 µL CO_2 L^{-1}) in Free-Air-CO_2-Enrichment (FACE) facility, Jaguariúna, Southern Brazil. Trees of 'Catuaí IAC 99' were codified in dynamic multiscale-tree-graphs, from October 2015 to March 2016, following the VPlants methodology. Leaf emission was observed in a frequency of 15 to 20 days, covering 14 growth dates of irrigated (IRR) and non-irrigated (NI) plants, under actual (a[CO_2]) and elevated air [CO_2] (e[CO_2]). In order to integrate the effect of temperature on leaf appearance, the phyllochron was expressed as a function of accumulated thermal time (i.e. in growing degree-days – GDD, °C day $leaf^{-1}$). The phyllochron was estimated on five branching orders, regarding orthotropic trunks (1st order axes) and 2nd to 5th order plagiotropic axes.

The GDD required to emit one new phytomer varied depending on branching order. The 1st and 2nd order axes did not differ in phyllochron, requesting lower thermal time for one phytomer emission than the 3rd and 4th order ones. The thermal time requested for leaf pair emission on 1st order axes was strongly reduced under irrigation and e[CO_2], requiring in average 253 °C day $leaf^{-1}$ compared to 377 °C day $leaf^{-1}$ under a[CO_2]-NI. The request for leaf pair emission on 2nd and 3rd order axes was modified by their position over the vertical profile and water supply. The phyllochron on 2nd order axes was significantly lower under irrigation in the middle growth zone of orthotropic axes (50 – 60th ranks) and higher in zones that defined the initial and final 1st order ranks that born 2nd order axes. The requested GDD for leaf pair emission on 3rd order axes gradually reduced from the initial to the final 1st order ranks that born 3rd order axes.

The results suggest that the architecture development in coffee plants is hierarchically organized by the phyllochrons and modified by [CO_2] and water supplies. The phyllochron under FACE facility will be observed in longer period to improve the knowledge about complex orchestration in structural development of Arabica coffee plants under elevated air [CO_2] regarding the rates of phytomer growth and mortality.

Introduction

Air [CO_2] has increased from 1 to 1.8 µL CO_2 L^{-1} $year^{-1}$ (Hillel and Rosenzweig). Phyllochron is defined as a time interval between the appearances of two successive leaves (Erickson and Michelini, 1957). Growth patterns of successive leaves vary with plant ontogeny (Lemaire et al., 2009). The length of 2nd order axes showed that about 78% of vegetative growth in Arabica coffee occurs in the warm, rainy season (October to March, active growth), and 22% during the cool, dry season (April to September, reduced growth) – (Silva et al., 2004).

The hypothesis of this study was that the hierarchy over the same tree could have the impact on time intervals between two subsequent leaf appearances, while the elevated air [CO_2] and irrigation would modify the phyllochron in adult Arabica coffee plants.

Table 1: ANOVA p-values for effects of axes orders, concentration of CO_2 (actual~390 µL CO_2 L^{-1} and elevated = actual + 200 µL CO_2 L^{-1}) and irrigation on phyllochron (GDD, °C day $leaf^{-1}$) during the active Arabica coffee growth period (October 2015 to March 2016).

Factors	numDF	p-value
(Intercept)	1	**<.0001**
CO_2	1	0.6842
Irrigation	1	0.9408
Axes order	3	**0.0001**
CO_2: Irrigation	1	0.6996
CO_2: Axes order	3	0.3075
Irrigation: Axes order	3	0.3114
CO_2: Irrigation: Order	3	**0.0694**

Table 2: ANOVA p-values for effects of concentration of CO_2 (actual~390 µL CO_2 L^{-1} and elevated = actual + 200 µL CO_2 L^{-1}) and irrigation on phyllochron (GDD, °C day $leaf^{-1}$) over the axes order during the active Arabica coffee growth period (October 2015 to March 2016).

Factors	Order 1	Order 2	Order 3	Order 4
CO_2	**0.011**	0.4515	0.8779	0.4708
Irrigation	**0.042**	0.3246	0.8186	0.5759
CO_2: Irrigation	**0.073**	0.2138	0.6735	0.3359

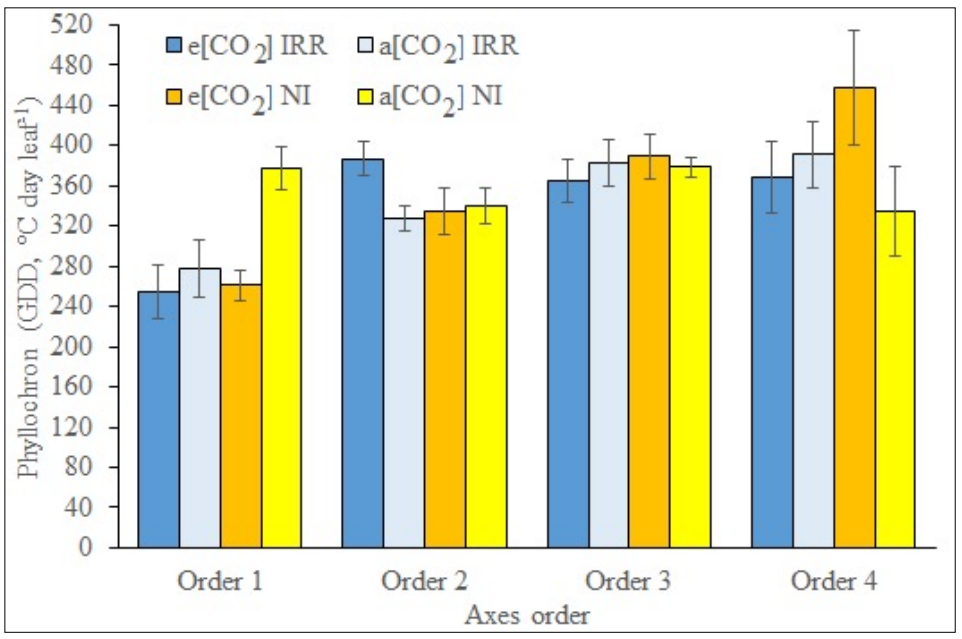

Figure 1: Mean values and standard error of phyllochron (GDD, °C day $leaf^{-1}$) during the active Arabica coffee growth period (October 2015 to March 2016). Impacts of axes order, concentration of CO_2 (actual~390 µL CO_2 L^{-1} and elevated = actual + 200 µL CO_2 L^{-1}) and irrigation (IRR – irrigated, NI – without irrigation) were observed.

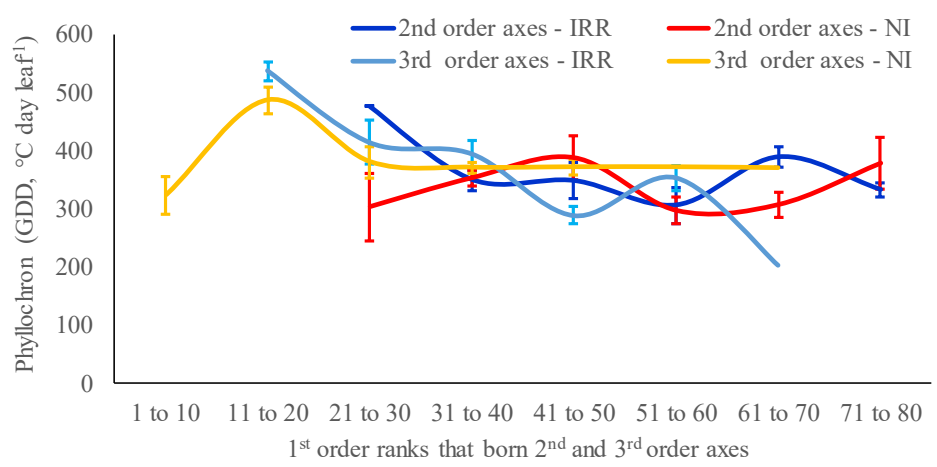

Figure 2: Mean and standard error for phyllochron (GDD, °C day $leaf^{-1}$) over the vertical profile (each 10 phytomers of 1st order axis). Impact of irrigation (IRR – irrigated, NI – without irrigation) during the active Arabica coffee growth period (October 2015 to March 2016).

The GDD required to emit one new phytomer varied depending on branching order (Table 1; Figure 1). The thermal time requested for leaf pair emission on 1st order axes was strongly reduced under irrigation and elevated [CO_2], requiring in average 253° C day $leaf^{-1}$ compared to 377° C day $leaf^{-1}$ under the treatment without irrigation and actual [CO_2] (Table 2; Figure 1). The request for leaf pair emission on 2nd and 3rd order axes was modified by their position over the vertical profile and water supply (Figure 2). The phyllochron on 2nd order axes was significantly lower under irrigation in the middle growth zone of orthotropic axes (50 – 60th ranks) and higher in zones that defined the initial and final 1st order ranks that born 2nd order axes. The requested GDD for leaf pair emission on 3rd order axes gradually reduced from the initial to the final 1st order ranks that born 3rd order axes.

Conclusion

The results suggest that the architecture development in coffee plants is hierarchically organized by the phyllochrons and modified by [CO_2] and water supplies. The phyllochron under FACE facility will be observed in longer period to improve the knowledge about complex orchestration in structural development of Arabica coffee plants under elevated air [CO_2] regarding the rates of phytomer growth and mortality.

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SOIL PROPERTIES UNDER ARABICA COFFEE CULTURE IN LONG-TERM FACE EXPERIMENT

ASIC PA201

FILIZOLA, Heloisa F.*, GONÇALVES, José Ricardo P.*, de SOUZA, Manoel D.*, RAKOCEVIC, Miroslava**

*Embrapa Environment, Jaguariúna, SP, Brazil; **Embrapa Agriculture Informatics and Institute of Biology, University of Campinas, Campinas, SP, Brazil, mimarako@unicamp.br.

Abstract

The hypothesis of this study was that the physical soil properties, both with soil N and C, would suffer modifications over the soil profile during Brazilian FACE experiment established to follow Arabica coffee and ecosystem responses to rising [CO_2].

Coffee plants were exposed to elevated air [CO_2] under FACE facility from winter 2011. Plants were grown under actual (~390 µL CO_2 L-1, named a[CO_2]), and elevated air [CO_2] (actual + 200 µL CO_2 L-1, named e[CO_2]). The soil was collected in all 12 octagonal plots exposed to e[CO_2] and a[CO_2] in January 2013, and in two particular plots in July 2015.

Values of soil porosity were reduced from 2013 to 2015, and reductions were more expressed under a[CO_2] than e[CO_2]. The soil density was higher under e[CO_2] than a[CO_2] in 2013, and situation was inversed in 2015.

Total C and N content in 2015 were higher under e[CO_2] than a[CO_2] in all observed depths with exception of the 0 - 5 cm where the response to air [CO_2] was inverted. In a case of C, this inversion was due to inorganic C content, since the organic C did not differ between two air [CO_2] treatments for this soil layer.

Introduction

- **Ecosystem responses to rising [CO_2] are a major source of uncertainty in climate change projections. The artificial facilities, as Free-Air-CO_2-Enrichment-systems (FACE) are suitable for investigating the plant responses and the effects of elevated [CO_2] on whole ecosystems. The hypothesis of this study was that the physical soil properties, both with soil N and C, would suffer modifications over the soil profile during Brazilian FACE experiment established to follow Arabica coffee and ecosystem responses to rising [CO_2].**

Material and Methods

- **Coffee plants were exposed to elevated air [CO_2] under FACE facility from winter 2011. Plants were grown under actual (~390 µL CO_2 L^{-1}, named a[CO_2]), and elevated air [CO_2] (actual + 200 µL CO_2 L^{-1}, named e[CO_2]). The soil was collected in all 12 octagonal plots exposed to e[CO_2] and a[CO_2] in January 2013, and in two particular plots in July 2015. The sampling in Kopeck rings included 5 - 7 soil depths from 0 - 60 cm, effectuated 30 cm from coffee lines.**
- **Granulometry analyses were performed by pipette method based on Stoke's law, bulk density and porosity were analyzed on tension table, total C and N content were determined on Leco Truspec CHN, while organic C was combusted in muffle furnace. Analyses were made in bi- and triplicates.**

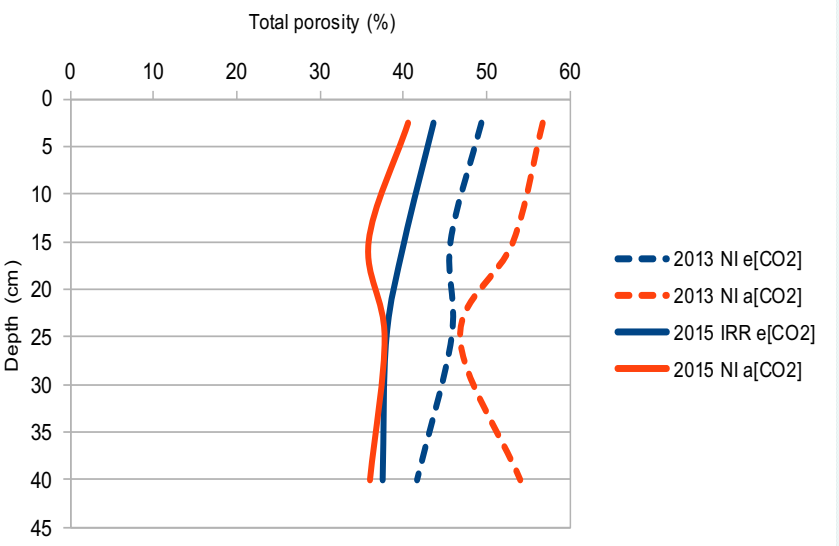

Figure 1: Total soil porosity variation in depth. IRR: irrigated; NI: without irrigation; e[CO_2]: elevated air [CO_2]; a[CO_2]: actual air [CO_2].

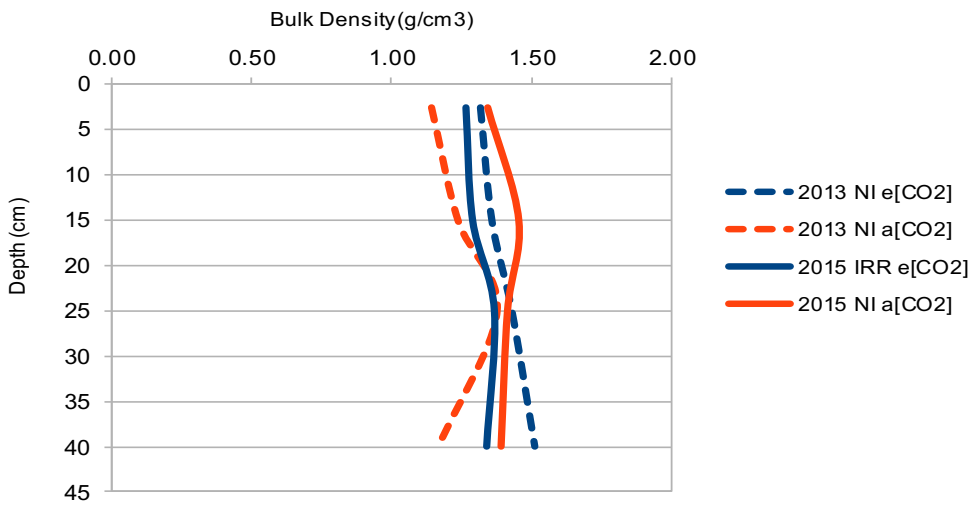

Figure 2: Bulk density variation in soil depth. IRR: irrigated; NI: without irrigation; e[CO_2]: elevated air [CO_2]; a[CO_2]: actual air [CO_2].

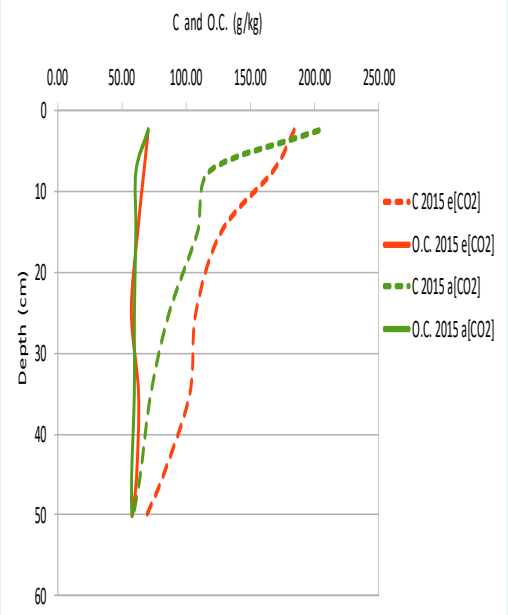

Figure 3: Variation in soil depth of total carbon (C), and organic carbon (O.C.) - e[CO_2]: elevated air [CO_2]; a[CO_2]: actual air [CO_2].

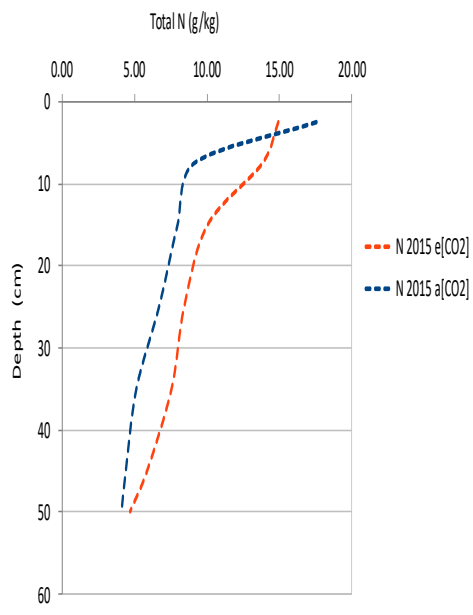

Figure 4: Nitrogen variation in soil depth - e[CO_2]: elevated air [CO_2]; a[CO_2]: actual air [CO_2].

- **Soil macro-, micro- and total-porosity (Fig.1) were higher under a[CO_2] than e[CO_2] in 2013. The situation was inverted in 2015, showing higher porosity under e[CO_2] than a[CO_2] that diminished gradually to 40 cm of depth. Values of soil porosity were reduced from 2013 to 2015, and reductions were more expressed under a[CO_2] than e[CO_2].**
- **The soil bulk density was higher under e[CO_2] than a[CO_2] in 2013, and situation was inversed in 2015 (Fig. 2). The compaction occurred in plowed layers (0 - 20 cm), because the soil management and weed control in octagonal rings were executed manually, while the physical structure in the beginning of 2013 still had the impacts of experiment establishment. The soil humidity was higher in e[CO_2] than a[CO_2] only in the dry winter period of 2015. In the dry winter period of 2015, the plant leaf area under e[CO_2] was about 50% lower than a[CO_2] (Rakocevic et al., in press), likely associated with more violent coffee leaf rust disease under e[CO_2].**
- **Total C (Fig. 3) and N content (Fig. 4) in 2015 were higher under e[CO_2] than a[CO_2] in all observed depths with exception of the 0 - 5 cm where the response to air [CO_2] was inverted. In a case of C, this inversion was due to inorganic C content, since the organic C did not differ between two air [CO_2] treatments for this soil layer (Fig. 3).**

Conclusions

- **After long-term experiment, soil physical properties were improved under e[CO_2] compared to a[CO_2].**
- **Lost leaf mass could better preserve the soil humidity, permitting quicker leaf C decomposition on soil surface and slower in deeper soil layers.**
- **The higher C and N contents over the soil profile under e[CO_2] suggest the presence of higher mass of microorganisms, small animals, and coffee roots formation and decomposition that have to be investigated in the future.**

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Article 210

AUTOMATION IN ECOLOGICAL ATTRIBUTES OF COFFEE LEAF RUST OCCURRENCE

CUNHA, Luiz Manoel S*, MEIRA, Carlos A. A.*, RAKOCEVIC, Miroslava**

* Embrapa Agriculture Informatics, Campinas, SP, Brazil; ** University of Campinas, SP, Brazil, mimarako@unicamp.br

Abstract

This work shows the application of six attributes selection methods in the building of predictive models, by using decision tree for analysis of coffee leaf rust occurrence disease (*Hemileia vastatrix* Berk. & Br). We used one complex data set contained 300 registers, divided in four data sub-sets, including meteorological data, periods of infection and infection rates. The scenario (model) of high fruit load and 5p.p (point percent) of leaf rust infection rate, which used the Wrapper method of selection that included 12, 15 and 16 features, had the highest accuracy of prediction (71%, 71% and 81%). The lowest accuracy in prediction of coffee leaf rust disease (59.3%) was obtained for high load and high (10p.p) leaf rust infection rate.

Introduction

The occurrence of coffee leaf rust disease (*Hemileia vastatrix* Berk. & Br.) (Zambolim, 2016) has the impact on leaf drop and consequently, on coffee bean production. Diverse mathematical and computational methods, as are decision trees and ensembles, are used to model the prediction of coffee leaf rust related to web of ecological data. The features for models are selected based on the expert knowledge, including meteorological data, periods of infection and infection rate, and derived combined features. The hypothesis of our study was that the methods of automated selection can improve the model accuracy compared to selection based only on expert knowledge (EKS). The aim this works was select the key attributes using automated methods for the building of predictive models for coffee leaf rust by decision tree (Hall et al., 2009).

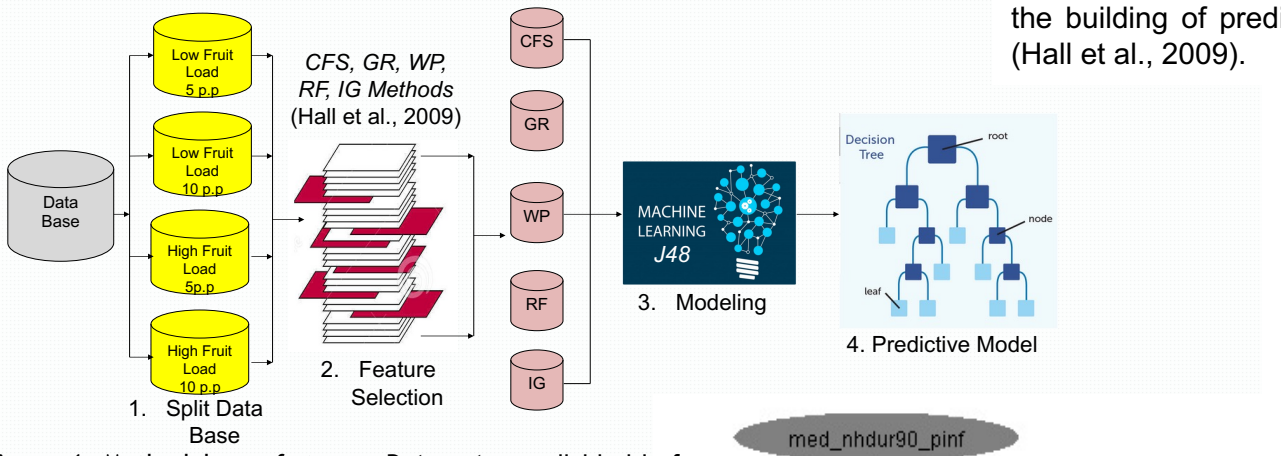

Figure 1: Methodology of stages. Data set was divided in four data sub-sets (1). Feature automated selection (2) was based on CFS, GR, WP, RF and IG methods. Tree decision modeling (3) produced the predictive models (4).

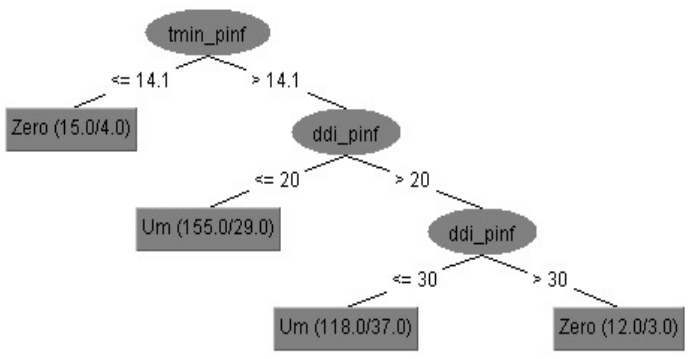

Figure 3: Model of intermediate accuracy with two automated selected features (the average daily minimum temperatures at the infection period – tmin_pinf and number of unfavorable days of infection at the infection point – ddi_pinf. The features are represented by elliptic forms in model flux chart.

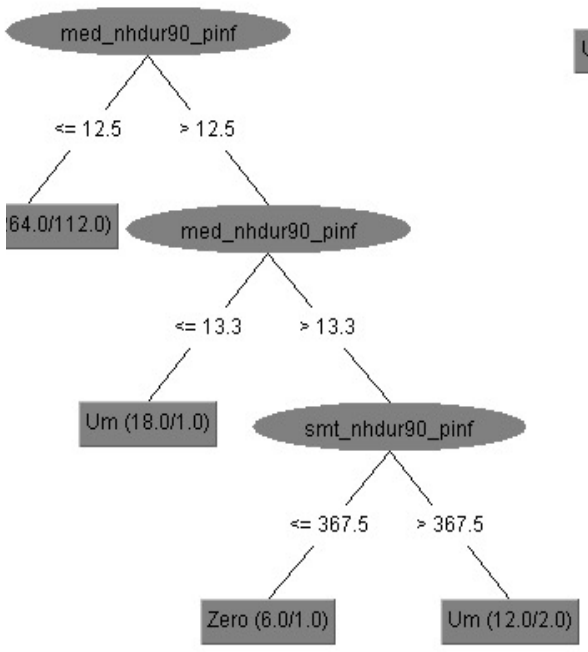

Figure 4: Model of low accuracy with two automated selected features (average number of daily hours with relative humidity ≥90% at the infection point - med_nhdur90_pinf) and sum of number of daily hours with relative humidity ≥90% at the infection point – smt nhdur90 _pinf) . The features are represented by elliptic forms in model flux chart.

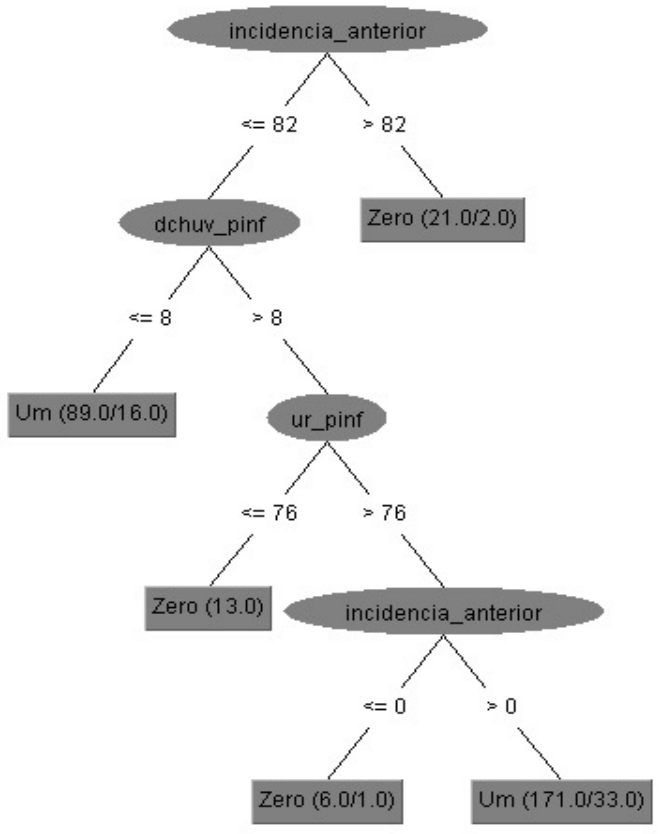

Figure 2: Model of high accuracy with three automated selected features (previous incidence of crop value – incidencia_aterior, days of rain during the infection period – dchuv_pinf and average relative air humidity – ur_pinf). The features are represented by elliptic forms in model flux chart.

Results and Discussion

Using the decision tree methodology and key attributes selection (Figure 1) predictive models of varied accuracy for coffee leaf rust were created (Figures 2 – 4). The inclusion of automated methods of feature selection, allowed that even scenarios with lower number of selected features (two or three – Figures 2-4) produced models of higher accuracy than those with all 16 features selected by EKS. The scenario of high fruit load and 5p.p of leaf rust infection rate, using WP method of selection with 12, 15 and 16 features had the highest accuracy of prediction (71%, 71.7% and 81% respectively). The most distinguished features were the incidence of crop value in the previous month (Figure 2), the average daily minimum temperatures at the infection period (Figure 3), the number of unfavorable days of infection at the infection point (Figure 3) and the average relative humidity at the infection period (Figure 2). The lowest accuracy in prediction of coffee leaf rust disease (59.3%) was obtained for high load and high (10p.p) leaf rust infection rate.

Conclusion

The feature selection by mathematical methods can improve the accuracy of decision tree models in prediction of coffee leaf rust disease and contribute to building more understandable prediction models. In future model development, leaf rust disease and meteorological data from wider Arabica coffee production areas, as well as other derived combined features, as growth degree days, and regrouping of normal *versus* excessive year impacts, as those of El Niño and La Niña effects, will be considered for fuzzy system predictions.

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GENETIC DIVERSITY OF COFFEE GERMPLASMS BY ISSR MARKERS

PB200

YAN, lin , HUANG, lifang*,WANG, xiaoyang*,DONG, yunping*,ZHOU, hua**, CHEN jinhuan***, CHEN, peng*.*

Spice and Beverage Crops Research Institute, CATAS, Wanning, Hannan,China, Dehong Institute of Tropical Agriculture in Yunnan Province, Ruli, Yunnan, China,***Institute of Tropical and Sub-tropical Cash Crops, YAAS,Baoshan,Yunnan,China.*

Abstract

To assess the genetic diversity of coffee germplasm accessions and some taxonomic problems of *Coffea* genus, the genetic diversity of 159 coffee accessions was analyzed using 19 inter-simple sequence repeat (ISSR) markers. Based on a clustering graph using PUGMA, 159 resources could be divided into 3 groups at the level of 0.65. Nine accessions in C.excelsa and C. liberica were in the first group. All of accessions in C arabica were in the second Group. A total of 33 accessions in C. Canephora and one accessions from Fujian were in the third Group.

Introduction

Coffee (*coffea* spp.) is one of the main economic crops in tropic China, and has a significant contribution to Chinese economy. However, there is little information on the genetic diversity of coffee varieties in China. In this study, the genetic diversity were evaluated among coffee genetic resources by ISSR marker technique.This would provide an important scientific basis for coffee germplasm identification, conservation and molecular breeding of coffee.

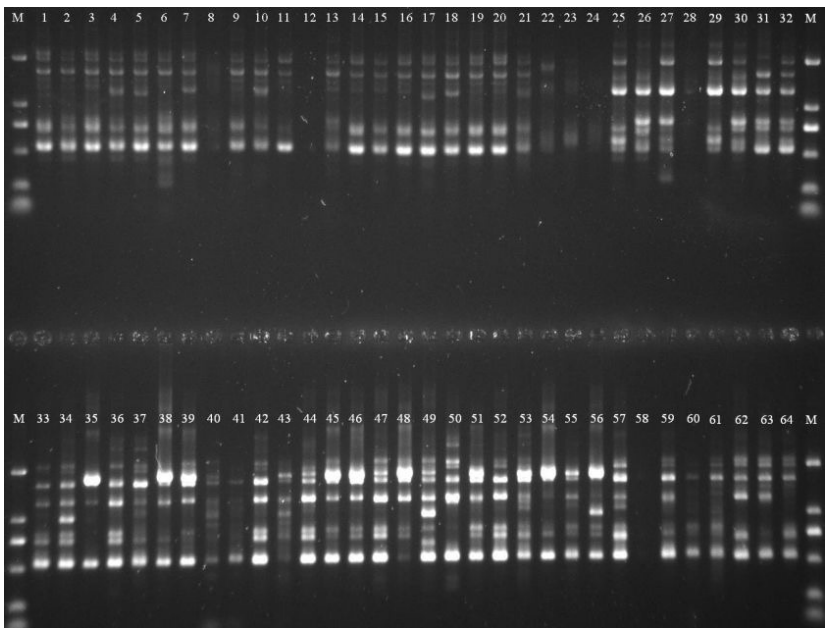

Figure 1: ISSR fingerprinting of the coffee germplasm resources with primer UBC843. M is a DL2000 Ladder DNA marker while lanes 1–64 are coffee accessions.

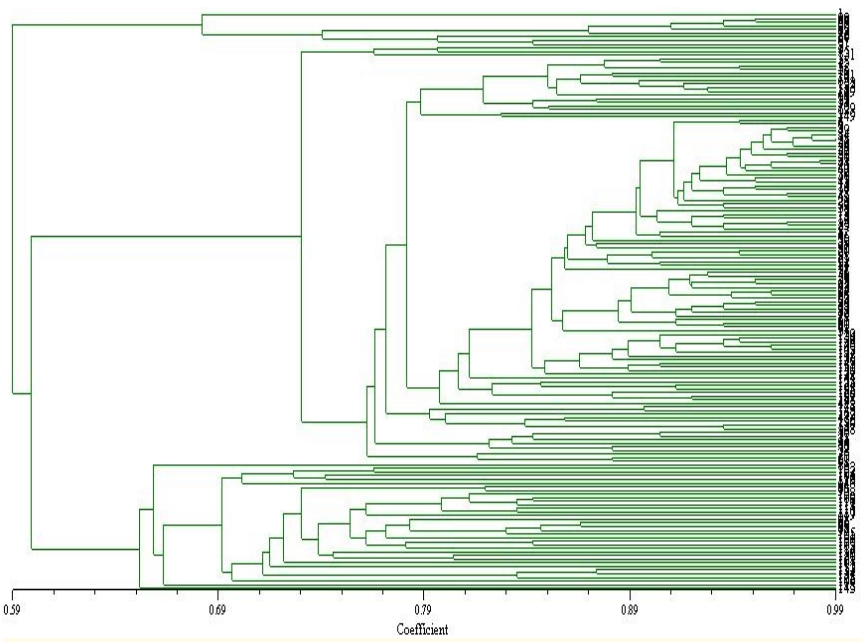

Figure 2: Clustr dendrogram of 159 coffee accessions based on ISSR

Main Text

Amplifications across the genus: According to the 100 pairs of ISSR primers, the 19 effective primer pairs were selected to amplify 159 coffee accessions. A total of 145 fragments were amplified ranging from 0.2-2.1kb, and the number of fragments per primer ranged from 4 to 15 with a mean of 7.6 fragments per primer. 128 out of 145 fragments were polymorphic and the rate of polymorphic reached 88.3%.

Genetic diversity analysis: Based on a clustering graph using PUGMA, 159 resources could be divided into 3 groups at the level of 0.65 of similarity coefficient. Nine accessions in *C. excelsa* and *C. liberica* were in the first group. All of accessions in C. arabica were in the second Group. A total of 33 accessions in *C. Canephora* and one accessions from Fujian were in the third Group. At 0.63 level of similarity coefficient, *C. arabica* and *C. Canephora* are classified into the same cluster, this proves that the phylogenetic relationship between them is relatively closer.

Conclusion

The coffee genetic relationship of species could be easily divided, a genetic diversity existed and taxonomic status of resources had no correlation with geographical origin. There were large genetic differences among species (*C. arabica, C. canephora, and C. liberica*), and the small genetic differences within species, especially within Coffea arabica. The ISSR markers was an available method to study the genetic diversity of Coffea germplasm resources.

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PB204

PHENOTYPIC VARIABILITY OF CULTIVATED Coffea canephora and WILD COFFEE IN KAGERA REGION, TANZANIA

NG'HOMA Nyabisi, M[1], KUSOLWA Paul[2], MAMIRO P[2], and KILAMBO Deusdedit L[1]
[1] Tanzania Coffee Research Institute (TaCRI) Lyamungu, P.O.Box 3004 Moshi, Tanzania
[2] Department of Crop Science and Horticulture, P.O.Box 3005, Sokoine University of Agriculture, Morogoro, Tanzania mm

Abstract

Characterization of crops by using morphological traits is one of techniques has been used by breeders to discriminate/ differentiate varieties. A number of descriptors have been developed to differentiate genotypes of the same species. In this study leaf length, width, petiole length, stipule aristae length, fruit length, thickness, width, fresh dry ratio, out turn ratio, proportion of dry cherries to green beans, yields, bean sizes, and 100 bean weights were used to assess the variability of genotypes of 104 cultivated *C. canephora* and 20 wild coffee. Variations were observed in the lengths of stipule aristae, leaf petiole, fruits, fruits, width, fruit thickness, and proportions of fresh cherries to green beans, bean sizes, coffee indicating the highest genetic diversity of cultivated robusta and wild coffee and 100 bean weights among the genotypes cultivated *C. canephora* and wilding. Heritability analysis revealed that most of morphological traits *C. canephora* and wild coffee had the highest heritability percent

Introduction

MorpMorphological characterizations for crops have been used by scientists and gene bank curators for discriminations of germplasm materials based on phenotypic expressions using internationally accepted descriptors. Characterization descriptors show distinctive characters of accessions and the highly heritable characters that are easily seen by eyes and are equally expressed in all environments. Study of the morphological characters provides an efficient useful tool to select the most important characters to be considered in breeding programme.For coffee a number of descriptors have been developed to study the variations among coffee germplasm accessions (IPGRI, 1996; Walyaro, 2006; Sureshkumar *et al*.., 2013) The present study was conducted to assess the diversity of cultivated C. canephora and wild coffee using the morphological traits.

Materials and methods

An experiment was conducted for three years consecutively at Tanzania Coffee Research Institute (Maruku substation in Kagera region in Tanzania. Data were collected from 104 accessions of cultivated *C. canephora* and 20 accessions of wild coffee. Coffee characters assessed included Lengths of stipule aristae, leaves, leaf petioles, fruit, seeds widths of leaves, fruits, seeds, thickness of fruits, seeds, yields, fresh to dry cherries, out turn ratios, bean sizes and 100 bean weights.. Data were subjected to one way of analysis of variance (ANOVA-1), principal components analysis (PCA) and broad sense heritability using quantitative traits loci / Linkage associations by using the Gen Stat statistical software packages..

Results and Discussion

Variations ($P \leq 0.001$) in the lengths of stipule aristae, leaf, leaf petioles and fruits were observed among 124 genotypes. Significant variations ($P \leq 0.001$) were observed in the width of leaves, fruits, and seeds, yields, out turn ratio, proportion of fresh to dry cherries, proportion of dry cherries to green beans, bean sizes and 100 seed weights of 104 cultivated *C. canephora* and wild coffee genotypes. Heritability analysis revealed that most of morphological traits evaluated had high heritability percent. The overall heritability per morphological trait was: stipule length (67.43%), leaf length (72.72%), leaf width (58.39%), leaf petiole (66.61%), fruit length (79.11%), fruit width (82.93%), fruit thickness (76.14%), yield 18.145% and the proportion of dry cherries to green beans (31.92%) .

Figure 1: Morphological characteristics of wild coffee from Bushenyi forest

Figure 2: Morphological characteristics of selected cultivated C. canephora

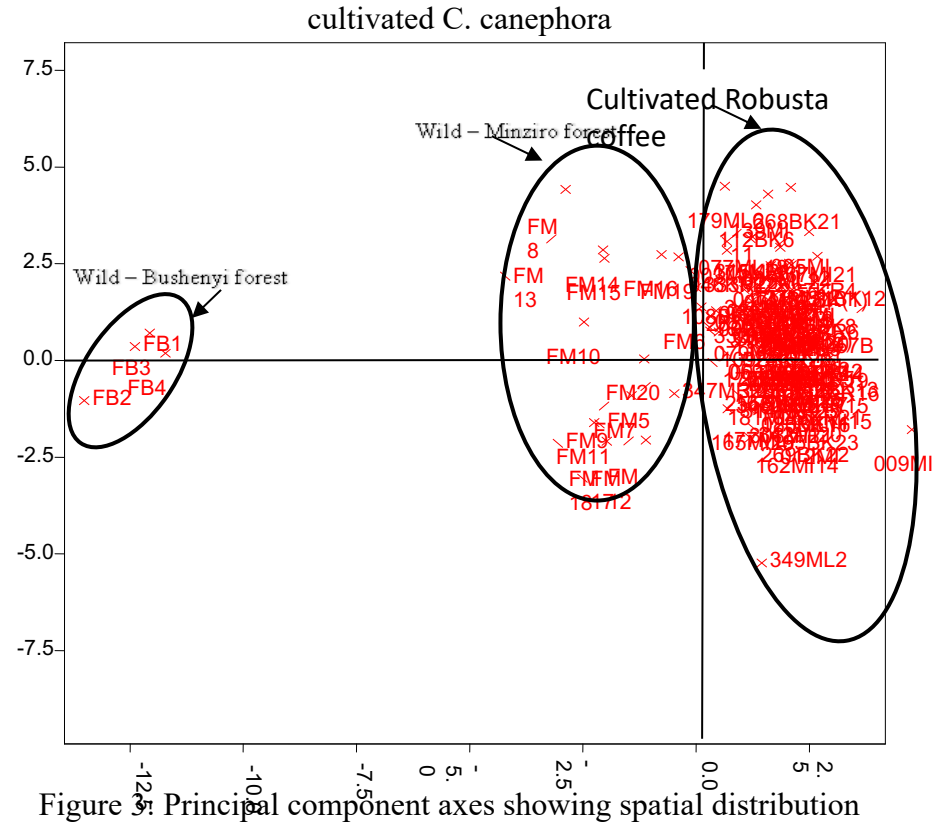

Figure 3: Principal component axes showing spatial distribution of 104 cultivated robusta and 20 wild coffee genotypes based on morpho-agronomic traits

The overall results indicated high variations of the morphological traits among the assessed coffee species. The results of this study suggest that uses of morphological traits in characterizing genotypes of crop including cultivated *C. canephora* and wild coffee species are still useful in crop improvement programme. Therefore, morphological traits are still vital in characterizing the large genomic diversity of coffee within the population in order to identify traits of interest such as agronomic, quality and yields which may be further evaluated using DNA markers for selecting appropriate parents for the development of superior genotypes to reduce selection cycles during developing varieties.

Conclusion

.The highest variations of morphological traits observed in this study revealed the highest genetic diversity of cultivated *C. canephora* and wild coffee species. With these findings, there is a need of further investigating genetic diversity of closely related genotypes by using single nucleotides polymorphism in order to identify and carry out genomic mapping of genes of interest which will be used for coffee improvement programme in future.

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Differential expression of WRKY transcription factors in the leaves of *Coffea canephora* var. S274 during salinity and drought.

PB-205

GIRIDHAR Parvatam, AVINASH Kumar, SIMMI Puthanoor *S.*

Plant Cell Biotechnology Department, CSIR-Central Food Technological Research Institute, Mysore – 570 020, Karnataka, India (parvatamg@yahoo.com)

RATIONALE

***Coffea canephora* is grown under low altitude and has a characteristic shallow rooting system that confers it with greater tolerance to water-deficit. However, the high quality tetraploid *C. arabica* is predicted to be the most affected by the increasing temperature due to the prevailing problems of global climatic change. Hence, it becomes important to study the mechanisms of water-deficit stress tolerance in the diploid species for scientific conclusions and further extrapolations to the tetraploid species. WRKY family of plant-specific transcription factors are known to play a very important role in biotic and abiotic stress response in plants. However, the role of WRKYs in osmotic stress response in perennial tree species is not well documented or studied.**

METHODS

One year old *C. canephora* seedlings of greenhouse acclimatized plants were treated with 20 or 200 mM NaCl for salinity and 1.5 or 15 % PEG-6000 for drought exposure in liquid Hoagland's hydroponic medium. RNA was isolated from leaves after 24 and 48 hours. Differential expression of plant-specific WRKYs was performed.

RESULTS

The study includes the differential expression of 29 WRKY-like ESTs. The ESTs showing best hit to *AtWRKY40/18/60*, *21*, *40* and *28/8/71* increased in salinity and drought whereas; homologue of *AtWRKY22* was responsive to only drought. The homologue of *AtWRKY28/71/23* exhibited down-regulation during salinity and drought whereas homologue of *AtWRKY69/65/35* showed down-regulation in only drought. In this study, *C. canpehora* homologue of *AtWRKY69* and *AtWRKY19* indicated over-expression in salinity, but decrease in drought.

Caffeine content is known to alleviate during salinity and drought stress without much change in transcript profiles of the caffeine biosynthetic genes. Increased caffeine degradation is predicted to be the reason possibly due to alteration of nitrogen utilization during salinity and drought stress in plants.

Introduction

- **Adverse effects of global climatic change pose a great threat to sustainable coffee cultivation (Mofatto et al. 2016). Among the main is the increasing problems of water-deficit:**
- **Since drought is a widespread limiting factor affecting the flowering and bean development and subsequently coffee yield (Da Matta et al. 2006), there is a need to study the molecular mechanism of water-deficit stress.**
- **Periods of drought become more pronounced as a consequence of shifts in the geographical regions of cultivation leading to environmental, economic and social problems.**
- **Although many tolerant varieties are available in both species, *C. canephora* are generally more tolerant to water-deficit stress compared to *C. arabica*.**
- **Water-deficit manifested by either salinity stress or drought conditions lead to low levels of absorbable water in the soil. Though the plant responses to salinity involve ion toxicity response, they coincide with drought response in terms of activation of Abscisic acid (ABA)-dependant and ABA-independent pathways. Abscisic acid is a plant hormone that act by regulating gene expression of drought responsive genes.**
- **WRKY transcription factors belong to one of the tenth largest plant-specific transcription factor family and recently implicated in mediating both the ABA-dependant and independent responses. They have been effective in utilization for transgenic drought resistant plants.**
- **The main interest of this study was to profile the WRKY transcripts during salinity and drought response in *C. canephora* plants. A comprehensive analysis of expression profile of water-deficit stress responsive WRKY factors could be extrapolated for utilization to the comparative studies for the susceptible *C. arabica*.**
- **According to our recent study, transcription of caffeine biosynthetic genes during water-deficit stress did not correlate with reduction in caffeine content. This observation was attributed to increased caffeine degradation via theophylline route (Kumar et al., 2015).**

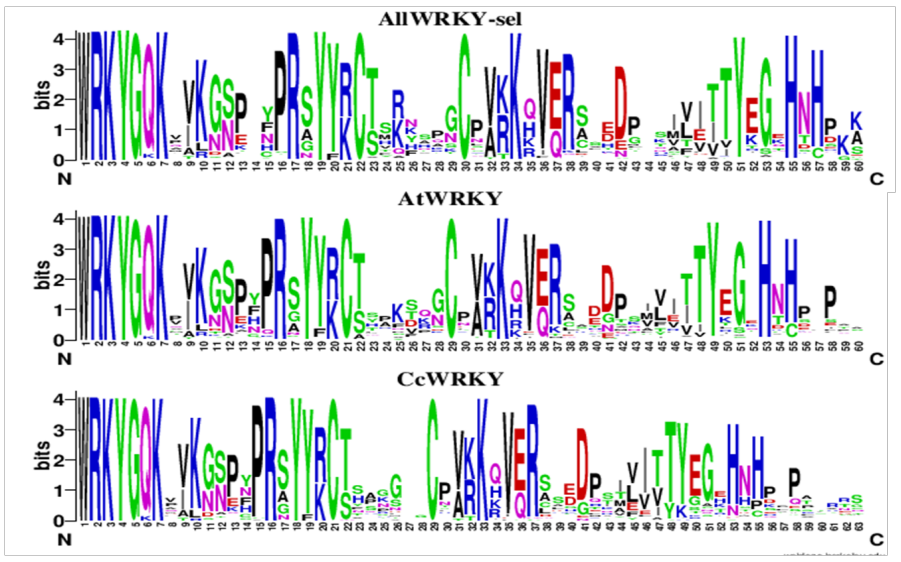


Figure 1: Consensus of the 60 amino acid domain in WRKY across plant taxons (A)*, in *Arabidopsis thaliana* (B) and in *Coffea canephora* (C)..

*All WRKY-sel: comprise sequences from *Arabidopsis thaliana* (70 sequences), *Glycine max* (334 sequences), *Coffea canephora* (49 sequences), *Nicotiana tabacum* (263 sequences), *Vitis vinifera* (68 sequences) and *Lycopersicon esculentum* (91 sequences)

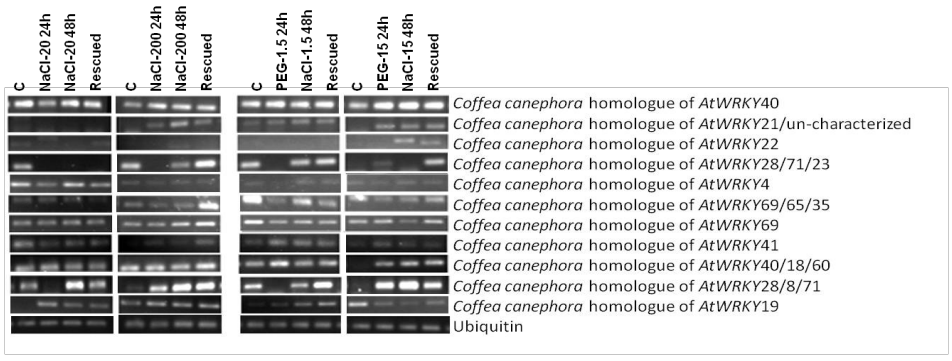
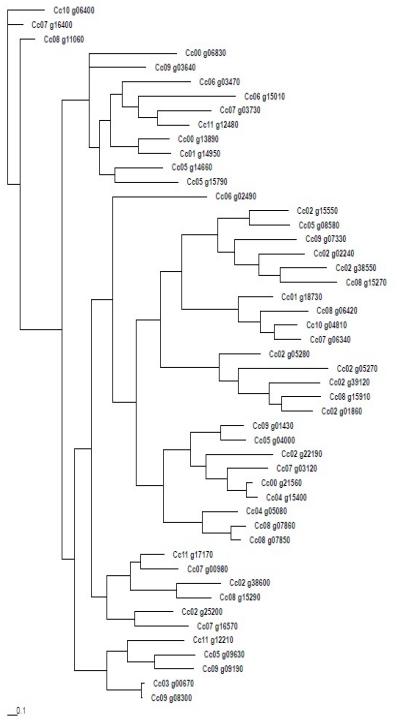


Figure 2: Expression of selected WRKYs in salinity and drought response.

***C**=control untreated plants: **NaCl-20**= plants treated for 24 or 48 h in 20mM NaCl: **NaCl-200**= plants treated for 24 or 48 h in 200mM NaCl: **PEG-1.5**= plants treated for 24 or 48 h in 1.5% PEG-6000: **PEG-15**= plants treated for 24 or 48 h in 15% PEG-6000: and **Rescued**=plant rescued in normal Hoagland's medium for 48h after a 48 h exposure to respective stress conditions.



Sl. No.	Genomic Annotation and gene ID	SGN Annotation similarity search	Gene structure and Genome annotated term	RPKM from coffee genome browser
1.	| Symbols: WRKY40, ATWRKY40 | WRKY DNA-binding protein 40 | chr1:30383834-30385356 FORWARD LENGTH=302 | Symbols: WRKY18, ATWRKY18 | WRKY DNA-binding protein 18 | chr4:15383296-15384812 FORWARD LENGTH=310 | Symbols: WRKY60, ATWRKY60 | WRKY DNA-binding protein 60 | chr2:10629812-10631095 FORWARD LENGTH=271	*AtWRKY40*	5 exons, 4 introns (Coffea canephora homologue of AtWRKY40)	
2.	-No Hits-	*AtWRKY21*	(Coffea canephora homologue of AtWRKY21/un-characterized)	
3.	Symbols: WRKY22, ATWRKY22 | WRKY family transcription factor | chr4:522839-524129 REVERSE LENGTH=298 Symbols: WRKY27, ATWRKY27 | WRKY DNA-binding protein 27 | chr5:21410996-21412218 FORWARD LENGTH=348 Symbols: WRKY29, ATWRKY29 | WRKY family transcription factor | chr4:12291831-12293088 FORWARD LENGTH=304	*WRKY 23 [(Populus tomentosa x P. bolleana) x P. tomentosa], AtWRKY22*	3 exons, 2 introns (Coffea canephora homologue of AtWRKY22)	
4.	Symbols: WRKY28, ATWRKY28 | WRKY DNA-binding protein 28 | chr4:10061508-10062691 FORWARD LENGTH=318 Symbols: WRKY71, ATWRKY71 | WRKY DNA-binding protein 71 | chr1:10454482-10455652 FORWARD LENGTH=282 Symbols: WRKY23, ATWRKY23 | WRKY DNA-binding protein 23 | chr2:19405045-19406446 REVERSE LENGTH=337	*AtWRKY28* (1e-38; 50.47)	3 exons, 2 introns (Coffea canephora homologue of AtWRKY28/71/23)	
5.	Symbols: WRKY4 | WRKY DNA-binding protein 4 | chr1:4776622-4779043 FORWARD LENGTH=514 Symbols: WRKY3 | WRKY DNA-binding protein 3 | chr2:1014724-1016936 REVERSE LENGTH=513 Symbols: WRKY20, ATWRKY20 | WRKY family transcription factor family protein | chr4:13437298-13440693 REVERSE LENGTH=557	*AtWRKY4* (8e-36), *Vitis vinefera WRKY2* (8e-46)	4 exons, 3 introns (Coffea canephora homologue of AtWRKY4)	
6.	Symbols: WRKY69, ATWRKY69 | WRKY DNA-binding protein 69 | chr3:21715192-21716766 FORWARD LENGTH=272 Symbols: WRKY65, ATWRKY65 | WRKY DNA-binding protein 65 | chr1:10236589-10237467 FORWARD LENGTH=259 Symbols: WRKY35, MEE24, AtWRKY35 | WRKY DNA-binding protein 35 | chr2:14693839-14696378 REVERSE LENGTH=427	*AtWRKY32* (1e-33)	5 exons, 4 introns (Coffea canephora homologue of AtWRKY69/65/35)	
7.	Symbols: WRKY69, ATWRKY69 | WRKY DNA-binding protein 69 | chr3:21715192-21716766 FORWARD LENGTH=272 Symbols: WRKY65, ATWRKY65 | WRKY DNA-binding protein 65 | chr1:10236589-10237467 FORWARD LENGTH=259 Symbols: WRKY35, MEE24, AtWRKY35 | WRKY DNA-binding protein 35 | chr2:14693839-14696378 REVERSE LENGTH=427	*AtWRKY69* (7e-44)	3 exons, 2 introns (Coffea canephora homologue of AtWRKY69)	
8.	Symbols: WRKY41, ATWRKY41 | WRKY family transcription factor | chr4:6759303-6760763 FORWARD LENGTH=313 Symbols: WRKY53, ATWRKY53 | WRKY family transcription factor | chr4:12392666-12393739 REVERSE LENGTH=324 Symbols: WRKY30, ATWRKY30 | WRKY DNA-binding protein 30 | chr5:8153416-8154633 REVERSE LENGTH=303		3 exons, 2 introns (Coffea canephora homologue of AtWRKY41)	
9.	Symbols: WRKY40, ATWRKY40 | WRKY DNA-binding protein 40 | chr1:30383834-30385356 FORWARD LENGTH=302 Symbols: WRKY18, ATWRKY18 | WRKY DNA-binding protein 18 | chr4:15383296-15384812 FORWARD LENGTH=310 Symbols: WRKY60, ATWRKY60 | WRKY DNA-binding protein 60 | chr2:10629812-10631095 FORWARD LENGTH=271		5 exons, 4 introns (Coffea canephora homologue of AtWRKY40/18/60)	
10.	Symbols: WRKY28, ATWRKY28 | WRKY DNA-binding protein 28 | chr4:10061508-10062691 FORWARD LENGTH=318 Symbols: WRKY8, ATWRKY8 | WRKY DNA-binding protein 8 | chr5:18801403-18803901 REVERSE LENGTH=326 Symbols: WRKY71, ATWRKY71 | WRKY DNA-binding protein 71 | chr1:10454482-10455652 FORWARD LENGTH=282		4 exons, 3 introns (Coffea canephora homologue of AtWRKY28/8/71)	

Figure 3: Phylogenetic and bioinformatic comparison of *C. canephora* WRKYs at SGN Unigene database and Coffee genome browser

Conclusion

- **Exogenous stress to high concentrations of sodium chloride (200mM) or Poly-Ethylene Glycol-6000 (15%) was detrimental to the plants and they succumbed to the stress within five to seven days post -treatment irrespective of whether the plants were rescued in normal Hoagland's medium or remained in the respective stress condition. The plants exhibited symptoms of black spots in leaves followed by necrotic lesions and drying away of the entire shoots. Thus, salinity and drought response was studied at 24 h and 48 h post-treatment followed by rescue in normal Hoagland's for the next 48 h.**
- **Differential expression of 29 WRKY-like ESTs in salinity (NaCl-20mM and NaCl-200mM) and drought (PEG-1.5% and PEG-15%) indicated 11 ESTs with differential expression profile during either or both of the stress treatments in the leaves of coffee seedlings.**
- **ESTs showing best hit to *AtWRKY40/18/60*, *21*, *40* and *28/8/71* exhibited increase in salinity and drought whereas; homologue of *AtWRKY22* was responsive to only drought. A homologue of *AtWRKY28/71/23* exhibited down-regulation during salinity and drought whereas homologue of *AtWRKY69/65/35* showed down-regulation in only drought. Amongst the analyzed WRKYs, the most notable ESTs responsive to salinity and drought stress is the coffee homologue of *AtWRKY40/18/60* and *AtWRKY28/8/71*. *Arabidopsis* plants transformed with multiple expression cassette of *AtbHLH17* and *AtWRKY28* have been shown to have higher tolerance to NaCl, mannitol and oxidative stress (Babitha et al. 2013).**

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Effect of non-purine cytokinin 4-CPPU on somatic embryogenesis and accumulation of caffeine in *Coffea canephora* callus cultures

PB-206

SRIDEVI VADDADI*, GIRIDHAR PARVATAM

*N.N.S.V. College, Chirala-523155, A.P., India, Plant Cell Biotechnology Department, CSIR-Central Food Technological Research Institute, Mysore – 570020, KA, India (vaddadi_sridevi@yahoo.com)

Abstract

RATIONALE

Several synthetic and natural cytokinins in combination with auxins have been investigated for their potential role in induction of somatic embryogenesis in *Coffea* species. In recent years, the advantages of non-purine cytokinins in plant tissue culture and horticulture has been demonstrated. Developing a sustainable method for somatic embryogenesis and also to find out the role of 4-CPPU on caffeine in coffee is having importance to obtain quality plants.

METHODS

Non-purine cytiokinin 4-CPPU [N-(2-chloro-4-pyridyl) N-phenylurea] has been incorporated at 0.5 – 3.0 mg/l to MS medium containing 2% sucrose for induction of direct somatic embryogenesis and *in vitro* callus production. Somatic embryos produced were recorded after 8 wks and the caffeine content of callus cultures analysed to find out influence of 4-CPPU.

RESULTS

There was good response for direct somatic embryogenesis from cotyldonary leaf explants on MS medium containing 2.5 mg/l 4-CPPU wherein 58% explants responded with 25-36 somatic embryos per explants. However, hypocotyl explants exhibited moderate response (36%) with 15-20 somatic embryos per explant. Secondary somatic embryogenesis was noticed from these primary embryos upon sub-culturing onto medium comprising half strength MS with 0.5 mg/l IAA and 0.25 mg/l 4-CPPU. Maturation of embryos and regeneration into plantlets achieved on half strength MS medium. The highest concentrations of caffeine (0.34% DW) was recorded in the callus grown in the presence of 0.5 mg L^{-1} IAA and 0.5 mg L^{-1} 4-CPPU.

Introduction

- ❖Somatic embryogenesis is an important process of tissue culture of *Coffea* species.
- ❖The earlier established protocols have been used to obtain pure culture of homogenous material (explants), for mass production of in vitro plants and also for genetic transformation studies wherein a desired gene can be transferred through this process for developing transgenic plants.
- ❖Several synthetic and natural cytokinins in combination with auxins have been investigated for their potential role in induction of somatic embryogenesis in *Coffea* species (Santos-Briones and Hernández-Sotomayor 2006).
- ❖In recent years, the advantages of non-purine cytokinins in plant tissue culture and horticulture has been demonstrated (Vinayak et al., 2009; Sadki et al., 2015), wherein, 4-C CPPU [N-(2- Chloro-4- Pyridyl0 N-phenylurea] exhibited cytokinin like activity.
- ❖In coffee, often the response for somatic embryogenesis varies with the samples and also depends on growth regulator and its concentration in culture medium.
- ❖Moreover, this non – purine cytokinin is reported to be stable at culture medium sterilization temperatures. Developing a sustainable method for somatic embryogenesis and also to find out the role of 4-CPPU on caffeine in coffee is having importance to obtain quality plants (Roussos et al., 2011).
- ❖Similarly, caffeine (1,3,7 trimethyl xanthine) in coffee is one of the major secondary metabolite and helps to combat physical and biotic stress factors such as pathogens and predators (Kim *et al*., 2011).
- ❖Caffeine content in *in vitro* cultures such as callus and somatic embryos of coffee may influence further regeneration in to plantlets.
- ❖ In the present study the influence of 4-CPPU on somatic embryogenesis in robusta coffee (*C. canephora* P. ex. Fr. variety robusta) was examined.

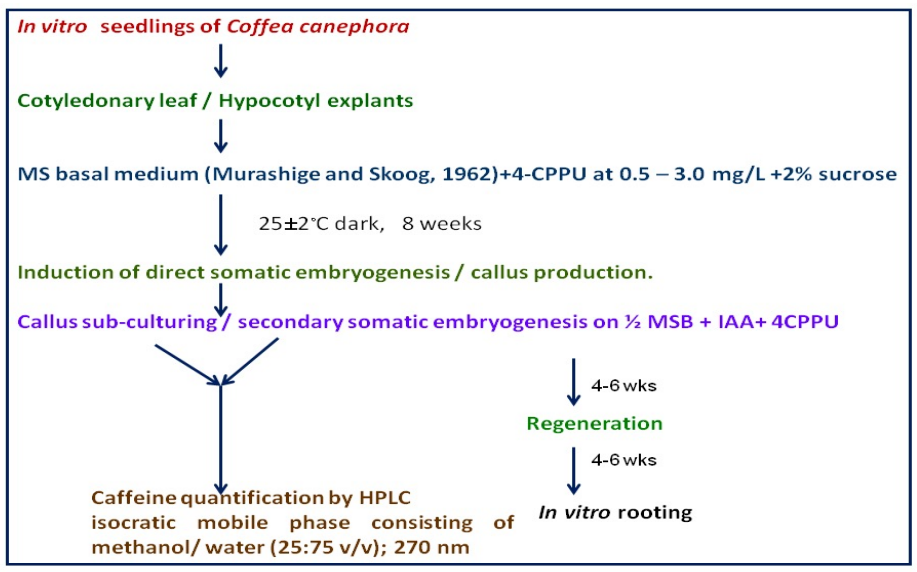

Fig.1 Details pertaining to Experiment and analysis

Table.1 Induction of direct somatic embryogenesis and callusing from different explants of *C. canephora*

4-CPPU conc. mg/l	% Response from explants for somatic embryogenesis		% explants showing callus initiation	
	Cotyledon	Hypocotyl	Cotyledon	Hypocotyl
0.5	-	-	-	-
1.0	10	-	-	-
1.5	14	12	+	+
2.0	22	18	+	+
2.5	58	36	++	++
3.0	28	28	+	+

Medium = MSB + 2% sucrose+ 4CPPU; Number of respective explants inoculated =100; values are mean ± SD of response recorded explants.

Table. 2 Caffeine content in *in vitro* cultures of *C. canephora*

IAA+ 4-CPPU (mg/l)	Caffeine content (mg/100g dry wt.)		Regenerated plant leaves
	Callus	Somatic embryos**	
0.5 + 0.25	280±14.6	218±18.2	
0.5 + 0.50	340±25.4	250±13.4	335±11.6
0.5 + 1.0	336±20.8	230±19.4	
0.5 + 2.0	328±18.8	210±10.3	
MSB (control)*	180±16.5	195±12.4	

* No further proliferation of cultures; ** Secondary somatic embryos *** on half-strength MSB after 8 weeks, values are mean ± SD of three analyses

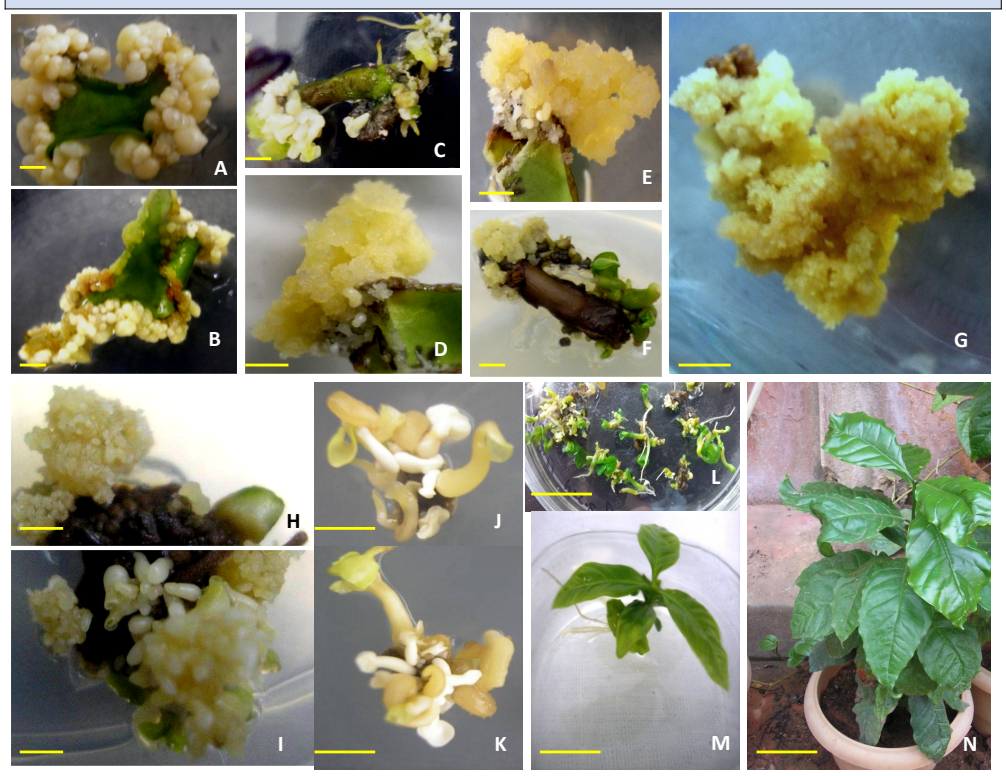

Fig.1 Somatic embryogenesis and callus induction in *Coffea canephora* CxR
A , B) Direct somatic embryogenesis from cotyledonary leaf explants (*bar* 10mm) C)Direct somatic embryogenesis from hypocotyl explants (*bar* 10mm) D) Callus induction from cotyledonary leaf explant (*bar* 10mm) E, F) Callus induction along with somatic embryos from cotyledonary leaf and hypocotyl explants (*bar* 10mm) G) Callus proliferation upon subculturing 0.5 mg/l IAA and 0.5 mg/l 4-CPPU comprising medium (*bar* 20mm) H, I) Secondary somatic embryogenesis in presence of 0.5 mg/l IAA and 1.0 mg/l 4-CPPU containing medium (*bar* 10mm) J, K, L) Regeneration somatic embryos *(bar* 10, 30mm) L) *In vitro* rooting of regenerated plantlet on ½ MSB medium (bar 5cm) M) Six months old Potted plant (*bar* 11 cm)

Highlights:

1. Direct somatic embryogenesis from cotyldonary leaf explants and hypocotyls explants was evident in presence of optimized concentrations of 4-CPPU on MS medium, however the response varies with the explants used.
2. Overall, hypocotyl explants exhibited sluggish response for somatic embryos production. A maximum of 30.7± 2.17 and 15.91 ± 2.6 somatic embryos were induced from cotyledon and hypocotyl explants respectively.
3. Moderate callusing was noticed from both types of explants and it was found to be best in presence of 2.5 mg/l 4-CPPU on MS medium comprising 2% sucrose.
4. Subsequent proliferation of callus and secondary somatic embryogenesis respectively could be possible in presence of IAA and 4-CPPU on half strength MS medium with B5 vitamins.
5. HPLC analysis of Caffeine content of callus and somatic embryos reveals 5-15% variation in respective cultures grown on medium with different combination of IAA and 4-CPPU.
6. As diverse array of plant hormones act together at the cellular level to produce physiological and morphological effects on plant growth, morphology and yield (Kalia et al., 2016), the outcome of the present investigation is important.

Conclusion

This is the first report about the influence of 4-CPPU on somatic embryogenesis in coffee and caffeine content in different tissues.. In the present study, in presence of optimized levels of 4CPPU with IAA abnormalities were found minimal. Although callus mediated somatic embryogenesis has not been pursued further, the data pertaining to the caffeine content of callus cultures will throw some light on influence of 4-CPPU on caffeine biosynthetic pathway which needs to be investigated. Hence, this novel response due to non-purine has to be further studied at molecular and cellular level to understand the role of this hormonal stress on organogenesis and triggering of caffeine pathway genes.

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Reference Genes Suitability for qRT-PCR Studies in *Coffea* spp. Plants Grown at High CO_2 and Temperature

Martins, M.Q.[1,2], Fortunato, A.S.[1], Goulao, L.F. [1], Rodrigues, W.P.[1,3], Partelli, F.L.[2], Campostrini, E.[3], Lidon, F.J.[4], DaMatta, F.M.[5], Ramalho, J.C.[1,4], Ribeiro-Barros, A.I.[1,4]

[1] PlantStress&Biodiversity, LEAF, DRAT, Instituto Superior de Agronomia, Universidade de Lisboa, Oeiras, Portugal

[2] CEUNES, DCAB, UFES, São Mateus and PPGPV, CCA, UFES, Alegre, ES, Brazil

[3] CCTA, Universidade Estadual do Norte Fluminense, RJ, Brazil

[4] DCT, Fac. Ciências e Tecnologia, Universidade NOVA de Lisboa, Portugal

[5] Dept. Biologia Vegetal, Universidade Federal de Viçosa, MG, Brazil

Introduction

Some climate change scenarios point to increases in the global temperature (*ca*. 3.7 to 4.8 °C) and CO_2 levels (*ca*. 800-1150 µL L^{-1}) in 2100 [1], with dramatic impacts in plant productivity and survival. Analysis by RT-qPCR is a reliable method to study and validate these effects at the gene expression level, provided the appropriate set up of key parameters, such as reference genes, is assured regarding the species dependent responses to specific environmental conditions. In this study we identified the most stable reference genes to assess gene expression in *Coffea* spp. plants grown at high atmospheric CO_2 (700 µL L^{-1}) and submitted to supra-optimal temperature conditions (up to 42 ºC).

Material and Methods

Plants of *Coffea arabica* L. (cv. Icatu and IPR 108) and *C. canephora* cv. Conilon Clone 153 (CL 153), *ca*. 1.5 years of age, were grown for 10 months in 28 L pots in walk-in chambers, under environmental controlled conditions of temperature (25/20 ºC, day/night), irradiance (*ca*. 700-800 µmol m^{-2} s^{-1}), RH (75%), photoperiod (12 h), and 380 or 700 µL CO_2 L^{-1} in air. Thereafter, temperature was increased (0.5 ºC day^{-1}) from 25/20 ºC to 42/34 ºC. Ten candidate genes were analyzed: GAPDH, EF-1A, ELF-4A, CYCL, ACT, DNAJ, S15, MDH, a-TUB, UBQ2, following studies in *Coffea* spp. [2].

Results and Discussion

The stability of 10 candidate genes (CG; **Table 1**) was assessed by qPCR in 24 cDNA samples combined with the algorithms GeNorm, NormFinder and BestKeeper as well as the coefficient of variation (CV%). The final ranking was performed using the statistical package RankAggreg [2]. MDH was the most stable gene for all comparison groups, i.e. genotypes, [CO_2] conditions, temperature stress, multiple stress and total stress (**Table 2**). Actin and S15 ranked in the top five positions; while α-TUB and CYCL were amongst the least stables. Regarding the minimum number of reference genes for qRT-PCR studies, pairwise variation (V) indicates that two genes are enough for normalization of gene expression studies, since V-value <0.15 for V2/V3 (**Fig 1**).

Table 1 – **Candidate reference genes.**

Gene Symbol	Gene Name	Acession Number
UBQ2	Ubiquitin-conjugating enzyme E2	GR984245
α-Tub	Alpha-tubulin	GT009437
S15	40S ribosomal protein S15	GR987196
DNAJ	Plant DNA J protein	GR986679
MDH	Malate dehydrogenase	GW464198.1
Act	Actin	GT000704
eIF-4A	Eukaryotic initiation factor 4α	GT71729
Cycl	Cyclophilin	GT007167
EF-1A	Elongation factor 1α	GR996930
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase	DV692958
UBQ	Ubiquitin	AF297089

Figure 2 - **Determination of the optimal number of genes reference evaluated by standardization Pairwise.**

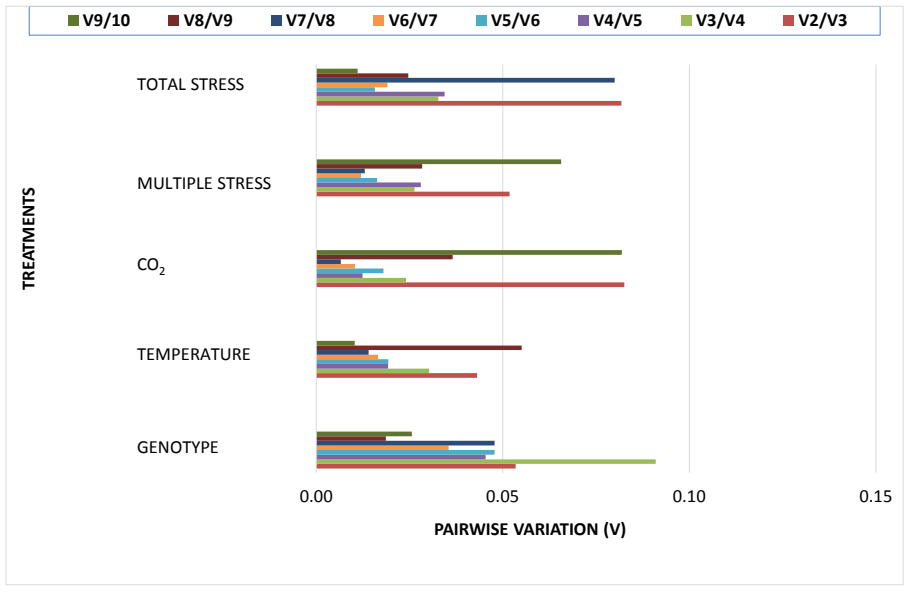

Table 2 - **Overall ranking of the most stable genes within each treatment.**

Genotypes		Temperature		CO_2		Multiple stress		Total stress	
MDH	1.68	MDH	1.86	MDH	1.68	MDH	1.41	MDH	1.41
S15	1.86	UBQE2	1.86	S15	1.78	ELF-4A	2.21	ACT	1.57
ACT	2.99	DNAJ	2.11	UBQE2	2.71	DNAJ	2.63	S15	2.78
DNAJ	3.34	ACT	3.46	ACT	2.83	S15	2.78	DNAJ	3.94
ELF-4A	3.83	S15	3.98	ELF-4A	4.40	ACT	4.40	ELF-4A	4.95
UBQE2	5.05	ELF-4A	6.24	GAPDH	6.82	GAPDH	6.64	UBQE2	5.05
GAPDH	7.00	GAPDH	6.74	EF-1A	7.00	UBQE2	6.74	GAPDH	7.00
CYCL	8.00	EF-1A	8.24	a-TUB	8.00	CYCL	8.00	CYCL	8.00
EF-1A	9.00	CYCL	8.74	CYCL	8.80	EF-1A	9.15	a-TUB	9.24
a-TUB	10.00	a-TUB	10.00	DNAJ	9.00	a-TUB	9.24	EF-1A	9.74

Conclusions

The use of the MIQE guidelines is determinant for accurate qRT-PCR studies in *Coffea* spp., as the expression stability of conventional housekeeping genes varied considerably under the experimental conditions tested. At least two reference genes (*MDH* and preferably *Actin* or S15) should be used for normalization of gene expression in qRT-PCR studies in *Coffea* genotypes exposed to increasing temperature and [CO_2] conditions, although for specific heat stress conditions MDH could be used with UBQE2.

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Heat Stress Relieving Mechanisms In *Coffea arabica* cv. Icatu and Their Relation to Increased Air

Lidon, F.C.[1,*], Martins, M.Q.[2,3], Rodrigues, W.P.[2,4], Rodrigues, A.P.[2], Fortunato, A.S.[2], Leitão, A.E.[1,2], Pais, I.P.[2,5], Semedo, J.N.[2,5], Silva, M.J.[1,2], Colwell, F.[2], Martins, L.D.[6], Tomaz, M.A.[6], Partelli, F.L.[3], Campostrini, E.[4], Reboredo, F.H.[1], Pessoa, M.F.[1], Scotti-Campos, P.[2,5], Ribeiro-Barros, A.I.[1,2], DaMatta, F.M.[7], Ramalho, J.C.[1,2]

[1] DCT, Fac. Ciências e Tecnologia, Universidade NOVA de Lisboa, Portugal

[2] PlantStress&Biodiversity, LEAF, DRAT, Instituto Superior de Agronomia, Universidade de Lisboa, Oeiras, Portugal

[3, 6] CEUNES, DCAB, UFES, São Mateus and PPGPV, CCA, UFES, Alegre, ES, Brazil

[4] CCTA, Universidade Estadual do Norte Fluminense, RJ, Brazil

[5] UBRG, Instituto Nacional de Investigação Agrária e Veterinária, Portugal

[7] Dept. Biologia Vegetal, Universidade Federal de Viçosa, MG, Brazil

Introduction

According to several studies based on a model approach, climate changes and global warming will cause strong impacts on coffee crop, by reducing adequate cultivation areas, yield and biodiversity (particularly in *Coffea arabica*), with implications for the entire coffee chain of value. However, recent works showed that high [CO_2] can strengthen coffee C-assimilation metabolism and the whole plant status [1,2], mitigating heat stress impact [3]. Therefore, heat response mechanisms at leaf level were evaluated, in order to unveil the positive role of high [CO_2] on coffee heat tolerance [4].

Material and Methods

Plant Material and Growth Conditions

Plants of *Coffea arabica* L. cv. Icatu, *ca*. 1.5 years of age, were grown for 10 months in 28 L pots in walk-in growth chambers, under environmental controlled conditions of temperature (25/20 ºC, day/night), irradiance (*ca*. 700-800 µmol m^{-2} s^{-1}), RH (75%), photoperiod (12 h), and 380 or 700 µL CO_2 L^{-1} in air. Thereafter, temperature was increased (0.5 ºC day^{-1}) from 25/20 ºC to 42/34 ºC. Plant responses at leaf level were assessed at 25/20 ºC, 31/25 ºC, 37/30 ºC and 42/34 ºC.

Methods

Evaluation was focused on protective molecules (α-tocopherol, HSP70 and RFOs - raffinose and stachyose) and antioxidant enzymes activities (superoxide dismutase, Cu,Zn-SOD; ascorbate peroxidase, APX; glutathione reductase, GRed; catalase, CAT), as well as on the expression of genes related to such protective molecules, as described in [4].

Results and Discussion

At the highest temperature (42/34 ºC) it was observed that (**Table 1**):

1) the protective molecules **α-tocopherol**, **HSP70** and the **RFO raffinose** increased in both CO_2 conditions;

2) among antioxidant enzymes, **ascorbate peroxidase** (**APX**) was the most heat sensitive with activity reductions of 90% or higher in both [CO_2];

3) **catalase** and **glutathione reductase** (GR) activities decreased at normal [CO_2], but were **enhanced under high [CO_2]**.

Table 1 – Cellular content of the non-enzyme antioxidants (α-tocopherol, HSP70, Raffinose) and chloroplastic maximal activities of enzyme antioxidants Cu,Zn-superoxide dismutase (Cu,Zn-SOD), ascorbate peroxidase (APX), glutathione reductase (GR), and cellular catalase, under 380 or 700 µLCO_2 L^{-1}, at control (25/20 ºC, day/night) and supra-optimal temperatures of 37/30 ºC and 42/34 ºC. Values represent the mean ± SE (n=4-6); different letters express significant differences between temperatures for the same [CO_2] (a, b, c), or between [CO_2] for each temperature (A, B). In bold, strongly increased values; in red decreased values.

Compound	[CO_2] (µL L^{-1})	Temperature (day/night) 25/20 ºC			37/30 ºC			42/34 ºC		
α-Tocopherol	380	0.284 ± 0.036	cA		**0.458 ± 0.036**	bA		**1.013 ± 0.036**	aA	
(mg g^{-1} DW)	700	0.379 ± 0.036	bcA		**0.489 ± 0.029**	bA		**0.716 ± 0.036**	aB	
HSP70	380	0.818 ± 0.047	bA		**1.699 ± 0.047**	aB		**1.897 ± 0.060**	aA	
(µg g^{-1} DW)	700	0.710 ± 0.040	cA		**3.264 ± 0.084**	aA		**1.774 ± 0.056**	bA	
Raffinose	380	4.06 ± 0.13	bA		**10.71 ± 0.09**	aA		**11.07 ± 0.22**	aB	
(mg g^{-1} DW)	700	3.62 ± 0.18	dA		**8.84 ± 0.29**	bB		**13.14 ± 0.15**	aA	
Cu,Zn-SOD	380	523 ± 4	bB		431 ± 14	cA		602 ± 9	aA	
(Units g^{-1} DW)	700	648 ± 11	aA		413 ± 3	cA		498 ± 9	bB	
APX	380	11.04 ± 1.66	aA		10.22 ± 0.96	aA		**1.32 ± 0.22**	bA	
(mmol ASC min^{-1} g^{-1} DW)	700	14.88 ± 0.89	aA		10.97 ± 1.70	bA		**1.07 ± 0.28**	cA	
GR	380	1.179 ± 0.019	bA		1.816 ± 0.221	aA		0.395 ± 0.053	cB	
(µmol NADPH min^{-1} g^{-1} DW)	700	0.777 ± 0.084	bB		1.019 ± 0.118	abB		**1.105 ± 0.037**	aA	
Catalase	380	7.62 ± 1.47	bA		17.72 ± 3.69	aA		5.37 ± 1.27	bB	
(µmol H_2O_2 min^{-1} g^{-1} DW)	700	5.67 ± 1.19	bA		10.34 ± 0.88	aB		**12.77 ± 0.54**	aA	

Table 2 – Real-time PCR expression studies represent **n** fold relative to the control of temperature and CO_2 (25/20 ºC, 380 µL CO_2 L^{-1}), under 380 or 700 µLCO_2 L^{-1}, at supra-optimal temperatures of 31/25 ºC, 37/30 ºC and 42/34 ºC. Data are from genes of 70 kDa heat shock-related protein from chloroplastic stroma (*HSP70*), early light-induced protein (*ELIP*), 20 kDa chaperonin from chloroplast (*Chape 20*), Chaperonin CPN60 (*Chape 60*), all related to protective proteins; the genes of catalase isozyme 1 (*CAT*), Cu,Zn superoxide dismutase (*CuSOD2*), ascorbate peroxidase from cytoplasm (*APX Cyt*) and chloroplast (*APX Chl*), are related to antioxidative enzymes. Original expression values for each gene resulted from the mean ± SE (n=6-9), from 3 independent biological assays. * indicate the presence of statistical significance (in bold are strongly increased values).

Temperature (day/night)	[CO_2] (µL L^{-1})	Gene Expression Relative to Control Temperature and CO_2 (25/20 ºC, 380 µL L^{-1}) *HSP70*	*ELIP*	*Chape 20*	*Chape 60*	*CAT*	*CuSOD2*	*APX Cyt*	*APX Chl*
31/25°C	380	0.95	2.33	1.93	1.18	1.88*	1.00	0.46*	0.72
	700	0.87	2.70	1.74	1.32	1.88*	0.95	1.15	0.91
37/30 °C	380	**2.70***	**4.71***	**4.00***	**2.83***	**1.84***	**1.39***	0.50	0.71
	700	**2.74***	**7.38***	**3.29***	**3.43***	**2.86***	**2.13***	1.57	**2.87***
42/34°C	380	**4.96***	**9.28***	**7.40***	**14.63***	**1.75***	**2.88***	**7.94***	1.48
	700	**9.36***	**12.16***	**13.52***	**34.19***	1.28	**3.75***	**24.49***	**5.04***

Besides:

4) at 42/34 ºC, the upregulated expression of genes related to protective molecules (HSP70, ELIP, Chaperonins 20 and 60) was mostly driven by temperature. In fact, up-regulated expression was observed, in both [CO_2]. However, a much higher extent was observed under enhanced [CO_2].

5) A similar expression trend was observed for *APX Cyt*, *APX Chl* and *CuSOD2* genes, but, again, high [CO_2] promoted higher increases at 42 ºC (except catalase) (**Table 2**).

Conclusions

The observed patterns of most protective molecules and related gene expression, together with a higher photochemical energy use [3], likely favoured ROS control [4], mitigating thermal impact and emphasizing the role of high [CO_2] on coffee plant acclimation ability under future warming scenarios.

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[1] PlantStress&Biodiversity, LEAF, DRAT, Instituto Superior de Agronomia, Universidade de Lisboa, Oeiras, Portugal

[2] DCT, Fac. Ciências e Tecnologia, Universidade NOVA de Lisboa, Portugal

[3, 5] CEUNES, DCAB, UFES, São Mateus and PPGPV, CCA, UFES, Alegre, ES, Brazil

[4] CCTA, Universidade Estadual do Norte Fluminense, RJ, Brazil

[6] URBG, Instituto Nacional de Investigação Agrária e Veterinária, Portugal

[7] Dept. Biologia Vegetal, Universidade Federal de Viçosa, MG, Brazil

Introduction

Using IPCC estimates, it were predicted strong reductions of adequate areas for the coffee crop, particularly of *Coffea arabica*, with large implications for the entire coffee value chain.

This clearly shows the need to understand heat impact, considering both the plant and the quality of the beans. It must also be considered the possible role of increased air [CO_2] level that was found to be a key aspect for heat tolerance at leaf level in *Coffea* spp. [1,2].

In this context, it were studied the individual and combined impacts of high [CO_2] and supra-optimal temperature levels on the bean of *Coffea arabica* cv. Icatu, considering their chemical characterization, with particular emphasis on compounds with known impact on the quality of the beverage (*e.g.*, caffeine, trigonelline, chlorogenic acids and hydroxycinnamic acids).

Material and Methods

Experimental conditions

Coffea arabica cv. Icatu plants, *ca*. 2.5 years of age, were grown in 80 L pots, in walk-in growth chambers, under environmental controlled conditions of temperature (25/20 ºC, day/night), irradiance (700-800 µmol m^{-2} s^{-1}), RH (75%), photoperiod (12 h), and 380 (**380-plants**) or 700 (**700-plants**) µL CO_2 L^{-1} in air. Thereafter, temperature was **gradually** increased from 25/20 ºC to 40/30 ºC along 6 months. Coffee berries were yielded under adequate maturation stage, at **25/20 ºC** and within the range of **30-35 ºC** and **36-40 ºC**.

Bean characterization

Determinations include titratable acidity [3], total phenol content [4], total soluble solids [5], chlorogenic acids (3-, 4- and 5-CQA), caffeine, trigonelline, caffeic acid and *p*-coumaric acid [6].

Results and Discussion

Table 1 – Values for total phenol content, titratable acidity, and total soluble solids, in coffee beans yielded at 25, 30-35 and 36-40 ºC (diurnal temperatures). For each parameter, the mean values ± SE (n=4-6) followed by different letters express significant differences between temperatures for the same CO_2 treatment (a, b, c), or between CO_2 treatments for each temperature (A, B)

Parameter	[CO_2] (µL L^{-1})	Bean Harvest Diurnal Temperature: 25 ºC				30-35 ºC				36-40 ºC			
Total Phenol (mg GAE g^{-1} DW)	380	44.1	±	1.8	aA	37.9	±	1.0	bB	45.5	±	2.8	aA
	700	35.8	±	1.1	cB	49.4	±	2.1	aA	42.0	±	0.8	bA
Titratable acidity (mL NaOH 0.1 N g^{-1} DW)	380	1.95	±	0.07	aA	2.00	±	0.11	aA	2.09	±	0.17	aB
	700	2.09	±	0.10	bA	2.29	±	0.08	aA	2.62	±	0.07	aA
Total Soluble Solids (% DW)	380	34.5	±	0.9	bA	38.4	±	1.3	aA	37.6	±	1.2	abA
	700	33.0	±	1.2	aA	35.4	±	0.5	aA	33.5	±	0.8	aB

Table 2 – Values for total and individual CQAs, caffeic acid, caffeine, trigonelline and *p*-coumaric acid, in coffee beans yielded at 25, 30-35 and 36-40 ºC (diurnal temperatures). For each parameter, the mean values ± SE (n=5-8) followed by different letters express significant differences between temperatures for the same CO_2 treatment (a, b, c), or between CO_2 treatments for each temperature (A, B)

Parameter	[CO_2] (µL L^{-1})	Bean Harvest Diurnal Temperature: 25 ºC				30-35 ºC				36-40 ºC			
5-CQA (mg g^{-1} DW)	380	53.5	±	0.6	aA	46.0	±	1.8	bB	43.7	±	1.6	bA
	700	50.9	±	1.7	aA	53.5	±	1.2	aA	48.1	±	0.6	aA
4-CQA (mg g^{-1} DW)	380	3.48	±	0.10	cA	4.77	±	0.32	bA	7.78	±	0.24	aA
	700	3.50	±	0.24	cA	5.64	±	0.26	bA	7.44	±	0.34	aA
3-CQA (mg g^{-1} DW)	380	2.58	±	0.04	cA	4.67	±	0.54	bA	10.56	±	0.16	aA
	700	3.05	±	0.27	cA	6.07	±	0.31	bA	9.87	±	0.29	aA
Total CQAs (mg g^{-1} DW)	380	59.6	±	0.5	abA	55.7	±	2.1	bB	62.7	±	2.2	aA
	700	57.2	±	2.0	bA	64.9	±	1.1	aA	65.4	±	0.7	aA
Caffeic acid (mg g^{-1} DW)	380	1.34	±	0.07	aA	1.05	±	0.12	abB	0.66	±	0.07	bB
	700	1.32	±	0.12	abA	1.41	±	0.08	aA	1.04	±	0.10	bA
Caffeine (mg g^{-1} DW)	380	21.8	±	1.4	aA	19.1	±	0.4	aA	19.1	±	0.7	aA
	700	17.7	±	1.1	aB	15.4	±	0.9	aB	12.0	±	0.6	bB
Trigonelline (mg g^{-1} DW)	380	13.6	±	0.8	cA	16.9	±	0.9	bA	20.9	±	1.4	aA
	700	12.1	±	0.9	bA	16.4	±	0.7	aA	17.9	±	0.5	aB
p-Coumaric acid (mg g^{-1} DW)	380	2.25	±	0.14	aA	1.80	±	0.13	abA	1.33	±	0.10	bA
	700	1.98	±	0.14	aA	1.58	±	0.10	abA	1.17	±	0.08	bA

A) At **control temperature** (25/20 ºC), the high air [CO_2] did not change most parameters, with only **total phenol** (**Table 1**) and **caffeine** (**Table 2**) showing decreases.

B) At **control [CO_2]** (380 µL L^{-1}) temperature promoted the rise in **total soluble solids**, **3-CQA**, **4 CQA** (and **total CQAs**) and **trigonelline**, whereas **5-CQA**, **caffeic acid** and ***p*-coumaric acid** followed an opposite trend.

C) Under the **simultaneously exposure to high growth [CO_2] and temperature increase**, it can be observed that some responses changed, when compared to their single effects. In fact, high [CO_2]:

1) exacerbated the increases (**acidity**) and decreases (**caffeine**) under supra-optimal temperatures,

2) cancelled the heat effect on **total soluble solids**,

3) mitigated heat promoted increases (**trigonelline**) and decreases (**caffeic acid**).

Moreover, high growth [CO_2]:

4) did not significantly change **CQAs** contents when compared to beans of the 380-plants when collected at 36-40 ºC, although a tendency to higher values were obtained in the case of **5-CQA**.

5) did not change the reduction trend of *p*-coumaric acid, promoted by temperature.

Trigonelline is a precursor of the volatile compounds that contribute to the aroma and taste of roasted coffee, whereas thermal decomposition of chlorogenic acids gives origin to several compounds, which contribute to flavour, acidity and astringency of coffee drink [7].

Complementing the data, showing that high [CO_2] can mitigate the heat impact at leaf level and strenght plant vigour [1,2], these results points for a **complex interaction** relation between high growth [CO_2] and supra-optimal temperatures at bean level, with a likely impact on its final chemical composition and, therefore, on its quality regarding the beverage to be obtained.

Conclusions

1)Temperature had a greater impact than high [CO_2] promoting a global change at chemical level.

2) High [CO_2] might mitigate some temperature driven effects in some compounds, although can exacerbate acidity and caffeine changes.

3) A complex interaction relation between high growth [CO_2] and supra-optimal temperatures was observed at bean level, with a likely impact on the beverage to be obtained.

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[2] Martins MQ *et al*. (2016) *Frontiers in Plant Science*, 7: art 947.
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Acknowledgements

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PB 210

Impact of Elevated CO_2 and Temperature in the Transcriptome of *Coffea* spp.

Fortunato, A.S.[1], Bohn, A.[2], Goulao, L.[1], Ribeiro-Barros, A.I.[1,3], Rodrigues, W.P.[1,4], Martins, M.Q.[1,5], Partelli, F.L.[5], Campostrini, E.[4], Leitão, A.E.[1,3], Lidon, F.C.[3], DaMatta, F.M.[5], Ramalho, J.C.[1,3]

[1] PlantStress&Biodiversity, LEAF, DRAT, Instituto Superior de Agronomia, Universidade de Lisboa, Oeiras, Portugal

[2] Instituto de Tecnologia Química e Biológica António Xavier, Universidade NOVA de Lisboa, Oeiras, Portugal

[3] DCT, Fac. Ciências e Tecnologia, Universidade NOVA de Lisboa, Portugal

[4] CCTA, Universidade Estadual do Norte Fluminense, RJ, Brazil

[5] CEUNES, DCAB, UFES, São Mateus and PPGPV, CCA, UFES, Alegre, ES, Brazil

[6] Dept. Biologia Vegetal, Universidade Federal de Viçosa, MG, Brazil

Introduction

Discussions about the impact of global climate changes have dominated many major scientific events due to their foreseeable consequences on growth, productivity and quality of agricultural products. In this context, coffee crop was estimated to be seriously affected in the future, raising serious socio-economic concerns and challenges. These predictions are mainly based on temperature increase, but it was recently shown that effects of increased [CO_2] should also be considered [1-3]. This highlights the need to develop new tools to evaluate and select genotypes with improved adaptability to multi-environmental changes. In this study we performed a large-scale transcriptome analysis of *Coffea* spp. leaves from plants subjected to predicted changes on environmental conditions.

Material and Methods

Plant Material and Growth Conditions

Plants of *Coffea arabica* L. cv. Icatu and *C. canephora* cv. Conilon Clone 153 (CL 153), *ca*. 1.5 years of age, were grown for 10 months in 28 L pots in walk-in chambers, under environmental controlled conditions of temperature (25/20 ºC, day/night), irradiance (*ca*. 700-800 µmol m^{-2} s^{-1}), RH (75%), photoperiod (12 h), and 380 or 700 µL CO_2 L^{-1} in air. Thereafter, temperature was increased (0.5 °C day^{-1}) from 25/20 °C to 42/34 °C.

Methods

Total RNA was isolated from leaves of both genotypes, subjected to 380 or 700 µL CO_2 L^{-1}, and 25/20°C, 37/30°C, and 42/34 °C. mRNA libraries were constructed with the Illumina "RNA-seq sample prep" kit (Illumina, San Diego, CA) and sequenced separately on a Hiseq 2000 (Illumina) at the MGX platform (MGX-Montpellier GenomiX, www.mgx.cnrs.fr/). High-quality reads were sequenced and assembled into a reference genome of *C. canephora* [4] (www.coffee-genome.org/coffeacanephora). Data analysis was performed with tools from the R Language and the Cytoscape plug-ins *Bingo* and *Enrichment Map*.

Results and Discussion

Global analysis (Fig. 1)

In total 25574 uni-sequences were retrieved from both genotypes. From these, 18409 corresponded to differentially expressed genes (DEG) under at least one temperature condition. The number of DEG increased with the enhancement of temperature and [CO_2]. Genotype specific genes were about 50% of all DEG. While temperature generally affected more genes in Icatu than CL 153, the single effect of [CO_2] impacted more genes in CL 153.

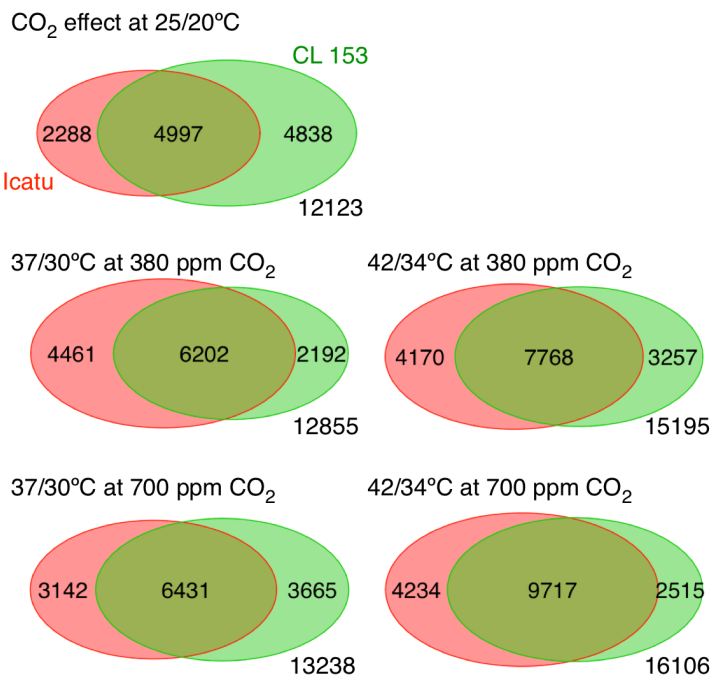

Figure 1 - Number of differentially expressed genes per genotype, temperature and [CO_2]

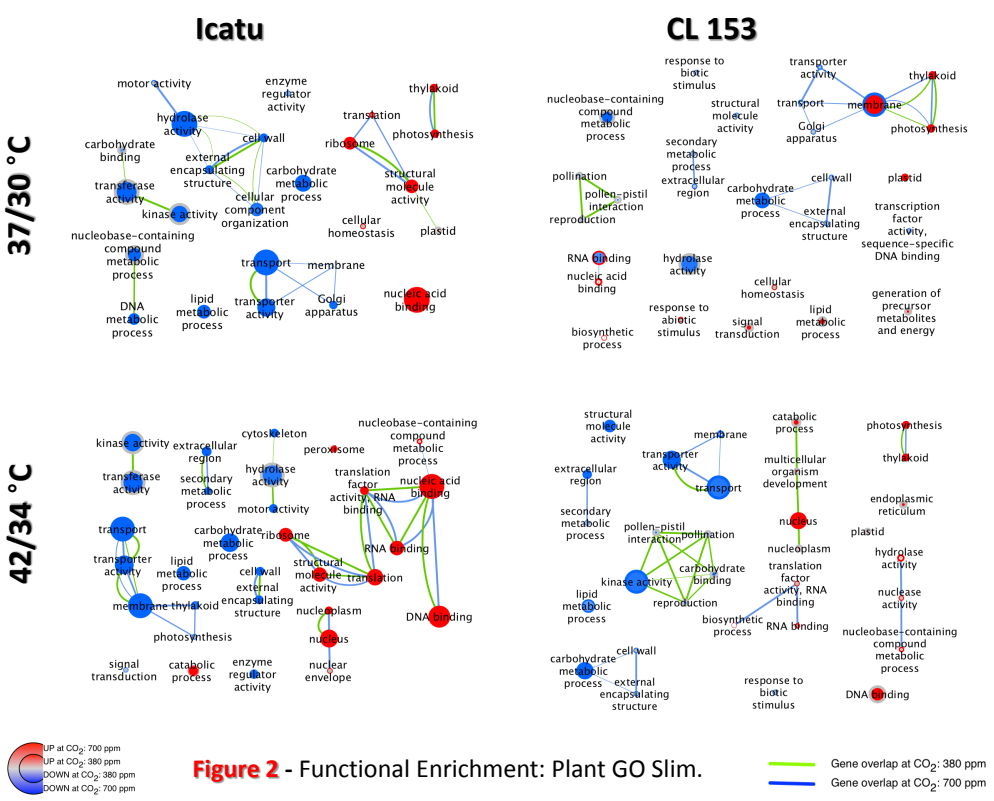

Figure 2 - Functional Enrichment: Plant GO Slim.

Effect of temperature and [CO_2] on differential expression (Fig. 2)

In general, preliminary exploitation of the large transcriptional data set showed that the main down-regulated functions by temperature and [CO_2] were transferase and kinase activity, carbohydrate metabolism, transport, and cell wall biosynthesis, while the main up-regulated functions were nucleic acid binding; translational machinery, and structural molecule activity (Icatu). On the other hand, photosynthesis (including thylakoids), and membranes presented a mixed (up and down-regulation) expression profile. The affected pathways were also genotype-dependent. A thorough expression analysis of dominant genes in each gene set will unravel in detail the estimated response patterns promoted by likely future environmental warming and [CO_2] conditions (in progress).

Conclusions

High temperature and [CO_2] enhance differential expression in both genotypes, involving a wide range of processes, functions and components. In view of this complex interactions between environmental factors, identification of major pathways related to stress acclimation is in progress.

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[1] Ghini R, *et al*. (2015) *Climatic Change*, 132:307-320. DOI: 10.1007/s10584-015-1422-2.
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The 26th International Conference on Coffee Science
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[1] UBRG, Instituto Nacional de Investigação Agrária e Veterinária, Portugal

[2] DCT, Fac. Ciências e Tecnologia, Universidade NOVA de Lisboa, Portugal

[3] PlantStress&Biodiversity, LEAF, DRAT, Instituto Superior de Agronomia, Universidade de Lisboa, Oeiras, Portugal

[4] CCTA, Universidade Estadual do Norte Fluminense, RJ, Brazil

[5, 6] CEUNES, DCAB, UFES, São Mateus and PPGPV, CCA, UFES, Alegre, ES, Brazil

[7] cE3c, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

[8] Dept. Biologia Vegetal, Universidade Federal de Viçosa, MG, Brazil

Introduction

According to Intergovernmental Panel on Climate Change (IPCC), relevant climate changes will occur in a near future, having major agricultural implications regarding important crops, such as coffee. Based on global warming predictions, modeling studies estimate severe reductions of biodiversity (particularly in *Coffea arabica),* of adequate cultivation areas and yield, therefore affecting the whole coffee chain of value. However, despite several recent works [1,2,3], real biological effects of supra-optimal temperatures and high [CO_2] are still poorly understanded. Membrane lipid dynamics is crucial to plant adaptation to abiotic stresses, including coffee [4]. In this context, this work describes lipid changes induced by heat and high [CO_2] in chloroplast membranes of *C. arabica* cv. Icatu.

Material and Methods

Plant Material and Growth Conditions

Plants (*ca*. 1.5 years) of *Coffea arabica* L. cv. Icatu were grown for 10 months in 28 L pots in walk-in chambers, under controlled temperature (25/20 ºC, day/night), irradiance (*ca*. 700-800 µmol m^{-2} s^{-1}), RH (75%), photoperiod (12 h), and 380 or 700 µL CO_2 L^{-1} in air. Thereafter, temperature was increased (0.5 ºC day^{-1}) from 25/20 ºC to 42/34 ºC.

Methods

Lipid analysis were performed in chloroplast membranes according to [4] in leaf samples collected at 25/20 ºC, 31/25 ºC, 37/30 ºC and 42/34 ºC. Lipid classes were separated by thin layer chromatography using two solvent systems. Lipid bands were scraped off, saponified and methylated for individual fatty acids (FAs) analysis by gas-liquid chromatography (Varian, CP-3380). Individual FAs and lipid classes were identified by comparison with known standards. Total fatty acids (TFAs) values are the sum of individual FAs. The double bond index (DBI) was calculated as DBI = [(% monoenes + 2 x % dienes + 3 x % trienes / (% saturated FAs)].

Results and Discussion

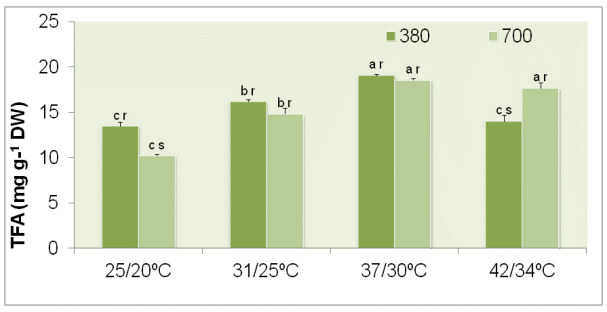

Fig. 1 – Effect of [CO_2] (µL L^{-1}) and temperature on total fatty acids (TFA) of chloroplast membranes. Values are means ± SE (n=3); significant differences between temperatures for the same [CO_2] (a,b,c), or between [CO_2] for each temperature (r,s).

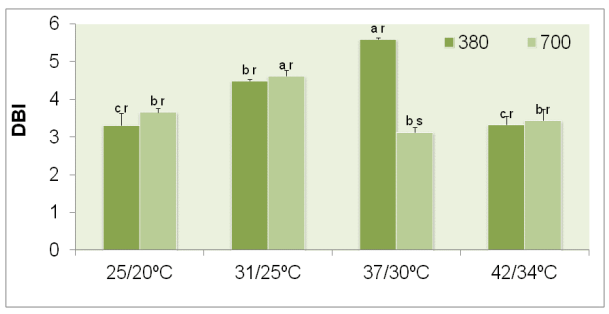

Fig. 2 – Effect of [CO_2] (µL L^{-1}) and temperature on double bond index (DBI) of chloroplast membranes. Values are means ± SE (n=3); significant differences between temperatures for the same [CO_2] (a,b,c), or between [CO_2] for each temperature (r,s).

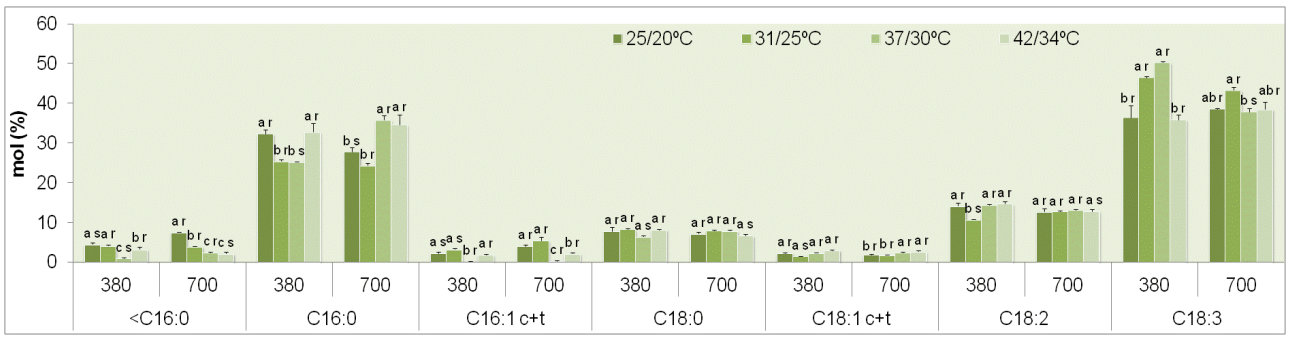

Fig. 3 – Effect of [CO_2] (µL L^{-1}) and temperatures on fatty acids composition of chloroplast membranes. Values are means ± SE (n=3); significant differences between temperatures for the same [CO_2] (a,b,c), or between [CO_2] for each temperature (r,s).

- TFA increased until 37/30 ºC under both [CO_2] (**Fig. 1**), mainly as a result of galactolipids rise (**Fig. 4**). At 42/34 ºC TFA stabilized at high [CO_2], but they decreased at normal [CO_2] as compared to 37/30 ºC.
- At 25/20 ºC, a higher galactolipids/phospholipids ratio occurred under high [CO_2] due to more abundant DGDG (digalactosyldiacylglycerol) and lower PC (phosphatidylcholine) (**Fig. 4**).
- Until 37/30 ºC increased unsaturation (DBI) (**Fig. 2**) was particularly stimulated by temperature under normal [CO_2], due to membrane enrichment in the most abundant PUFA, linolenic acid (C18:3) (**Fig. 3**).

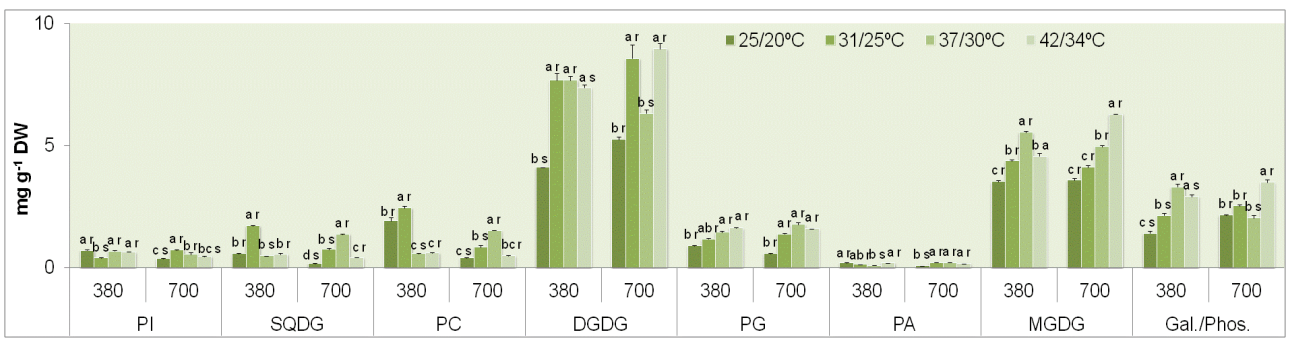

Fig. 4 – Effect of [CO_2] (µL L^{-1}) and temperatures on lipid classes and galactolipids/phospholipids ratio of chloroplast membranes. Values are means ± SE (n=3); significant differences between temperatures for the same [CO_2] (a,b,c), or between [CO_2] for each temperature (r,s).
PI - Phosphatidylinositol; PC – Phosphatidylcholine; SQDG - Sulfoquinovosyl diacylglycerol; DGDG – Digalactosyldiacylglycerol; PG – Phosphatidylglycerol; PA - Phosphatidic acid; MGDG – Monogalactosyldiacylglycerol; Galactolipids – sum of DGDG and MGDG; Phospholipids – sum of PI, PC, PG and PA.

Conclusions

In plants subjected to 42/34 ºC and high [CO_2], enhanced galactolipids/phospholipids ratio combined to stable TFA and DBI lowering might have accounted to the previously reported maintenance of chloroplast membrane functioning and performance [5,6].

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DIVERSITY AND GENETIC ANALYSIS OF BEAN MORPHOLOGY AND QUALITY COMPOUNDS IN ARABICA COFFEE (*COFFEA ARABICA* L.)

PB212

TRAN, Hue T.M.[ab*], VARGAS, Carlos Alberto Cordero[c], LEE, Slade L.[ad], FURTADO, Agnelo[a], SMYTH, Heather[a], HENRY, Robert[a]

[a] QAAFI/The University of Queensland, Australia, [b] WASI, Vietnam, [c] CATIE, Costa Rica, [d] Southern Cross University, Australia

*Presenting author: t.tran22@uq.edu.au

Introduction

The narrow genetic base of commercial arabica resulting from a genetic bottle neck in domestication and self-pollination has been well documented (Teressa et al., 2010 and references therein), raising the need for new diverse germplasm sources. Diverse genetic materials are also critical for the success of association studies to identify genes and markers linked to quality traits.

Beans of 232 diverse arabica coffee accessions originating from 27 countries were harvested from the germplasm collection at CATIE in Costa Rica. Non-volatiles and volatiles were analysed by HPLC and GCMS (SIDA combined with HS-SPME), respectively. XP-GWAS method (Yang et al., 2015) was applied to identify SNPs affecting these compounds.

Table 1. Variation in green bean physical quality measured in 232 arabica coffee accessions

Variable	Min	Max	Mean	CV (%)	Lsd 0.05	Literature
W100 (g)	10.16	23.13	15.53	12.20	0.54	9.77 - 21.82
Length (L) (mm)	6.64	13.20	9.62	7.73	1.12	8.19 - 11.04
Width (W) (mm)	5.97	8.08	6.83	5.43	0.59	6.11 - 8.27
Ratio L/W	1.11	1.75	1.41	7.68	0.15	1.33 – 1.35
Thickness (mm)	3.27	5.18	3.95	7.04	0.49	4.60 - 5.13
Bulk density (kg/m^3)	454	679	600	4.57	20.86	635 - 707

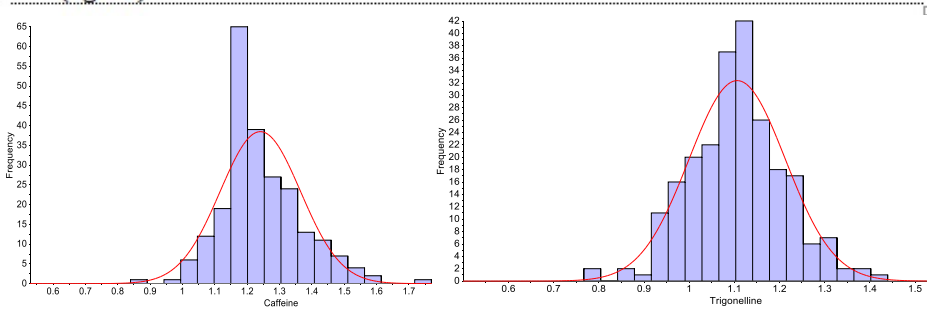

Figure 1. The distribution of caffeine (left) and trigonelline (right) content in the arabica coffee population (n = 232).

Table 2. Pearson correlation between bean morphology, non-volatiles and volatiles (n = 35)

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)
(2)	-0.13																						
(3)	0.14	-0.20																					
(4)	0.25	-0.41	0.38																				
(5)	0.07	0.46	-0.10	-0.08																			
(6)	-0.57	0.35	-0.19	-0.15	0.20																		
(7)	0.06	-0.16	-0.03	-0.10	-0.10	-0.23																	
(8)	0.21	-0.22	0.04	-0.01	-0.11	-0.42	0.76																
(9)	0.12	0.00	-0.02	-0.22	-0.14	-0.22	0.66	0.73															
(10)	-0.02	0.09	-0.16	-0.44	-0.14	-0.18	0.63	0.69	**0.91**														
(11)	-0.06	0.04	-0.09	-0.28	-0.21	-0.10	0.66	0.62	**0.94**	**0.91**													
(12)	-0.24	0.39	-0.32	-0.68	0.07	0.12	0.29	0.26	0.62	0.75	0.62												
(13)	-0.19	0.44	-0.41	-0.76	0.11	0.14	0.16	0.06	0.45	0.64	0.49	**0.90**											
(14)	0.25	-0.21	0.30	-0.07	0.01	-0.15	0.27	0.44	0.18	0.23	0.12	0.14	0.09										
(15)	0.17	-0.29	0.27	0.00	0.03	-0.16	0.45	0.50	0.37	0.29	0.23	0.13	0.05	0.54									
(16)	0.01	0.04	0.15	-0.12	0.07	-0.08	0.47	0.60	0.80	0.76	0.70	0.64	0.44	0.35	0.50								
(17)	-0.05	0.03	0.09	0.11	0.15	0.00	0.37	0.49	0.57	0.52	0.51	0.47	0.17	0.28	0.29	**0.81**							
(18)	-0.05	-0.01	0.10	0.23	0.20	0.04	0.26	0.32	0.44	0.38	0.38	0.39	0.11	0.17	0.18	0.70	**0.95**						
(19)	0.05	0.14	0.16	-0.20	0.06	0.06	0.21	0.22	0.59	0.53	0.50	0.58	0.47	0.21	0.37	0.75	0.53	0.47					
(20)	-0.07	0.10	0.02	-0.11	0.13	-0.01	0.43	0.50	0.73	0.72	0.69	0.68	0.45	0.26	0.35	**0.92**	**0.93**	**0.87**	0.70				
(21)	0.33	-0.42	0.51	0.46	0.04	-0.19	-0.18	-0.22	-0.24	-0.36	-0.29	-0.44	-0.39	0.22	0.18	-0.07	-0.09	0.05	0.02	-0.14			
(22)	0.05	-0.33	0.46	0.18	-0.18	-0.01	0.30	0.34	0.25	0.18	0.18	-0.03	-0.27	0.29	0.41	0.45	0.42	0.34	0.31	0.37	0.23		
(23)	-0.11	-0.23	0.27	0.04	-0.24	0.03	0.40	0.22	0.36	0.26	0.35	0.18	0.02	0.02	0.44	0.44	0.30	0.25	0.39	0.38	0.18	0.75	
(24)	-0.05	-0.15	0.18	0.07	0.06	-0.21	0.47	0.53	0.47	0.42	0.47	0.28	0.09	0.24	0.30	0.48	0.35	0.31	0.28	0.43	-0.05	0.31	0.35

Bean morphology; Non-volatiles; Ketones; Aldehydes; Phenolic compounds; Norisoprenoids; Pyrazines; (Terpenes)

(1) Weight of 100 bean (2) Bean moisture loss during roasting (3) Roasted bean density (4) Bean roasting colour (5) caffeine (6) trigonelline (7) 2,3-butanedione (8) 2,3-pentanedione (9) 2-methylpropanal (10) 3-methylbutanal (11) 2-methylbutanal (12) guaiacol (13) 4-ethylguaiacol (14) 4-vinylguaiacol (15) beta damascenone (16) 2,5-dimethylpyrazine (17) 2-ethyl-3,6-dimethylpyrazine (18) 2,3-diethyl-5-methylpyrazine (19) 2,3-dimethylpyrazine (20) 2-ethyl-3,5-dimethylpyrazine (21) 3-isobutyl-2-methoxypyrazine (22) linalool (23) geraniol (24) limonene (values highlighted in red are different from 0 with a significance level $P = 0.001$. Values in bold are highly significant correlation ($r > 0.80$).

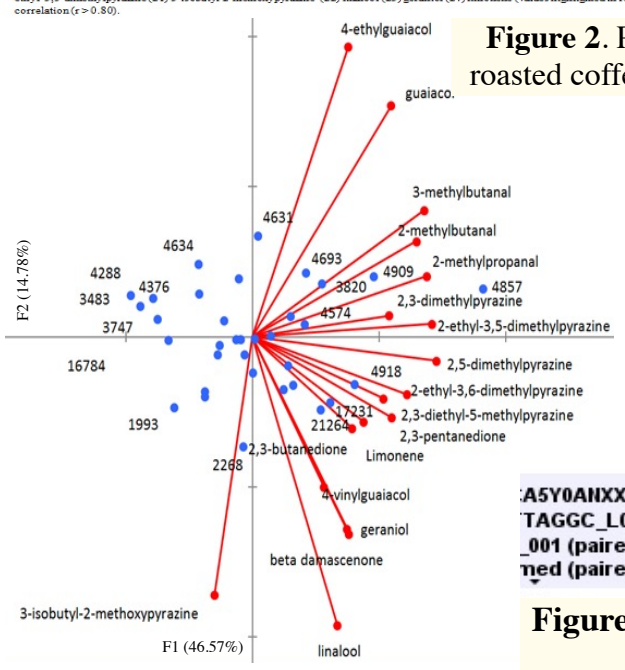

Figure 2. PCA of 18 volatiles measured in roasted coffee bean for 35 arabica accessions

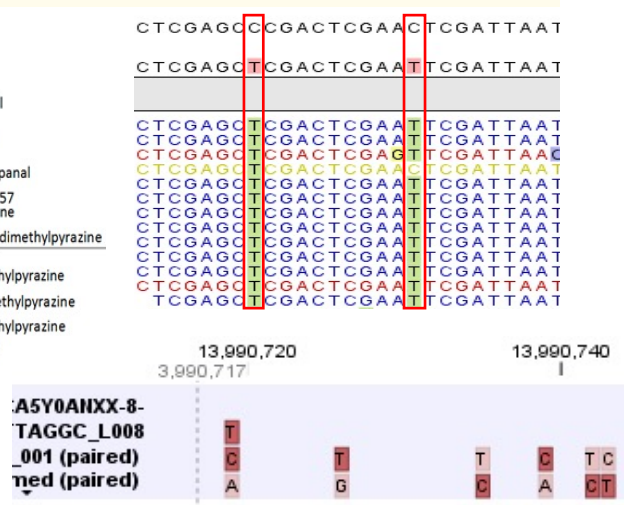

Figure 3. Identification of SNPs affecting caffeine and trigonelline.

Findings: Substantial variation was observed for bean morphology including weight of 100 beans, bean length, width and thickness, and bulk density (Table 1). Non-volatiles including caffeine and trigonelline showed large variation in the range previously reported (Fig. 1). Targeted analysis of 18 volatiles from 35 accessions also showed significant variation (CV% from 13.50% for 4-vinylguaiacol to 62.13% for geraniol) and correlation (Fig. 2). No strong correlation was found between bean morphology and the levels of non-volatile or volatile compounds (Table 2). For each non-volatile, DNA bulks from accessions of two phenotypic extremes were subjected to WGS for identification of trait associated variants (Fig. 3).

Conclusions

The large genotypic variation observed for bean morphology and biochemical traits and the lack of strong correlations indicates that it should be possible to breed for desirable combinations of traits (i.e. large bean size, low caffeine, high trigonelline, and favourable volatiles). Identification of DNA markers affecting caffeine and trigonelline contents will assist arabica coffee breeding.

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Pathogenicity of Strains of *Mycena citricolor* from Different Hosts

PB213

GRANADOS-MONTERO, Milagro; AVELINO, Jacques; ARAUZ-CAVALLINI, Felipe; CASTRO-TANZI, Sebastian; UREÑA Natalia

Abstract

There are differences in pathogenicity of strains recovered from different coffee varieties and companion vegetation. The infection success (IS), gemmae production capacity by lesion, incubation and latent periods, and pathogenicity index (PI) were calculated for each strain. We hypothesized that these differences are physiological and are not due to genetic variation.

Introduction

Conventionally, coffee farmers try to control American leaf spot disease by applying treatments directed to residual inoculum present on old damaged coffee leaves. However, the effectiveness of these treatments is low, particularly in Niña years where the epidemics increase faster. Our hypothesis is that there is a "hidden inoculum" not controlled, living all the year, in the companion vegetation (weeds and shade trees) which can efficiently contribute to the epidemic onset.

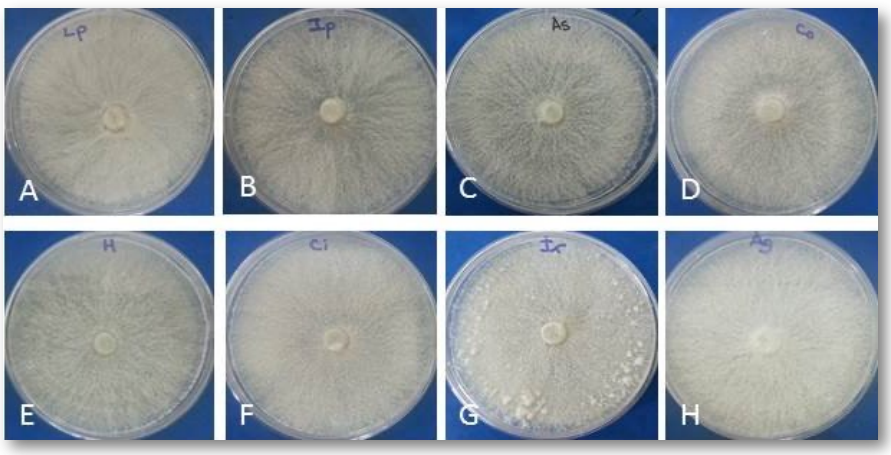

Figure 1. Eight strains of *M. citricolor* with 38 days. A) McSp B) McIn C) McCc D) McCo E) McPo F) McCv G) McIr H) McPa.

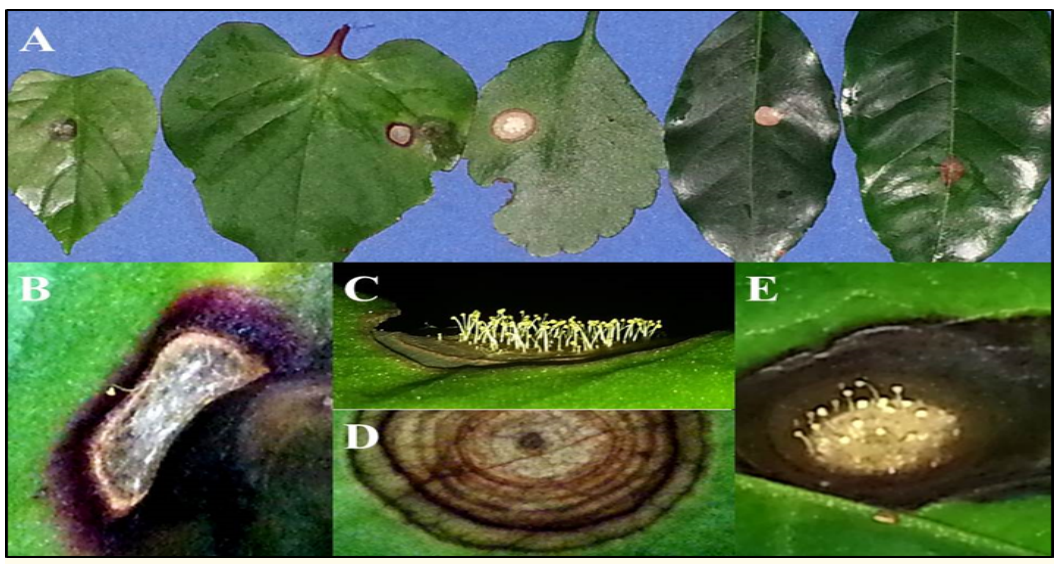

Figure 2. Lesiones by *M. citricolor* in different hosts. A) left to righ: *C. verticillata* (Cv), *A. cordifolia* (Ac), *B. calycinum* (Bc); Caturra (Ca) and Catimor (K) coffee varieties. B) *A. cordifolia* spot detail. C) Geminifers on *B. calycinum* lesion. D) Characteristic zonation in *B. calycinum* lesion. E) *C. verticillata* spot detail.

Main text

It collected leaves with symptoms or/and gemmae and were transported to the University of Costa Rica Phytopathology Lab for pathogen isolation or for direct inoculum picking. At least 15 gemmae were placed on the adaxial surface of healthy and fresh Caturra leaves of two years old. Were maintained into humid boxes at 20-21° C and 100% of relative humidity for 15 days, and at 24-25° C and approximatively 80% of relative humidity for other two weeks. The number of formed lesions, the mean diameter of each lesion and the quantity of geminifers produced were assessed each two days. The strain McK had a 100% IS, it was the first in producing lesions and started the geminifers production 6 days before McCa. The isolates with major PI were recovered from *Anredera cordifolia* (9.1), McK (9.9) and *Bryophyllum calycinum* (18.9). Caturra (McCa) only showed a PI of 3.67, reflecting a low pathogenicity.

Inoculum directly coming from field was more aggressive than inoculum coming from *in vitro* culture.

Conclusion

This work indicates that there are more sources of inoculum in coffee plantations than those normally expected. It is important to control this inoculum by applying a selective management of weeds, using less susceptible shade trees, and controlling isolated Catimors within Caturra plantations, particularly in localities with favorable conditions for the pathogen.

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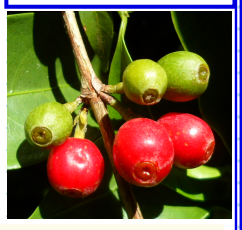
Divo, a new family of *Copia* LTR-Retrotransposons in coffee-trees

PB215

Dupeyron M‡*, Hamon P*, de Kochko A*, Crouzillat D §, and Guyot R‡
*Evolution des Génomes des Caféiers, UMR DIADE; ‡ CoffeeAdapt, UMR IMPE; IRD, centre IRD de Montpellier, BP 64501, Montpellier Cedex 5, France; § Nestle´ R&D Tours, Notre Dame d'Oe´, Tours, France
mathilde.dupeyron@ird.fr



Abstract
LTR-Retrotransposons (LTR-RTs, classe I transposable elements) are the main components of plant genomes. Numerous lineages and families have been described, leading to a well-established classification of elements. This profusion of LTR-RTs sequences and structures were enriched with the recent discovery of non-autonomous element structures, such as LARDS, TRIM and TR-GAG. With the available bioinformatics tools dedicated to the LTR-RTs identification and analysis, it became reasonable to performed annotation of such transposable elements at whole-genome scale. Here during the process of *Coffea* genomes annotation from the Arabica Coffee Genome Consortium (ACGC), we describe a novel family of *Copia* LTR-RT called *Divo*, belonging to the *Bianc*a lineage. *Divo* is restricted to dicotyledonous plant genomes, with a relatively low copy number in coffee-trees, *Arabidopsis* and grapevine. In *Coffea*, the presence of recently inserted and complete copies suggests that *Divo* might be still active, but with a different evolution between *Coffea arabica*, the only allotetraploid genome, and its two diploid progenitors, *Coffea canephora* and *Coffea eugenioides*. Altogether our results indicate that *Divo* is a novel *Copia* LTR-RTs family, ubiquitous in dicotyledonous plant genomes and can highlight evolutionary events in coffee-trees genomes.

A novel *Copia* LTR-RTs family

Divo is a typical *Copia* element, relatively short (~5kb), carrying Long Terminal Repeats (LTRs) at each extremity and *Gag* and *Pol* genes involved in the mobility of the element (Fig. 1). The Primer Binding Site (PBS) involved in the retrotranscription mechanism seems unusual for this family, as it does not correspond to any known tRNA. Full-length copies have been found in *C. canephora*, *C. arabica* and *C. eugenioides*. We searched for *Divo* copy number in these genomes as well as the number of solo-LTR, which are witnesses of LTR-RTs elimination by the genome (Table 1).

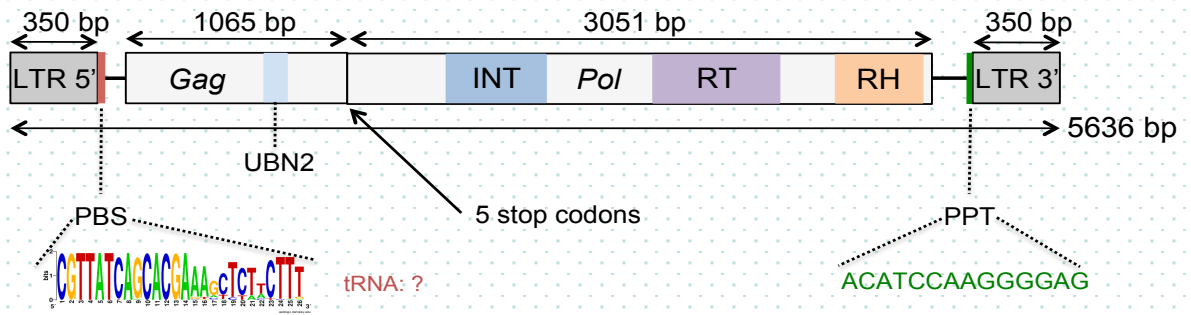


Figure 1: Structure of a *Divo* full-length copy found in *C. canephora*. The Weblogo® represents the putative PBS region of 41 *Divo* elements from *C. canephora*.

	Full-length copies (80% ID – 100% length)	Copies (80% ID – 80% length)	Partial copies (20% ID – 80% length)	Solo-LTRs	Total
C. canephora	41	129	212	142	524
C. arabica	37	204	351	201	793
C. eugenioides	20	132	223	336	711

Table 1: Assessment of *Divo* copy numbers in ACGC *C. canephora*, *C. arabica* and *C. eugenioides* genome sequences.

A recent but different amplification history

When a new copy inserts into the genome, LTR sequences are identical and are supposed to evolve separately, so the more they present a high divergence, the more the copy is old. We can though estimate an insertion age for each complete copy. *Divo* seems to have amplified its copy number twice in *C. eugenioides*, as we can see two small peaks between 1.5 and 2 Mya and 0.5 and 1 Mya, whereas a burst has occurred really recently in *C. canephora* (Fig. 2). *C. arabica* presents an intermediate pattern between its progenitors for the estimated ages of copy insertion but not for the copy numbers (Table 1).
Phylogenetic analyses with RT domains show that *Divo* belongs to the poorly known lineage *Bianca*. We can observe a separation of *Bianca* lineage in two families: *Divo* in dicotyledonous plants, the other family in monocotyledonous plants (Fig. 3).

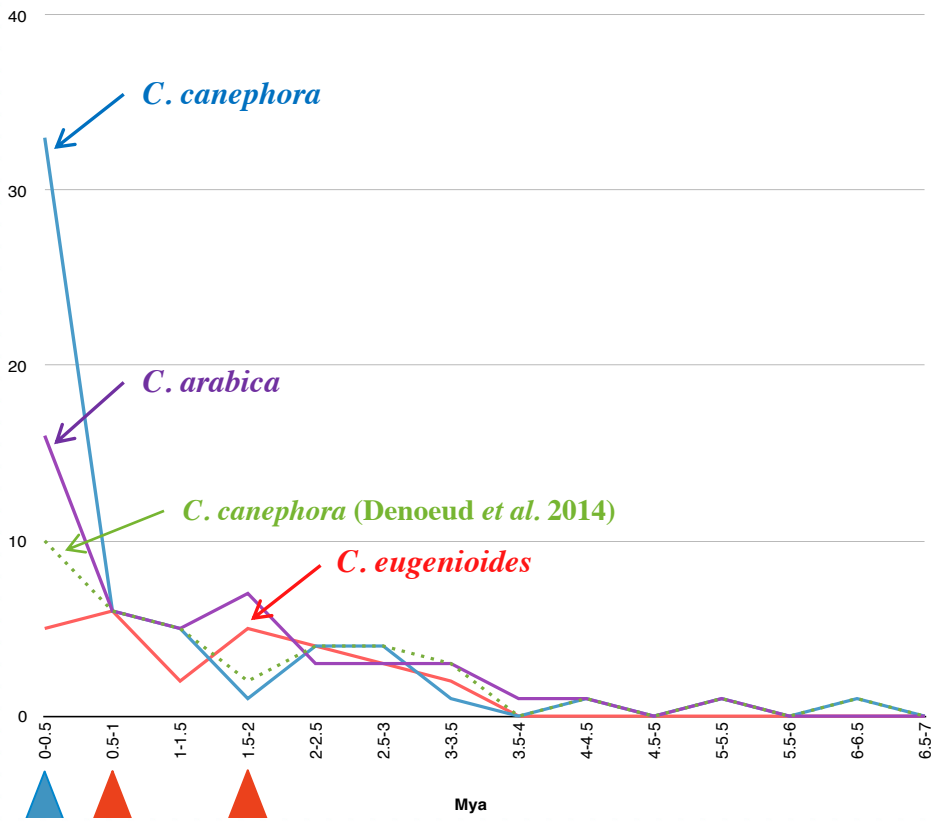


Figure 2: Estimated insertion ages of *Divo* full-length copies.

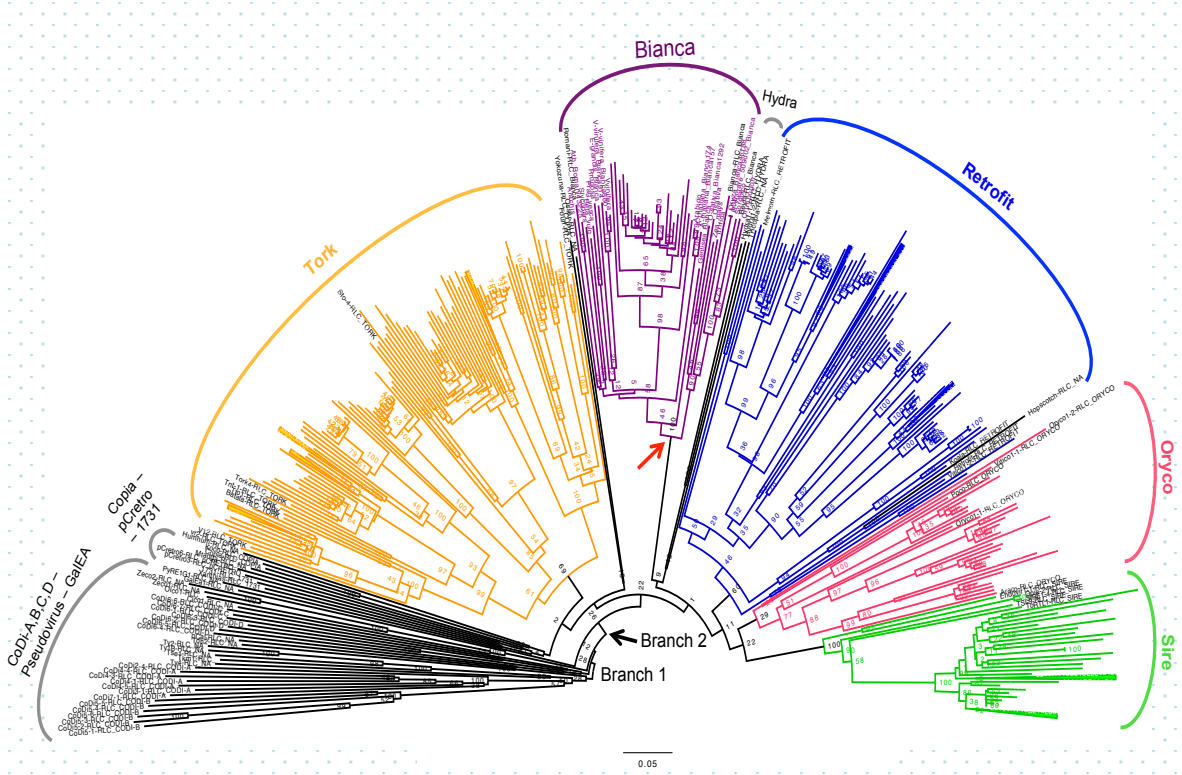


Figure 3: Reverse Transcriptase domain-based phylogenetic tree of *Copia* LTR-RTs in *C. arabica* genome and *Divo* copies in *C. arabica* and in others plant genus

Conclusion

Divo is a *Copia* LTR-RT (Fig. 1) potentially active in *Coffea* trees. The copy number differences between *C. canephora* and *C. eugenioides* genomes (Table 1) suggest a different accumulation and evolution of *Divo* in these two diploid species. *C. arabica,* the allotetraploid species, does not present the addition of the copy number of its two progenitors, suggesting reorganization of *Divo* elements during its recent formation, such as deletions. Moreover, *Divo* seems to be subjected to an intense elimination process in *C. eugenioides*, whereas it presents a really recent burst in *C. canephora* (Fig. 2), where it could therefore follow the genetic differentiation and had a great impact on the genome and gene functions. Our results also suggest that the *Bianca* lineage contains at least two families, *Divo* and *Bianca*. *Bianca* seems present only in Monocotyledonous plants, whereas *Divo* has been only found in Dicotyledonous ones (Fig. 3). Altogether, our results raise questions about a differential evolution of this TE lineage that accompanied *Coffea* evolution.

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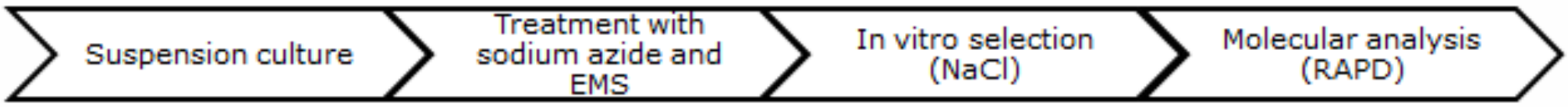
GENERATION OF CHEMICALLY INDUCED MUTATIONS USING EMBRYOGENIC COFFEE CELL SUSPENSIONS AND IN VITRO SELECTION FOR SALT TOLERANCE

PB216

BOLIVAR-GONZALEZ, Alejandro*, VALDEZ-MELARA- Marta F*, GATICA-ARIAS, Andrés M*
*Laboratorio Biotecnología de Plantas, Escuela de Biología, Universidad de Costa Rica, 2060 San Pedro, Costa Rica.
andres.gatica@ucr.ac.cr

Abstract
The objective of the present study was to induce mutation for salt tolerance using sodium azide and ethylmethanesulphonate (EMS) in embryogenic cell suspensions of coffee (*Coffea arabica* L. var. Catuaí), followed by cell line selection and subsequent plant regeneration. Determination of the optimal growth conditions for culture of embryogenic calli in liquid medium was the first step, three culture media were evaluated (CP ;van Boxtel & Berthouly, 1996), Teixeira *et al*, 2004 and Silva *et al*, 2000). It was determined that culture in flask with Teixeira liquid medium promoted the fastest calli proliferation and that the embryogenic regeneration was successfully achieves by culturing the calli in RITA ® systems with regeneration medium (van Boxtel & Berthouly, 1996). The medium lethal doses (LD_{50}) were determined for NaN_3 (5mM for 15 minutes) and for EMS (185.24 mM for 120 minutes). These doses were implemented in an *in vitro* selection protocol to determine the NaCl concentration that facilitated the identification of putative mutant cell lines. The NaCl concentration ended up being 150 mM. Finally, genetic variability was assessed and evaluated with RAPD markers; 50 bands were amplified and 22% of those bands were polymorphic. To our knowledge, this is the first report of in vitro selection of salt tolerant variants following sodium azide and EMS treatment of embryogenic coffee cell suspensions.



Introduction

Coffee represents the most important non-alcoholic beverage in the world economy. In the list of largest commodities in the international markets, it is currently ranked second, only behind oil. Coffee production sustainability and profitability are a growing problem. This is worsened by the complicated road that must be traveled to achieve successful conventionally bred varieties.
Crop improvement via mutagenesis is a powerful tool that adapts fairly well to the needs of a lot of coffee breeding programs. Mutagenesis can induce variability in genetically homogenous populations.

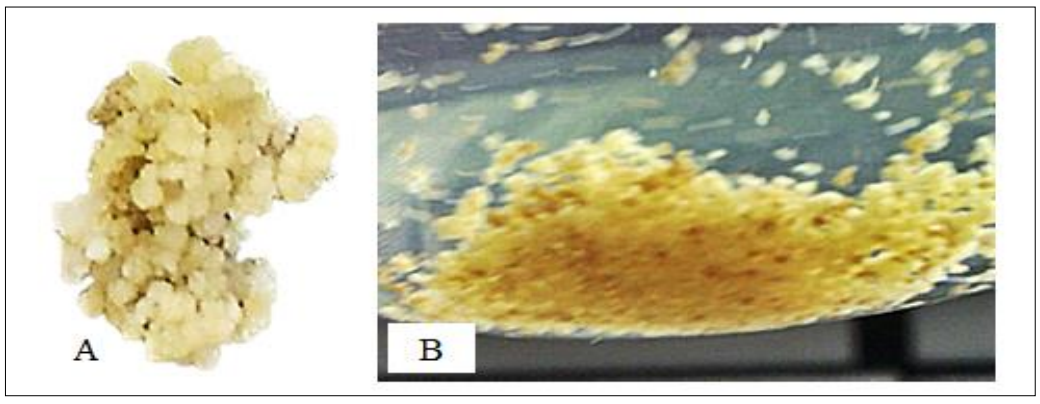


Figure 1. Embryogenic calli of coffee (*C. arabica* L. var. Catuaí). **a** Embryogenic friable calli obtained in C20 medium. **b** Embryogenic cell suspension culture growing in TEX liquid medium (Teixeira *et al.* 2004)

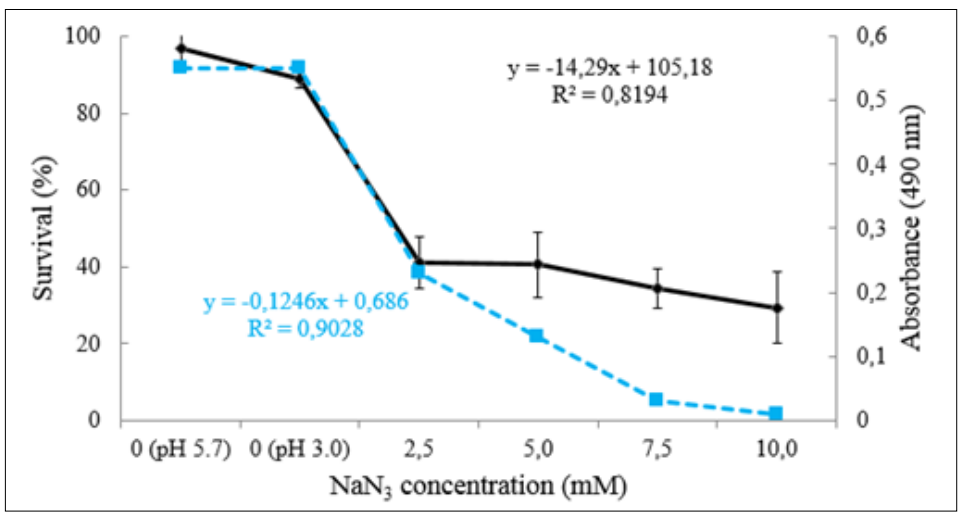


Figure 2. Effect of NaN_3 concentration on survival and viability of coffee (*C. arabica* L. var. Catuaí) embryogenic calli. **a** Survival percentage (solid line) and absorbance (490 nm) (dotted line) versus NaN_3 concentrations.

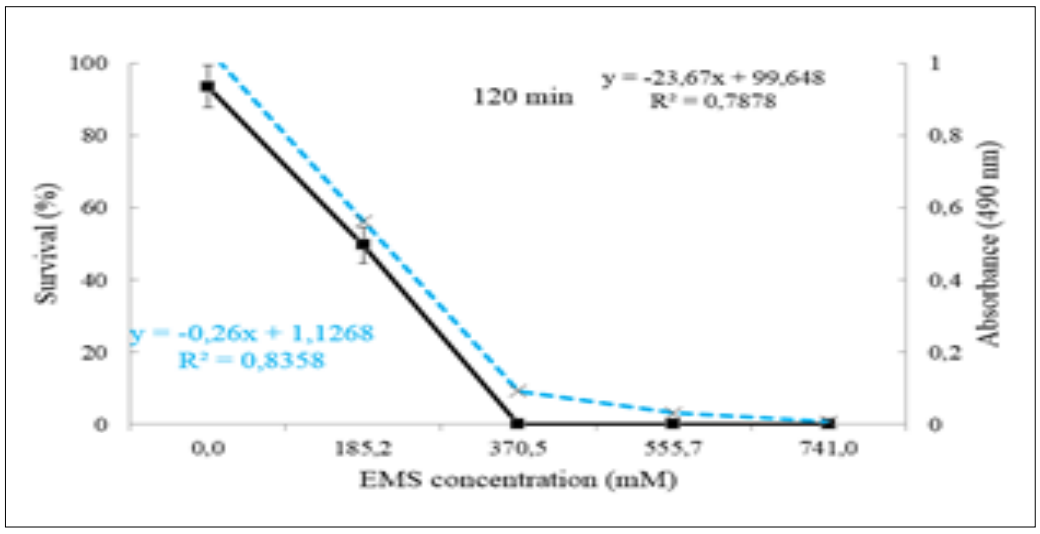


Figure 3. Effect of EMS concentration on survival and viability of coffee (*C. arabica* L. var. Catuaí) embryogenic calli. **a** Survival percentage (solid line) and absorbance (490 nm) (dotted line) after 60 min of exposure time to different EMS concentrations.

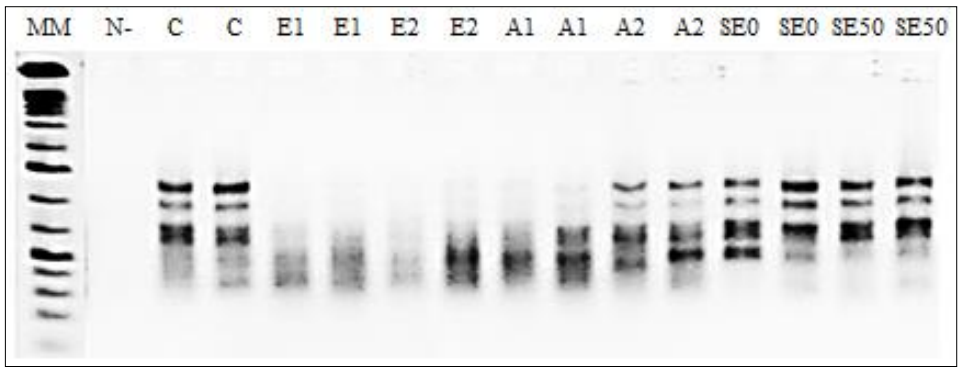


Figure 4. Band patterns obtained from the amplification of decameric primer OPB-02 in 7 genomic DNA samples of coffee (*C. arabica* L. var. Catuaí) embryogenic cell suspensions.

Conclusion

Our results indicate that embryogenic suspension cultures are suitable for sodium azide and EMS mutagenesis and provide the basis for the improvement of agriculturally important traits and to study gene function in coffee.

Acknowledgments

This study was financed by the University of Costa Rica and the Ministerio de Ciencia, Tecnología y Telecomunicaciones (MICITT) (project No. 111-B5-140)

Application of chemical mutagenesis to increase the resistance of coffee (*Cofeea arabica* L.) to leaf rust (*Hemileia vastatrix*)

VARGAS-SEGURA, Cesar*, SÁNCHEZ-AGUILAR, Karla**, TAPIA-FERNÁNDEZ, Ana**, ARAYA, Emmanuel***, VALDEZ-MELARA, Marta F*, GATICA-ARIAS, Andrés M*

*Laboratorio Biotecnología de Plantas, Escuela de Biología, Universidad de Costa Rica, 2060 San Pedro, Costa Rica ** Laboratorio de Investigación en Fitopatología, Universidad de Costa Rica, Sede del Atlántico *** Centro Nacional de Innovaciones Biotecnológicas (CENIBiot)
andres.gatica@ucr.ac.cr

PB217

Abstract

In order to induce genetic variability associated with characters for coffee rust resistance, coffee seeds (*Coffea arabica* cv. Catuaí) were treated by 8h with a solution of sodium azide (0, 50, 75, 100 and 125 mM) and ethyl methane sulfonate (EMS) (0, 1, 2, 3, 4 and 5% v/v). The LD_{50} values for sodium azide and EMS were between 50-75 mM and 2-3% v/v, respectively.

The age of a coffee leaf at the time of inoculation with rust affected the development of infection structures and sporulation. Therefore, leaves of one, two, three, four, five and six months old plants of the susceptible cultivars Caturra and Catuaí and the resistant cultivar CR-95 were inoculated with uredospores of *H. tastatrix* using a camel hairbrush. The inoculated plants were placed in the greenhouse at 22 ºC for 48 h in darkness and later were transferred at 26 ºC with a 12-h photoperiod. Preliminary results, demonstrated that Caturra is more susceptible than Catuaí; whereas CR-95 did not any symptom of the disease.

Finally, the induction of genetic variability in coffee seeds in response to the different sodium azide and EMS treatments was determined by AFLP (Amplified Fragment Length Polymorphism) analysis. The amplification of six AFLP primer combinations using a pool of plants obtained after mutagenic treatment with sodium azide allowed the identification of four polymorphism.

Introduction

Leaf rust, a disease caused by the fungus *Hemileia vastatrix*, is one of pest that causes most damage and economic impact in coffee. The rust fungus has affected 49% of the cultivated area of coffee in Central America and has forced the pruning of 28% of the coffee plantations in the region, despite the measures taken in each country to prevent this disease. In Costa Rica, the damage caused by rust have been severe and according to the Costa Rican Coffee Institute (ICAFE) this disease affect 64% of the country's coffee plantations.

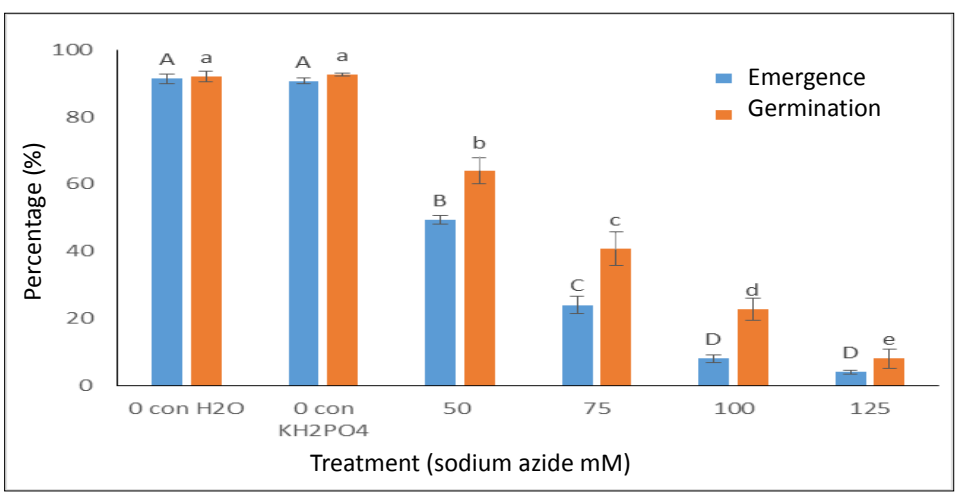


Figure 2. Effect of NaN_3 concentration on germination and emergence of coffee (*C. arabica* L. var. Catuaí) seeds.

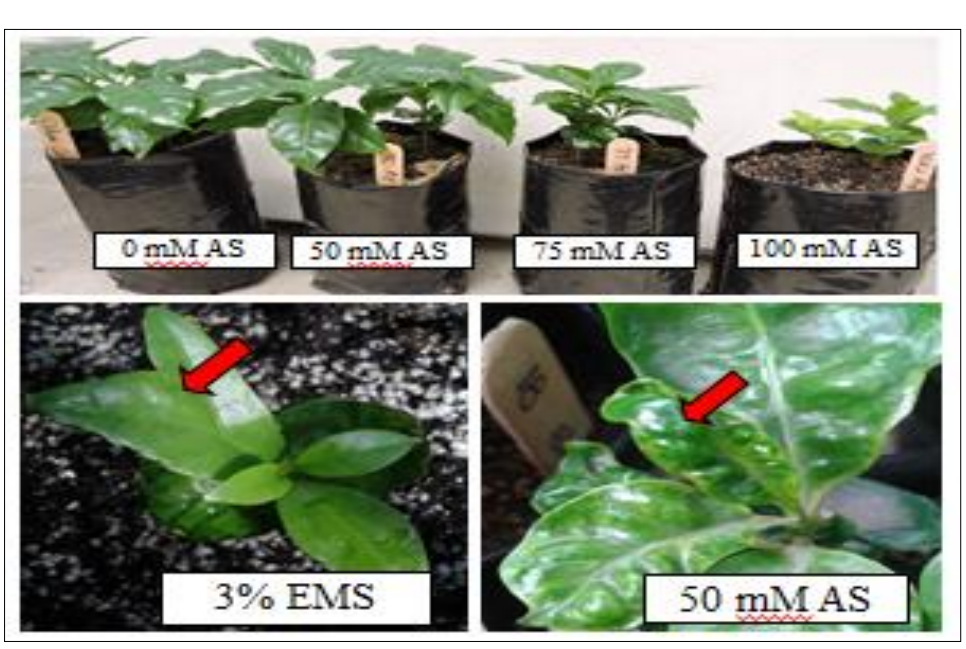


Figure 4. Coffee (*C. arabica* L. var. Catuaí) plants treated with different concentrations of sodium azide and EMS.

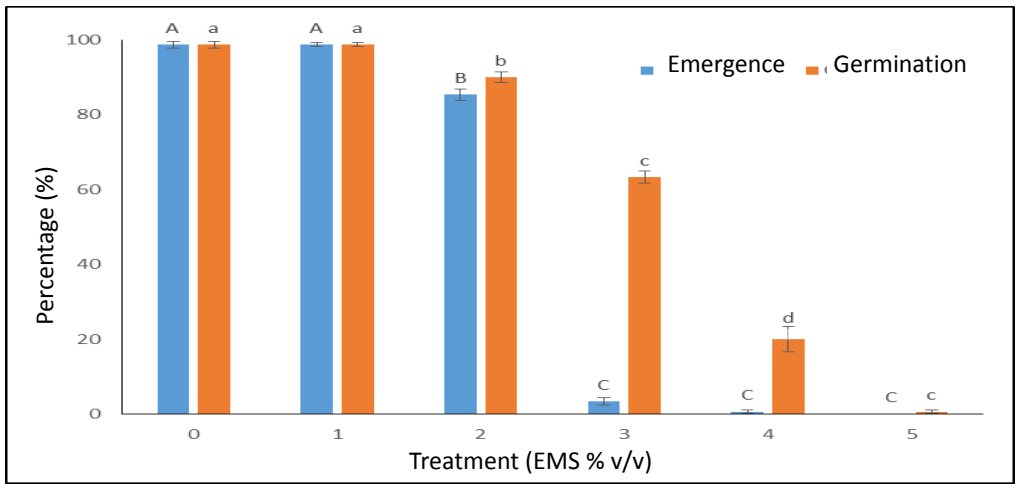


Figure 1. Effect of EMS concentration on germination and emergence of coffee (*C. arabica* L. var. Catuaí) seeds.

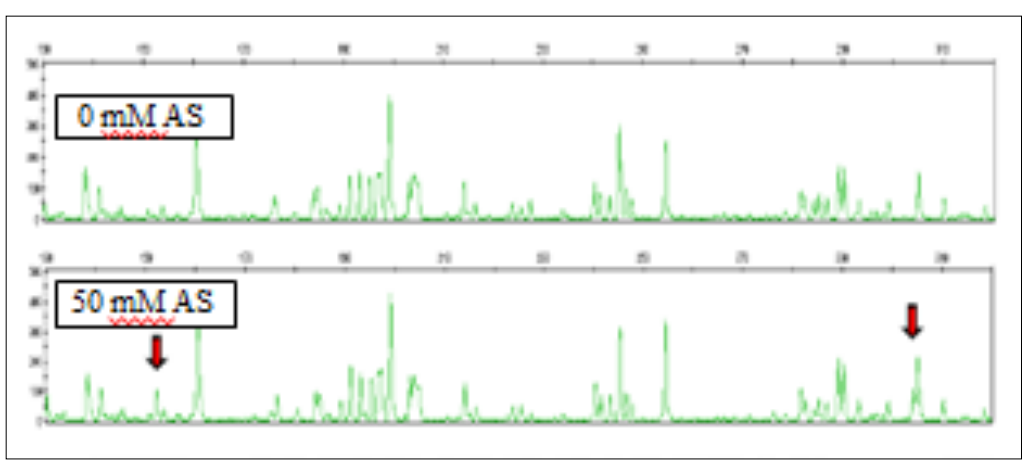


Figure 3. AFLP analyisis of coffee plants (*C. arabica* L. var. Catuaí) treated with sodium azide.

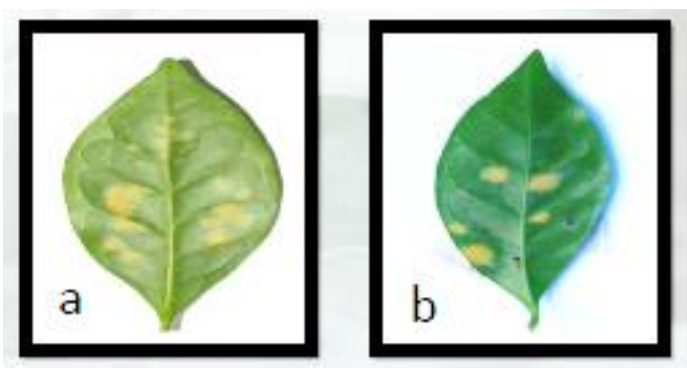


Figure 5. Two-months-old coffee (*C. arabica* L. var. Catuaí) leaves affected by rust (*Hemileia vastatrix*) (a) Caturra, (b) Catuaí.

Conclusion

Coffee breeding programs could use mutagenesis combined with screening methods and molecular markers as an additional tool to induce novel traits and produce new and improved coffee cultivars.

Acknowledgments

The University of Costa Rica and the Ministry of Science, Technology and Telecommunications (MICITT) financed this work.

Hybrid coffee (*Coffea arabica L.)* micro propagation in Ethiopia: status, challenges and prospects

Dereje Tamiru* and Elias Gebramariam*
***Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Center,**
Plant Biotechnology Research Section. Jimma, Ethiopia, P.O. Box 192 Email: tamiruf@hotmail.com

PB218

Abstract

Coffea arabica is the major agricultural commodity of Ethiopia and contributes about 20%-30% of the total export of the country. In spite of large genetic variation for improvement, the national average yield is very low (6 qt/ha). To improve production, three hybrids coffee varieties (Ababuna, Malko CH2 and Gawe) were developed by Jimma Agricultural Research Center, EIAR (Ethiopian Institute of Agricultural Research). However, the multiplication and distribution of these hybrid seedlings to the farmers and private sector has been a bottle neck. To alleviate this problem, plant tissue culture technique was adopted and established at Jimma Agricultural Research Center, EIAR. Since its inception in 2002, different plant tissue culture techniques such as nodal culture, direct somatic embryogenesis and indirect somatic embryogenesis were adopted. With Regard to nodal culture, nodal explants from in vitro derived orthotropic shoot was used and on average 2.7 shoots per node was achieved using BA as a plant growth regulator. Conversely, this technique is tedious, time consuming and difficult for mass propagation. Direct somatic embryogenesis was also tried as an alternative and it was possible to regenerate 13 somatic embryos from a single leaf explants (1 cm^2). However, this method is also not satisfactory to meet the desired goal. Likewise, using indirect somatic embryogenesis it has been possible to regenerate on avarage167/explants of somatic embryos per 1cm^2 leaf explants. Yet, the in vitro mass propagation of those coffee hybrids is by far below the required demand and the achieved result is by far below as compared to published results. The reason for this disparity is poor knowledge and technical skill, inadequate laboratory facilities and electric power shortage. This review summarizes the recent advances, challenges and achievement in hybrid coffee micro propagation in Ethiopia.

Introduction

❖Coffee is one of the major sources of export in Ethiopian economy as it contributes 25-30% of the Ethiopia's total export earnings (Abu Tefera, 2015). Beyond this, South-western Ethiopia is the natural habitat and primary center of diversity of *Coffea arabica* (Meyer,1965).

❖Despite the existence of genetic variation for improvement, the national average yield is very low (6 qt/ha).

❖In order to address this production constraints, Jimma Agricultural Research Center (JARC) developed three hybrid coffee cultivars Aba buna, MCH2 and Gawe with average yield of 23.8,24, and 26 qt/ha, respectively (Behailu et al., 2008).

❖However, those high yielding coffee varieties were not distributed to coffee growers, due to lack of efficient seedling production techniques.

❖Therefore, an economically feasible, clonally propagation method is needed to satisfy the growing demand of hybrid seed in order to make effective use of observed yield advantage.

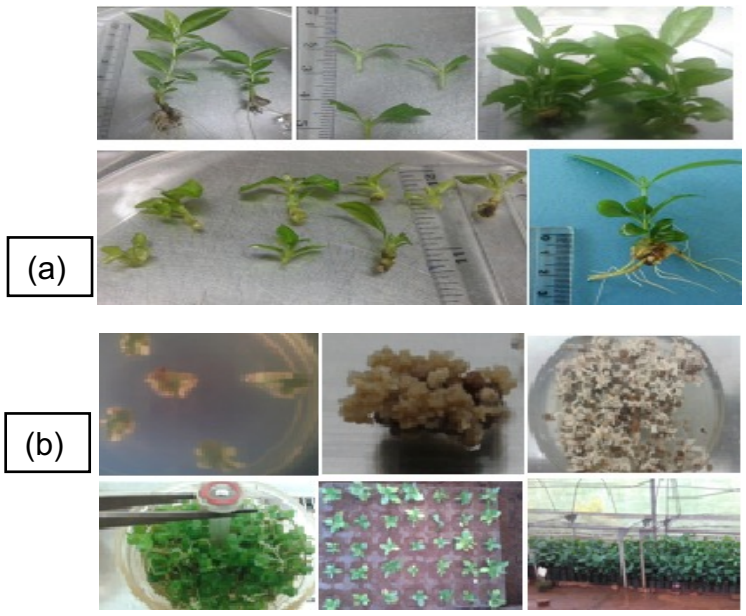


Figure 1: Coffee tissue culture (a) nodal culture (b) indirect somatic embryogenesis

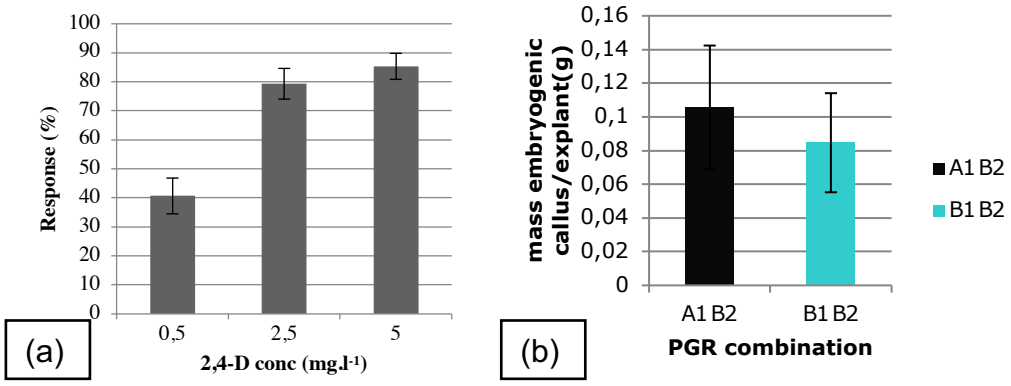


Figure 2 (a) Effect of 2,4-D on callus induction (b) Effect of different growth regulators on embryogenic callus production of (MCH2) hybrid *coffea arabica L.*

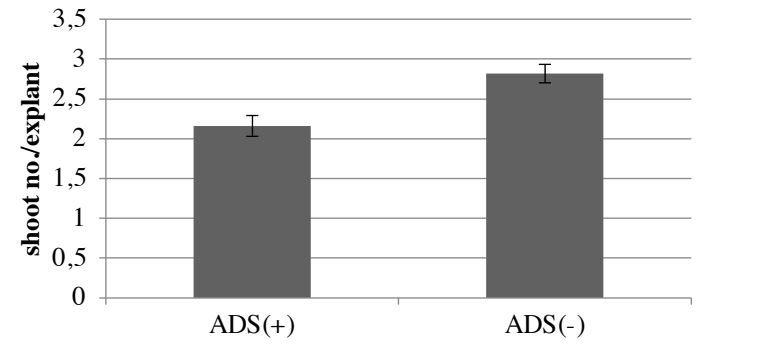


Figure 3: Effect of Adenine hemi sulfate on shoot multiplication of hybrid coffee (MCH2)

Discussion

❖Using nodal culture on average 2.7 shoots per node was gained. Supplementing of adenine hemi sulfate was affect the number and size of shoot regenerated.

❖ The rooting ability of 59.9 % was obtained from the shoots using ½ MS + 1.5 ml/L + 30g sucrose within two months.

❖Using direct somatic embryogenesis on average 13 somatic embryos / **1cm^2** leaf explants.

❖During pilot scale experiment,(indirect somatic embryogenesis) 11.42 g embryogenic callus were obtained and regenerated to 28,000 of somatic embryos (167/explants). Those somatic embryos were developed to 8000 plantlets up on first harvesting using temporary immersion bioreactor **RITA®**

❖Compared to the results reported elsewhere, our achievement is yet far below calling for more effort to achieve the desired goal. This is due to poor knowledge and technical skill, inadequate laboratory facilities.

Conclusion

•Indirect somatic embryogenesis provides an immense potential to multiply Ethiopian coffee (*Coffea arabica L.)* hybrids.

•Hybrid coffee *in vitro* plantlet production is still at an infant stage hence, strong emphasis should be given to build research capacity through enhancing human capacity (short and long term training), infrastructure (accredited tissue culture laboratory), technology and funding.

•Collaborative work should be encouraged with international coffee tissue culture laboratories for experience sharing.

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DIVERSITY, TRANSMISSION AND BIOLOGICAL FUNCTION OF ENDOPHYTES IN *MASCAROCOFFEA* SPECIES

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RAHARIMALALA Eva N.*, ANDRIAMIALIHARISOA Ravaka F., RATSIMBAZAFY Tojo T. N.**, MIASAMALAZA Mampionona Y.***, RAKOTOMALALA Jean Jacques R.*, HAMON Perla******

*Département de Recherches Agronomiques, FOFIFA, **Département de Biochimie et Biotechnologie, Université d'Antananarivo, ***Département de Biologie et Ecologie Végétales, Université d'Antananarivo, ****Institut de Recherche pour le Développement, Montpellier France

Abstract

Endophytes are bacteria or fungi living inside plant tissues without causing diseases symptoms (Wilson, 1995). They can synthesize secondary molecules which could transfer tolerance against abiotic and biotic stresses to the host plant. These molecules could be exploited for human health, agriculture, environment and industry.

Very few investigations on endophytes are reported for the genus *Coffea*. They concern only *Coffea arabica* and *C. canephora* (Vega et *al*., 2009). Our study is focused on the diversity, transmission and biological function of endophytes from five out 54 species belonging to *Mascarocoffea* (coffee-trees from Madagascar, Mascarenes and Comoros excluding the *Baracaoffea* group).

Tissue plants were surface-sterilized and bacteria were isolated using the medium Nutrient Agar (NA) and fungi using Potato Dextrose Agar (PDA); standard techniques are used for identification.

Fifty two endophytic bacteria isolated from three accessions of the group *Verae.* Many of them have the capacity to solubilize the inorganic phosphate. They could contribute to the growth of the host plant. *Actinomycetes* seems specific of *C. kianjavatensis* (A602).

About fungi, *Corticium* is present in all studied *Coffea*, while *Penicillium*, *Rhizoctonia*, *Pythium*, *Fusarium* and *Sclerotium* are the most frequent. The number of fungi transmitted vertically from seed to plantlet or by grafting is very restricted. Nevertheless, this phenomena seems to be linked to the reduction by *Penicillium* of the caffeine level in *C. canephora* grafted on *C. perrieri.* Several fungi from *C. perrieri* and *C. leroyi* have the capacity to synthesize monocaféoylquinic acid (AC3Q and AC4Q), chlorogenic acid (A5CQ), *o*-coumaric acid and coumarins. All these substances are present in the leaves of the host plant.

Our study is the first one focused on the importance of endophytes in *Mascarocoffea* coffees. It should be extended to all other *Coffea* (124) using more accurate techniques, especially molecular methods for the complete taxa determination of each endophyte, and modern apparatus for a better determination of secondary substances synthetized.

Introduction

Endophytes were defined by Wilson (1995) as bacteria or fungi living inside plant tissues without causing diseases symptoms.

Our study is focused on the diversity, transmission and biological function of endophytes from five *Mascarocoffea* species. These species are *Coffea homollei* (SZ), *C. kianjavatensis* (A213, A602), *C. leroyi* (A315), *C. farafanganensis* (A208) and *C. perrieri* (A305).

Endophytic bacteria and fungi were isolated from leaves by using medium Nutrient Agar (NA) and Potato Dextrose Agar (PDA) respectively.

Bacteria identification was based on their morphological appearance inside the media, their physiological and biochemical characteristics by using various types of medium.

Fungi identification was based on morphological characterization and microscopic identification.

About transmission, two cases were studied from seed to plantlet and from grafting.

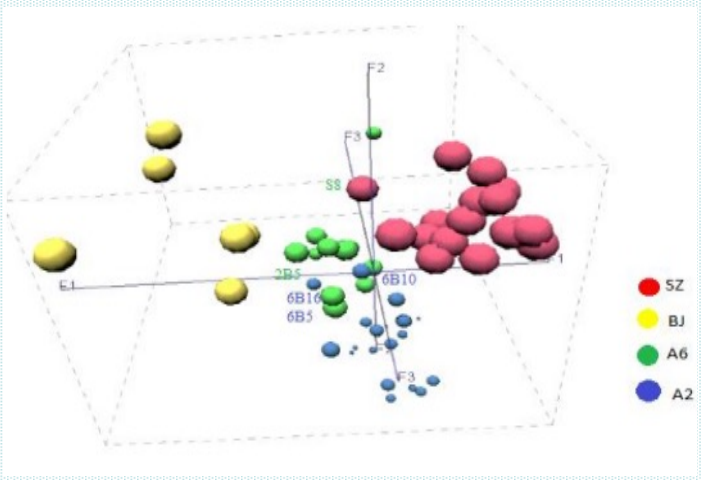

Figure 2: Spatial representation of a factorial analysis (AFD) showing bacteria groups isolated from the hosts plant

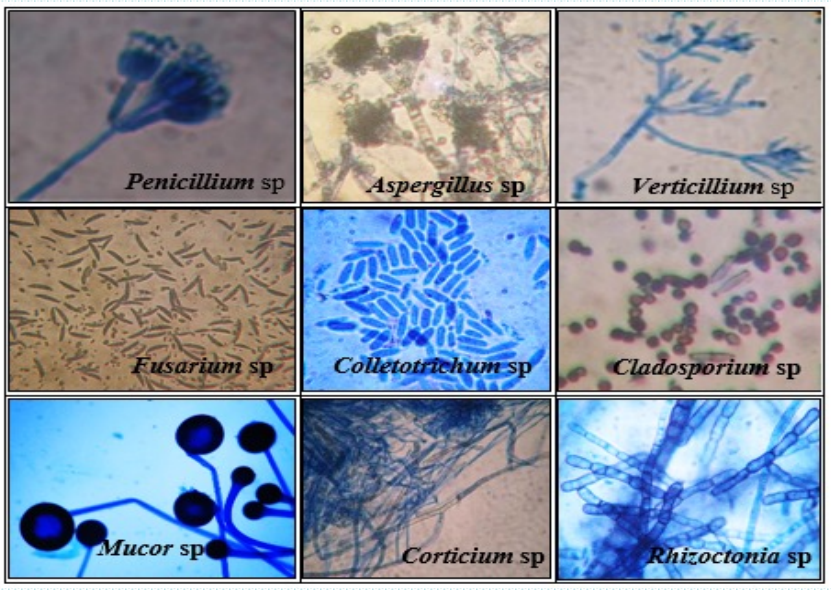

Figure 1: Types of endophyte fungi isolated

Table 1 : Fungi transmitted by grafting

Fungi	A602	A602/A305	A305
Pestalozzia sp.1	2	1	
Rhizoctonia sp.1	1	1	1
Rhizoctonia sp.2	2	1	1
Corticium sp.3		2	1
Mucor sp.1		2	1
Rhizoctonia sp.3	1		3
Penicillium sp.5	1		1

Fungi	K26	K26/A305	A305
Cladosporium sp.2	2	3	
Corticium sp.1	10	1	
Rhizoctonia sp.2	2	2	1
Penicillium sp.4		3	1
Verticillium sp.1		1	1
Rhizoctonia sp.1	3		1

Diversity :

Among the three studied accessions of the *Verae* group, 52 bacteria were isolated, of which 80% are Gram –. They were classified according to the host coffee plant. *Actinomycete* was isolated only from *C. kianjavatensis* A602.

About fungi, *Corticium* is the genus present in all studied coffee species, and *Penicillium*, *Rhizoctonia*, *Pythium*, *Fusarium* and *Sclerotium* are the most frequent.

Transmission :

The number of fungi transmitted vertically from seed to plantlet or by grafting is very restricted. *Penicillium* can be transmitted from the rootstock *C. perrieri* A305 into the grafted *C. canephora* K26. *Penicillium* is known to be able to degrade caffeine (Denis, 1996). This is probably the reason of the slight reduction of caffeine content by grafting (Charrier and Berthaud, 1975). *Verticillium* is present in the rootstock A305 and also in the grafted plant *C. canephora*.

Biological function :

32% of all the isolated bacteria from the *Verae* group are able to solubilise the inorganic phosphate. In-vitro tests show that (i) several bacteria isolated from wild coffee trees can degrade caffeine; (ii) the germination of *Hemilia vastatrix* is inhibited by some strains of *Verticillium* (Mahfud, and *al*., 2006). As *Verticillium spp* is widely present in *Mascarcoffea* species, is this fact could be linked to their strong resistance against rust? (iii) endophytic bacteria and fungi strains synthesized similar secondary metabolites existing in the host plant.

Conclusion

Bacteria endophytes may contribute to the nutrition of the host plant from their capacity to solubilise inorganic phosphate. Chromatographic analysis of synthesized products by endophytic bacteria and fungi from *Coffea perrieri* and *C. Leroy* show that these molecules could be the precursors of chemical substances accumulated by the host plant. The capacity of the bacteria isolated from the wild coffee to degrade the caffeine could be the reason of the free or lower caffeine in the host plant. The existence of the vertical transmission of the endophytes by grafting can explain the ecological adaptability of grafted plants.

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CONSTRUCTION OF DNA FINGERPRINTINGS OF COFFEE GERMPLASM RESOURCES WITH RAPD MOLECULAR MARKERS

PB221

*HUANG Lifang，DONG Yunping，WANG Xiaoyang，LIN Xingjun，SUN Yan，CHEN Peng，YAN Lin**

Spice and Beverage Crops Research Institute, Chinese Academy of Tropical Agricultural Sciences/ Key Laboratory of Genetic Resources Utilization of Spice and Beverage Crops, Ministry of Agriculture. Wanning, Hainan 571533，China.(email hlf032@163.com)

ABSTACT

The aim of this study is to quickly and accurately identify different cultivars (or lines) of coffee in which would construct a DNA fingerprint in different germplasm resources of coffee based on RAPD molecular marker. 100 RAPD primers were screened with 4 cultivars, indicating 10 of which are polymorphic. 10 RAPD primers were used to amplify 28coffee resources.

Introduction

Genetic diversity analysis of coffee germplasms by RAPD markers, construction of DNA fingerprints in coffee germplasm resources by the highest efficient primer.

28 coffee germplasm resources by RAPD amplification using 10 primers were obtained. By connecting the name of cultivars, the sampling sites, the name of the highest efficient primer and the RAPD data code, the DNA fingerprint codes of 28 coffee germplasm resources were built.

No.	Taxon	Name	No.	Taxon	Name
1	*C. liberica* Bull ex Hiern	Indian	15	*C. arabica* Linné	P3
2	*C. liberica* Bull ex Hiern	Shilongpo	16	*C. canephora* Pierre	26
3	*C. excelsa* Chevalier	Chalixiaoguo	17	*C. canephora* Pierre	Reyan 2
4	*C. excelsa* Chevalier	Chalizhongguo	18	*C. canephora* Pierre	27
5	*C. excelsa* Chevalier	Chalidaguo	19	*C. canephora* Pierre	24
6	*C. arabica* Linné	CATIMORCIFC7963	20	*C. canephora* Pierre	Reyan 1
7	*C. arabica* Linné	CATIMOR P88	21	*C. canephora* Pierre	24-10
8	*C. arabica* Linné	CCC24	22	*C. canephora* Pierre	Xing 34
9	*C. arabica* Linné	Brazil	23	*C. canephora* Pierre	Xing 33
10	*C. arabica* Linné	CATIMOR	24	*C. canephora* Pierre	Xing 32
11	*C. arabica* Linné	Typica	25	*C. canephora* Pierre	Xing 31
12	*C. arabica* Linné	Catimor dwarf	26	*C. canephora* Pierre	Xing 30
13	*C. arabica* Linné	Cameroon	27	*C. canephora* Pierre	Xing 29
14	*C. arabica* Linné	Papua New Guinea	28	*C. canephora* Pierre	Xing 28

Table 1 Coffee germplasm resources used in this study

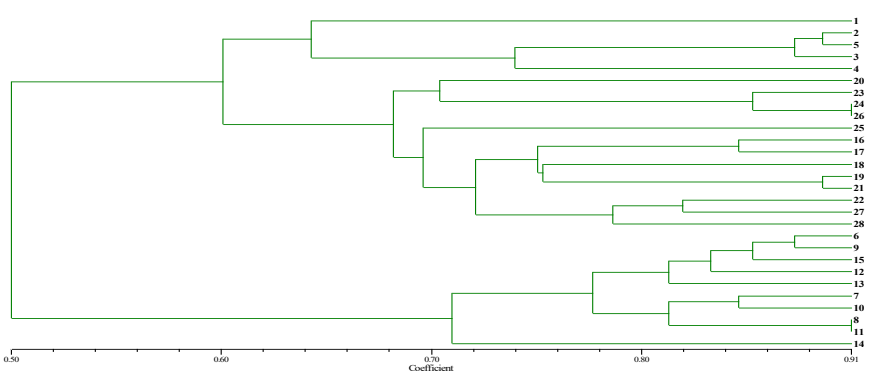

Fig. 1 UPGMA Tree of 28 coffee germplasm resources

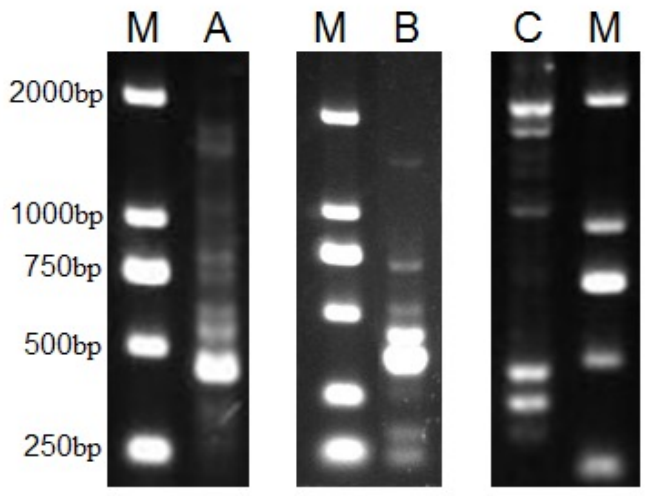

Fig. 2 The part of electrophoresis patterns of primer S8
Note: A, B, C respectively for Indian, Brazil, Xing 28. The same as Fig. 3. M: DL 2000 marker.

No.	RAPD data	No.	RAPD data
1	000000011100	15	101000101110
2	110001010000	16	010100000110
3	110001010100	17	100100010110
4	001000010100	18	100101001110
5	110101010100	19	100101111110
6	101000010110	20	001001000110
7	001000110110	21	100101101110
8	001000001110	22	110111000110
9	000000001110	23	001000001111
10	101000110110	24	001001001110
11	101000111110	25	100100111101
12	001000111110	26	001001001111
13	001010111110	27	000101000110
14	100001101110	28	110001000110

Table 3 The RAPD data of 28 coffee germplasm resources of primer S8

Main Text

The results showed that by using 10 reliable RAPD primers, 86 DNA fragments, among which 74 DNA bands were polymorphic, and the polymorphic proportion of DNA bands (PPB) was 86.05%, including the primer S8 with the richest polymorphic, and the average number of DNA bands amplified by each primer was 8. The genetic similarity coefficient of the 28 germplasm resources ranged from 0.338 to 0.905. It indicated that the efficiency of S8 was the highest by making the 10 RAPD primers, the primer can identify 28 coffee germplasm resources, and the DNA fingerprint codes of 28 coffee germplasm resources were built.

Conclusion

The construction of the DNA fingerprint of coffee germplasm resources could offer data support for the protection of the cultivars rights and interests.

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PB222

AN INTERACTIVE COFFEE SNP DATABASE

COULEE Manon, RIGOREAU Michel **, TOURNEBIZE Rémi*, CROUZILLAT Dominique**, DE KOCHKO Alexandre*, PONCET Valérie*, TRANCHANT-DUBREUIL Christine**

** IRD, UMR DIADE, Montpellier France, **Nestlé R&D, Tours, France*

Introduction

Studies of the genome using next-generation sequencing enable the identification of molecular markers such as single nucleotide polymorphisms (SNPs) that may be used by breeders to identify and trace genes when breeding new varieties. To enhance the potential utility of genome-wide SNPs for geneticists and breeders, we updated MoccaDB with a new web-based query interface to search and browse SNPs in addition to other genetic markers (SSR, RFLP, CAPS,etc..).

www.moccadb.ird.fr

We implemented new modules to include information and search tools related to SNPs, which allows access and visualization of the SNP information and locations both on linkage maps and reference genomes.

2564 markers
6 Coffea genetics maps
***Coffea canephora* genome**

Conclusion

Genome-wide SNP analysis is a promising tool to examine the genetic diversity of Coffee populations and genetic traits of scientific and economic importance. By combining information including SNPs and other genetic markers MoccaDB is an integrative web-portal providing a database of large-scale genome variation across coffee useful for both breeders and researchers .

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IS IT POSSIBLE TO OBTAIN EXCELLENT CUP QUALITY OF COFFEE GENOTYPES INTROGRESSED WITH HYBRID TIMOR (HT) AND BE BETTER TO ANOTHER TRADITIONAL VARIETIES?

PB225

ARRIETA, Noel*¨, CADENAS, Victor*, CORRALES, Allan*, RODRÍGUEZ, David*.
*Costa Rica Coffee Institute, Coffee Center Research (CICAFE).

ABSTRACT

This study is intended to determine in conditions agro ecological of Costa Rica can produce an excellent quality bean and beverage. Varieties resistance to CLR came from crosses by HdT and traditional (susceptible) like Caturra, Catuaí and another was included in this verification. Was used the cupping protocol of SCAA, 15 cuppers, and more of 200 samples from 7 coffee regions. The results indicate that there are conditions of climate, soil and management associated with genotype making to be able to get top quality beverage.

INTRODUCTION

The prestige that has the coffee of Costa Rica has no comparison with any other origin. The rust fungus severely affected the majority of coffee farmers and began the discussion internal Costa Rican coffee sectors of the possibility of change to varieties derived from HT. Recommendation technical renewing coffee plantations with varieties resistant to *H. vastatrix* is a prevailing fact. The discussion is based on knowing if these genotypes may have major cup quality. Growing coffee in different regions of Costa Rica, all farms with different climate, altitude and soil conditions.

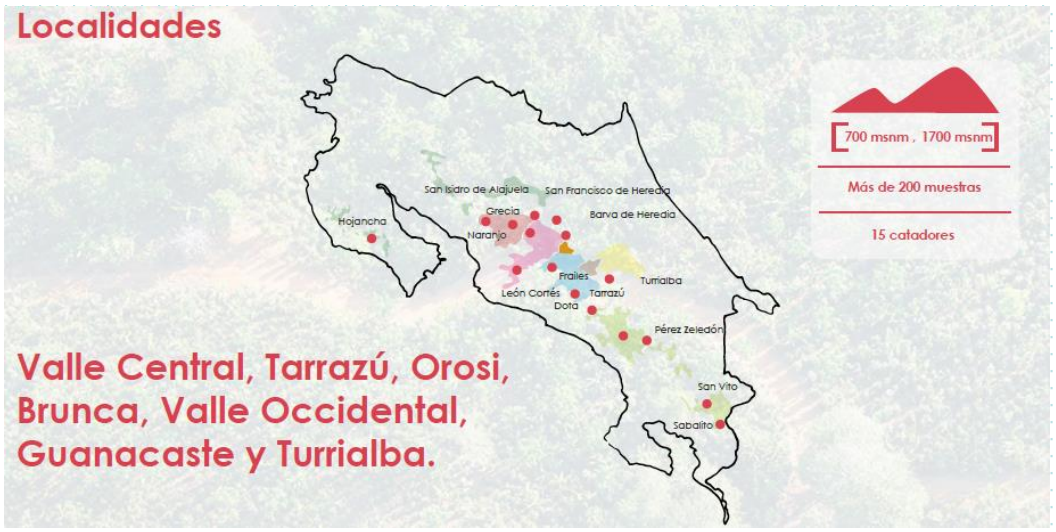


Figure 1: Location of the harvested samples origin.

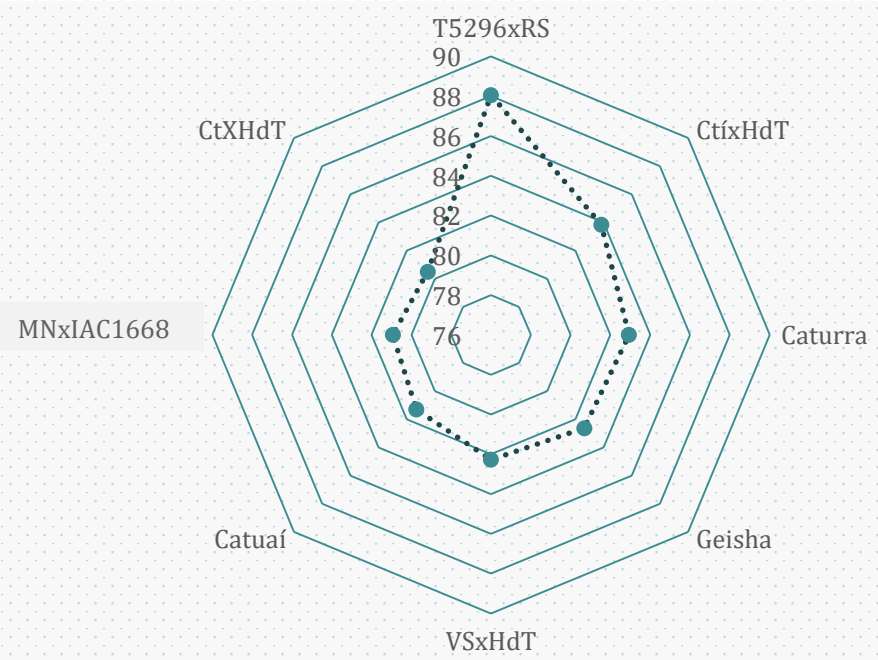
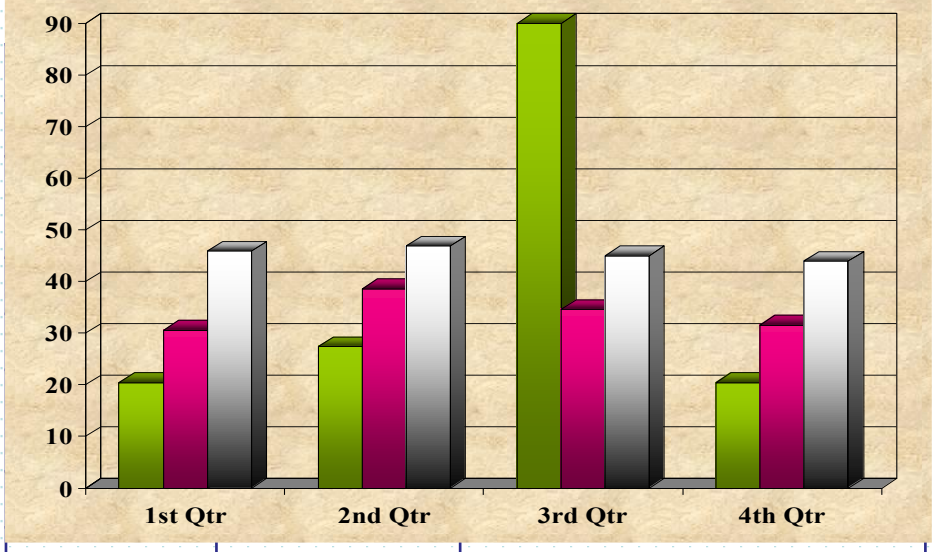


Figure 2: SCAA cup qualification of genotypes.

Table 1: List of genotypes used at the experiment



Sarchimor*	MNxIAC1668	Acaua MG1332
Catimor	CtxHdT	Oeiras MG6851, Castillo, CR95

RESULTS AND DISCUSSION

The genotypes used in the study had different origins genealogical so was expected The used sampling protocol was the proposed by SCAA, following strictly all the steps. Since, was evaluated the main attributes of the beverage (not show) as acidity, flavory, fragrancy, sweetness, etc., has made a simple static difference between medias for the cuppers qualification average. These results show the promising potential what have these introgressed HdT varieties.

CONCLUSION

The unique conditions in which coffee is grown in Costa Rica offers the possibility of producing excellent quality. It is possible to switch to varieties derived from HT without losing the prestige of quality that the country has, added to increase yield per area, resistance to pest and disease and adaptability. So, the coffee farmer can take the option to plant these genotypes with a technical support and data to certainly give a recommendation. Varieties as Centroamericano, Catigua MG2, Obatá red and Paraíso MG1 comply with the requirements to be a good option in severity damaged regions by CLR at the same time produce a excellent coffee beverage.

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¨Reference author for more information : narrieta@icafe.cr

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AN APPROACH TO GET ARABIC COFFEE GENOTYPES RESILIENT TO CLIMATE CHANGE, HIGHLY PRODUCTIVE AND EXCELLENT QUALITY OF CUP, THROUGH COMPREHENSIVE EVALUATION IN DIFFERENT AGRO-ECOLOGICAL CONDITIONS IN COSTA RICA.

PB226

ARRIETA, Noel*, *, FALLAS, Diego1, RODRÍGUEZ, David*.
*Costa Rica Coffee Institute, Coffee Center Research (CICAFE). 1University of Costa Rica.

ABSTRACT
Evaluate advanced lines from crosses with possible resilience to climate change with potential agronomic remarkable performance. Measure of the plant length, branches length, number of nodes, distance of internodes, diameter of plant, number of branches and qualification of vigor was the variables included in the analysis static to create a good correlation in create a "performance" plant in conditions adverse to growth coffee plants. Highly temperatures, hydric deficit, and super hydric conditions was the location included.

INTRODUCTION
Early evaluation of different progenies from crosses with HT is detailed in this paper. Agronomic performance methods are used during the first year of planting. In adverse temperature and abnormal rainfall conditions are put in test different genotypes. Discussed on the notable early development get the evaluated treatments. Adaptability to these conditions shows a result very good to continue evaluating for some years longer. All this gives hope of soon having a selection of a pure line with conditions of resilience to the adverse weather conditions in the near future we announced.

Genotype	Abbreviation	Varieties
Cavimor	CtíxHdT	Catigua MG2, Paraíso MG1, Pau Brasil, Araponga,
Caturra	Ct	Caturra
Sarchimor	VSxHdT	Obatá red, Obatá yellow, Tupi RN, Tupi, Marsellesa
Catuaí	Ctí	UFV2237, IAC99, IAC44
Sarchimor*	MNxIAC1668	Acaua MG1332
Catimor	CtxHdT	Oeiras MG6851, Castillo, CR95

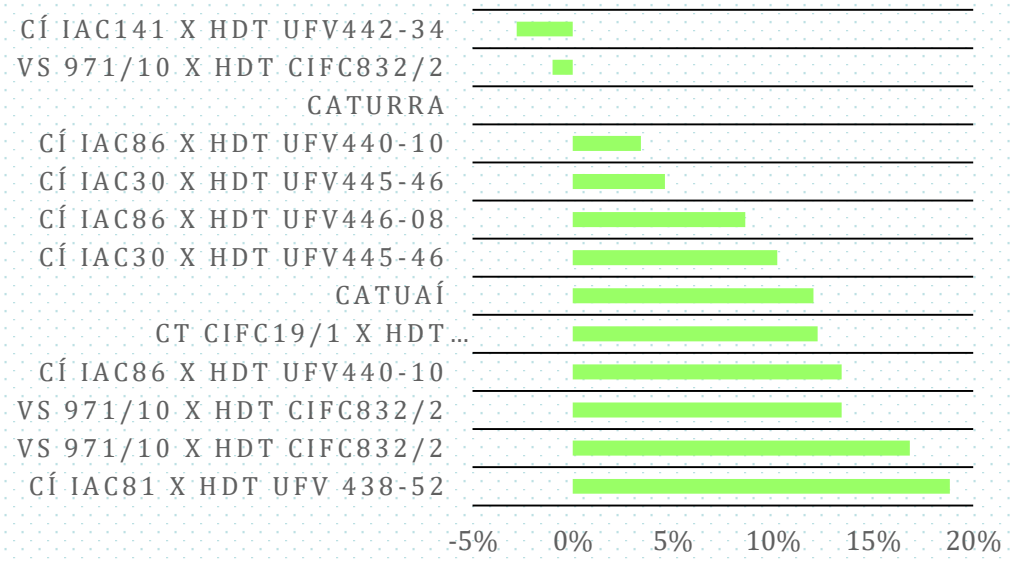

Figure 1: Percental difference relative between genotypes in agronomic performance.

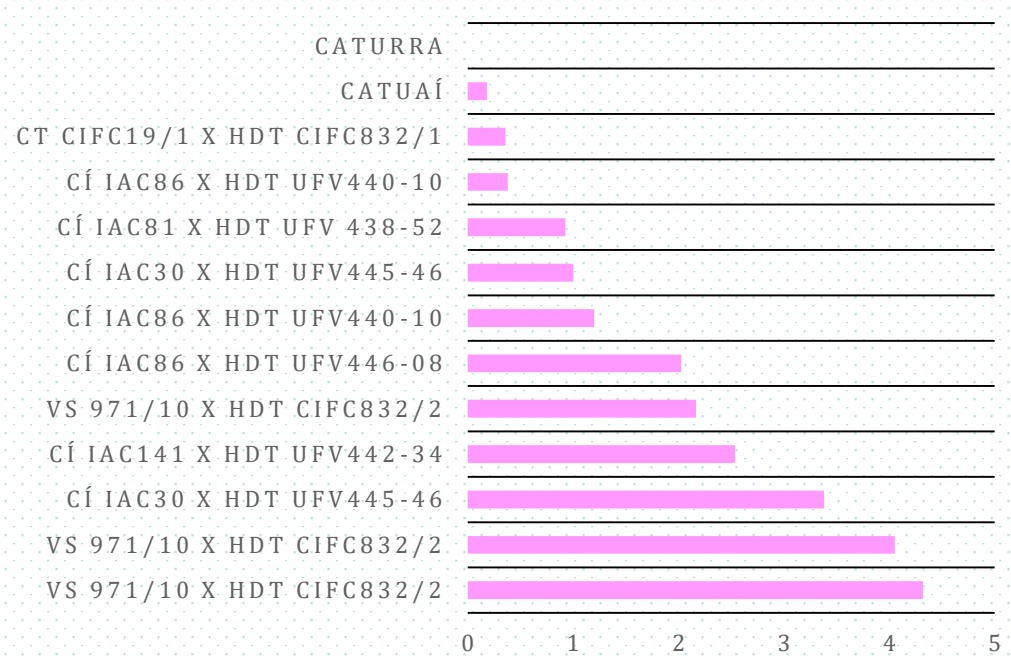

Figure 2: Vigor qualification of genotypes.

RESULTS AND DISCUSSION
First-year production showed the precocity of these genotypes in growth, productive stage and adaptability. The evaluated variables of plant development show that they had good adaptation to adverse conditions. In addition, data shows superiority with respect to comparators. Yields four times as much as the comparators reflects the enormous potential of the treatments.

CONCLUSION
The results show a great advance in soon having varieties adapted to the regions hotter and dry country. In addition, some of these selections showed have an excellent agronomic performance, early fruit production, acelerate growth and resistance to pest and disease and adaptability. So, the coffee farmer can take the option to plant these genotypes with a technical support and data to certainly give a recommendation. Varieties as Centroamericano, Catigua MG2, Obatá red and Paraíso MG1 comply with the requirements to be a good option in severity damaged regions by CLR at the same time produce a excellent coffee beverage.

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¨Reference author for more data: narrieta@icafe.cr

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Adaptation of *Coffea arabica* F1 hybrids to various environmental conditions

PB227

CHARMETANT, P.[1], THIMPSON M., M.[5], SILVA, B.[3], MARRACCINI, P.[1], VEIGA, A.[2], MOUEN, J.[5], ANDRADE, A.[4], PEREIRA, L.F.P.[4], LEROY, T.[1]

[1]Cirad, UMR AGAP, Montpellier, France [2]EMBRAPA Cerrados, Planaltina, Brazil, [3]IAPAR, Londrina, Brazil, [4]EMBRAPA Café, Brazil, [5]IRAD, Cameroon.

ABSTRACT

RATIONALE. The use of the genetic diversity of Coffea arabica is a source of high genetic gains for adaptation and coffee quality. First, we evaluated Ethiopian wild accessions and coffee varieties for their diversity and adaptation to various conditions, namely Cerrados and Northern Parana in Brazil, French Guiana, and Cameroon, with special attention to drought and high temperatures. We analyzed biochemical contents related to cup quality. We crossed a selection of progenitors with local cultivars. The oldest hybrid progenies are now two years old.

METHODS. To evaluate wild Ethiopian accessions and varieties we first used data obtained from collections established in various sites. From 2013 onwards, we crossed chosen plants with various dwarf cultivars. We established factorial hybrid trials at CPAC (Brazil, Cerrados), IAPAR (Parana), IRAD (Cameroon), and Cirad (French Guyana). Depending on the site, we use randomized design by tree, or by rows of 5 to 10 trees, with or without blocks. Stem diameter and height are the main criteria for vigor and compactness.

RESULTS. The genetic gain of F1 hybrids is generally positive over dwarf cultivars for vigor and height and over Ethiopian mothers for vigor only, with a significant effect of the mother factor. Hence a possibility to select Ethiopian female parents for their General Combining Ability.

CONCLUSIONS & PERSPECTIVES. We confirm the interest of *Coffea arabica* genetic resources to improve current cultivars in terms of vigor and of adaptation; maintaining plant compactness is possible. Cup quality will be checked. Further use of F1 hybrids for breeding may use vegetative propagation or backcrosses to commercial varieties. GWAS studies are ongoing.

Introduction

Even though it is relatively narrow, the genetic diversity of Coffea arabica is a source of high genetic gains in improving coffee varieties for adaptation and coffee quality. We evaluated Ethiopian wild accessions from CATIE for their diversity, for their adaptation to diverse conditions. We crossed a selection of progenitors with dwarf cultivars. We established the hybrids in Cerrados and Northern Parana (Brazil), in Cameroon, and in French Guiana. In the first stages we give special attention to growth, drought tolerance, and to high temperatures. The first hybrid progenies are now two years old.

Materials

We crossed 16 Ethiopian accessions with 7 dwarf cultivars. Of the latter, 3 are pure arabica lines. We succeeded in establishing 71 crosses and 14 selfings on the field (Table 1)

Methods

We evaluated growth parameters in nurseries and in experimental fields (Table 2) from 2014 to 2016. They are Stem diameter, Height, Number of nodes with leaves/branches, global vegetative vigor (1 to 5). We analyzed data using XLSTAT by Addinsoft.




Table 1 : List of crosses established

Mother\Father	DC1	DC2	DC3	DC4	DC5	DC6	DC7	Selfing
E01	3	1,2,3	3,4	2,1	3	2,1	2,1	1,2
E02	3	2,3	3,4	2,1	3	2,1	2,1	1,2
E03	3	3	3					
E04	3		3		3,4			
E05	3		3		3,4			
E06	3	3	3,4		3,4			
E07	3,4	1,2	3,4	1,2	3,4	1,2	1,2	2
E08	3		3		3			
E09	3,4	3	3,4		3,4			
E10		1,2		2		1,2	2	1,2
E11		1		1		1	1	1,2
E12	3		3,4		3			
E13	3,4		3,4		3,4			
E14	3	3	3,4		3,4			
E15			3		3			
E16		1,2		1,2		1,2	1,2	1,2
CM1		1,2		1,2		1,2	1,2	1,2
Selfing	3,4	1,2,3,4	3,4	1,2	3,4	1,2	1,2	

E = Ethiopian acession CM = variety from Cameroon DC = Dwarf Cultivar
1=CPAC 2=IRAD 3=IAPAR

Table 2: List of trials, experimental design

Trial	Experimental design	Reps
CPAC 2014	Tree by tree randomisation in 10 blocks	1 to 19
CPAC 2016	Tree by tree randomisation in 10 blocks	1 to 25
IRAD 2015	Rows of 10 plants in 2 blocks	2
IRAD 2016	Rows of 5 plants in 2 blocks	2
IAPAR 2016	Tree by tree randomisation	1 to 8
CIRAD 2015	Tree by tree randomisation	1 to 9

Table 3: ANOVA, growth parameters

ANOVA, Stem diameter

Source	DoF	Sum of Squares	Mean Square	F	Pr > F
Mother	18	11.913	0.662	2.284	**0.004**
Father	3	2.054	0.685	2.363	0.074
Mother x Father	17	6.185	0.364	1.255	0.232

ANOVA, Height

Source	DoF	Sum of Squares	Mean Square	F	Pr > F
Mother	18	555.146	30.841	4.521	**< 0.0001**
Father	3	46.583	15.528	2.276	0.083
Mother x Father	17	299.850	17.638	2.586	0.001

Table 4: Means, growth parameters

Mother	Height (cm)	N&K groups
E12	11.0	A
E04	9.4	AB
E02	9.1	AB
E06	8.7	AB
E07	8.7	AB
E15	8.6	AB
E09	8.5	AB
E13	8.4	AB
E03	8.1	AB
DC2S	**7.9**	**AB**
E14	7.8	AB
E01	7.1	AB
E08	7.0	AB
DC5S	**6.9**	**AB**
DC1S	**6.7**	**AB**
E05	5.8	B
DC3S	**5.7**	**B**

Mother	Stem Diameter (mm)	N&K 5% Groups
E12	4.2	A
DC5S	**3.8**	**AB**
E09	3.6	AB
E15	3.5	AB
E13	3.5	AB
E05	3.5	AB
E04	3.4	AB
E03	3.4	AB
E06	3.4	AB
E01	3.3	AB
E02	3.3	AB
E08	3.1	AB
E14	3.1	AB
DC1S	**3.0**	**AB**
E07	3.0	AB
DC2S	**2.9**	**AB**
DC3S	**2.5**	**B**

E : Ethiopian progenitor, DC : Dwarf Cultivar (control) Newman & Keuls test, p=0.005

Results and discussion

At this early stage, most data give clear evidence of increased vigor brought by hybridization over selected dwarf cultivars, especially regarding stem diameter and height (Table 4).

The effect of the female Ethiopian parent is always highly significant; the cultivar male parent does not have any significant effect (Table 3). Hence the need to continue selecting Ethiopian parents for their General Combining Ability, regarding important agronomic and quality criteria.

Heterosis was found in the same kind of F1 hybrids developed earlier in Central America; they showed a better adaptation to various environmental conditions, as compared with traditional varieties.

Conclusion

Coffea arabica genetic resources from Ethiopia are a valuable tool to improve current cultivars regarding vigor and adaptation to higher temperatures and to drought. Maintaining plant compactness is possible. Referring to earlier evaluation of such hybrids, cup quality may be maintained or improved. Further use of F1 hybrids for breeding may use vegetative propagation or backcrosses to commercial varieties. Ongoing GWAS studies may help the breeders to speed up the selection process.

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IN SILICO DESCRIPTION OF *Multi drug and toxic compound extrusion* (*MATE*) GENE FAMILY IN *Coffea canephora*

PB229

PINTO, Renan T*, CARDOSO, Thiago B*, ANDRADE, Alan C, PAIVA, Luciano V.***
*Federal University of Lavras, Lavras, MG, Brazil, **EMBRAPA CAFÉ.

Abstract

RATIONALE
In world parameters, coffee is an important commodity, regarding its to economical, medicinal and social relevance. *C. canephora* is beyond the cultivated species related to beverage production and is one parental of the hybrid *Coffea arabica*, which retains the majority of production. For the reasons of genetic improvement, it is important to identify and comprehend key genes on plant metabolic processes and one of the gene families widely studied for this purpose is the *MATE* family, which is composed by membrane transporter proteins and remains unexplored for *Coffea*.

METHODS
Thirty nine members of MATE family from different species, with several described functions were utilized as query to *blastp* algorithm analysis against a proteome database of *C. canephora.* The output redundancies were excluded and the sequences were checked against a RNA-seq database aiming to assess its predictions. From that, the protein sequences of known MATE members and putative *C. canephora* MATE members were aligned by ClustalW algorithm and the phylogenetic relations were estimated by with MEGA 6.0, using the Neighbor-joining method, with substitution mode of p-distance and bootstrap of 10,000 replicates.

RESULTS
Sixty putative MATE family members were identified on *C. canephora* proteome. The phylogenetic tree of the proteins can be divided in 8 groups with sequences of known MATEs sharing relevant similarity with *C. canephora* putative MATEs. Some of the groups have strong relation to protein functions, like vacuolar alkaloid accumulation, flavonoid transport and citrate exudation for aluminium tolerance, all of them with *C. canephora* putative MATE members.

CONCLUSIONS & PERSPECTIVES
Candidate genes that were identified are related to several metabolic processes involved on *Coffea* genetic improvement and can be characterized for the application on breeding programs.

Introduction

The *Multi drug and toxic compound extrusion* (*MATE*) family comprises key proteins to metabolic processes, and its respective genes can be interesting targets for genetic improvement of *Coffea* species. *In silico* characterization is the first step to study candidate genes for this purpose.

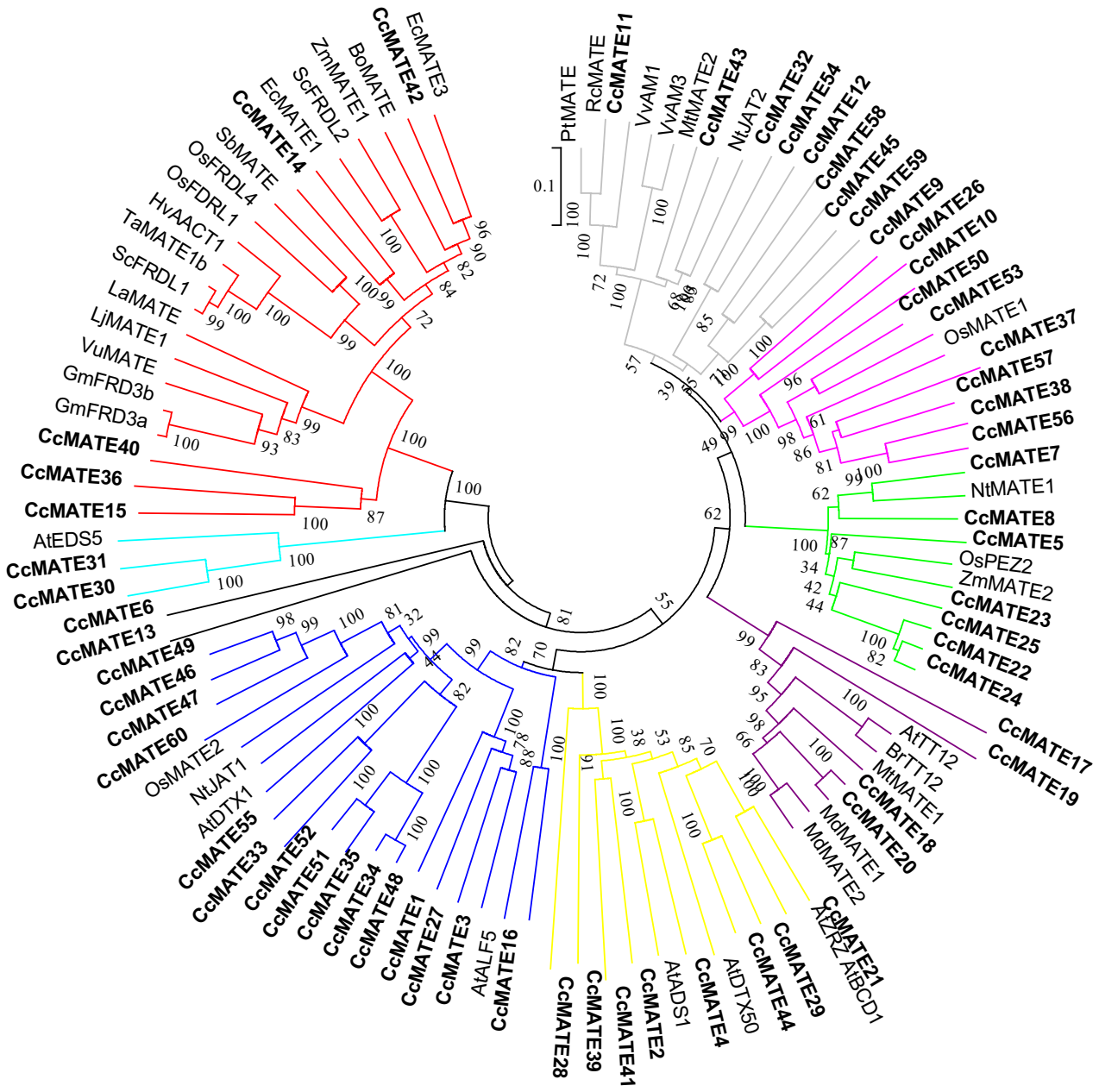

Figure 1: Phylogenetic tree of *MATE* gene family members of *C. canephora* with characterized members of other species.

Main Text

Throught an analysis with *blastp* algorithm, sixty putative *MATE* members were indentied in *C. canephora* and the genetic prediction of this candidates were refined by analysing them against a RNA-seq database. A phylogenetic tree was performed with addition of known MATE proteins from different species aiming to predict probable functions to *C. canephora* members. Some groups of the phylogenetic tree clustered MATE proteins characterized by specific metabolic functions, like flavonoid transport (purple and gray clusters), citrate efflux (red cluster) and salicilic acid transport (light blue cluster).

Conclusion

These putative MATE members identified in *Coffea canephora* are related to important features to coffee production and can be studied for application on breeding programs of *Coffea* species.

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PB230

EXPRESSION ANALYSIS OF *DREB* SUBFAMILY GENES IN LEAVES AND ROOTS OF *Coffea canephora* CONILON SUBJECTED TO DROUGHT

REICHEL, Tharyn*,, ALVES, Gabriel S.C.*,**, AQUINO, Sinara O.*,**, CARNEIRO, Fernanda A.*,**, ANDRADE, Alan C.**,***, MARRACCINI, Pierre**,****

*Federal University of Lavras, MG, Lavras, BRAZIL, **Embrapa Genetic Resources and Biotechnology, Brasilia, DF, BRAZIL, ***EMBRAPA Coffee-INOVACAFÉ, Lavras, BRAZIL, ****CIRAD, UMR AGAP, Montpellier, FRANCE

Abstract

The main objectives of this work were to analyze the expression of the different *DREB* (coding Dehydration Responsive Element Binding Proteins) genes found in the genome of *C. canephora* by reverse transcription-quantitative PCR (RT-qPCR) in leaves and roots of drought-tolerant and drought-susceptible clones of *C. canephora* grown in greenhouse and submitted or not to water limitation. Among the different subgroups (SGs) of *DREB* genes, expression of *Cc05_g06840* and *Cc08_g13960* appeared highly up-regulated by drought in leaves of drought-tolerant clones 14 and 73, but not in those of the drought-susceptible clone 22.

Introduction

We recently reported that the *CcDREB1D* (coding for a Dehydration Responsive Element Binding Protein) was a key candidate gene in the responses of coffee plants to drought (1, 2, 3). In order to precise the roles of *DREB* genes, this work analyzed the expression profiles of their different subgroups (4) by real-time quantitative PCR (RT-qPCR) in leaves and roots of drought-tolerant (D^T: 14, 73 and 120) and drought-susceptible (D^S: 22) clones of *C. canephora* Conilon grown in greenhouse and submitted (or not) to drought.

Methods

Clones of *C. canephora* grown in greenhouse were submitted (NI: non-irrigated, Ψ_{pd}= -3.0 MPa) or not (I: irrigated, Ψ_{pd}= -0.2 MPa) to drought (see PB233). For each clone and water condition, total RNAs were extracted, reverse-transcribed and tested by RT-qPCR (Fig. 1) using primer pairs of *Cc05_g06840* (SG-I) and *Cc08_g13960* (SG-III) (Table 1).

Gene Name	Primer names	Primer sequences
Cc05_g06840	Cc05_g06840-F	ACCCTCCAACTCCCCATGAC
	Cc05_g06840-R	TGGCAGCTCTGGGATGTACA
Cc08_g13960	Cc08_g13960-F	GCCCAAAGAGCCATCAATTC
	Cc08_g13960-R	CTTCCTCCCAGCTCGCTTCT
CcUBQ10	BUBI-F	AAGACAGCTTCAACAGAGTACAGCAT
	BUBI-R	GGCAGGACCTTGGCTGACTATA

Table 1. Primer pairs used for RT-qPCR assays. *DREB* gene names correspond to those available in the Coffee Genome Hub (http://coffee-genome.org)

Results and Discussion

RT-qPCR assays showed high up-regulated expression of *Cc05_g06840* (SG-I) and *Cc08_g13960* (SG-III) DREB-coding genes in leaves of D^T clones 14 and 73 submitted to drought, and to a lesser extend in those of D^T clone 120 (Fig. 1). However, expression of both genes did not increased upon drought in leaves of D^S clone 22.

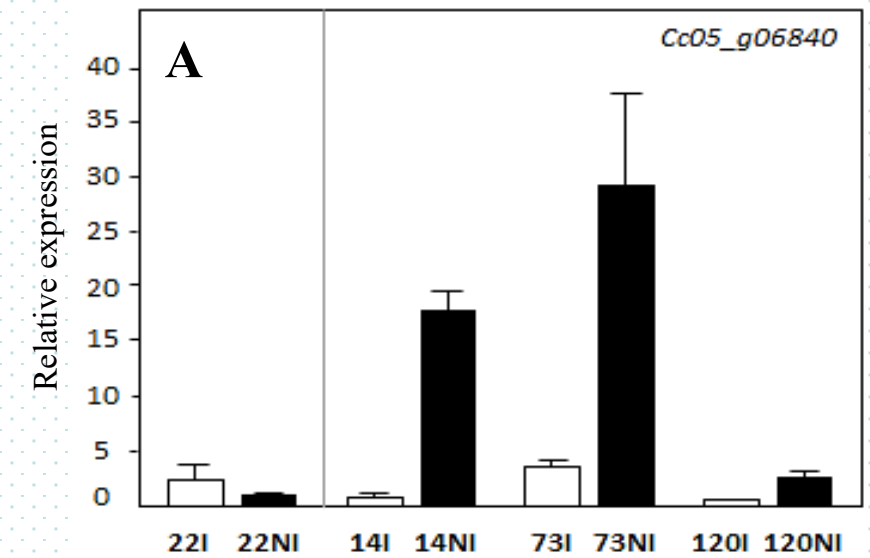

Figure 1. Expression profiles of *Cc05_g06840* (A) and *Cc08_g13960* (B) DREB-coding genes in leaves of D^S (22) and D^T (14, 73 and 120) clones of *C. canephora* grown with (I: control) or without (NI: drought) irrigation. In each case, values of relative expression (RE in arbitrary units) correspond to the mean of at least three technical repetitions ± SD. The expression of the *CcUBQ10* gene was used as a reference and the sample 14I as internal calibrator (RE = 1).

Conclusion

The fact that *Cc05_g06840* and *Cc08_g13960* DREB-coding genes showed up-regulated expression in leaves of D^T clones (mainly in 73) and not in those of D^S clone 22 highly suggests that they play a key role in response to drought of *C. canephora*.

Expression of other DREB-coding genes is undergoing.

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2- Vieira N.G., *et al.* (2013). Different molecular mechanisms account for drought tolerance in *Coffea canephora* var. Conilon. *Tropical Plant Biol.* 6 (4): 181–190.
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FUNCTIONAL ANALYSIS OF DIFFERENT PROMOTER HAPLOTYPES OF THE COFFEE (*COFFEA CANEPHORA*) *CcDREB1D* GENE THROUGH GENETIC TRANSFORMATION OF *NICOTIANA TABACUM*

PB231

AQUINO, Sinara O.*,, CARNEIRO, Fernanda A.*,**, RÊGO, Érica C. S.**, ALVES, Gabriel S. C.*, **, MARRACCINI, Pierre**,***, ANDRADE, Alan C.**,****
*Federal University of Lavras, MG, Lavras, BRAZIL, **Embrapa Genetic Resources and Biotechnology, Brasilia, DF, BRAZIL, ***CIRAD, UMR AGAP, Montpellier, FRANCE,
****EMBRAPA Coffee-INOVACAFÉ, Lavras, BRAZIL.

Abstract:

The regulation of HP15, HP16 and HP17 promoter haplotypes of *CcDREB1D* gene from *Coffea canephora* was studied by performing GUS staining in plants of transgenic *Nicotiana tabacum* subjected to dehydration, heat-shock and cold stress. The increase of GUS staining observed in T1 plantlets transformed by these promoters showed that all of them were upregulated to all tested abiotic stress confirming the importance of these sequences in controlling the expression of *CcDREB1D*.

Introduction

The sequencing of *CcDREB1D* (coding for a Dehydration Responsive Element Binding Protein) promoter regions revealed the presence of three haplotypes (HP15, HP16 and HP17) diverging by single nucleotide polymorphisms and insertions/deletions in the drought-tolerant clone 14 (D^T: HP15/HP16) and drought-susceptible clone 22 (D^S: HP15/HP17) of *C. canephora* Conilon (1). The full-length sequences of these promoters were cloned in the binary vector pBI101 upstream of the *uidA* (coding the *β*-glucuronidase, GUS) (Fig. 1) and transferred using by *A. tumefaciens* mediated transformation in tobacco to study their regulation by performing GUS staining in T1 transgenic plants subjected to different dehydration (DH, Fig. 2A), heat-shock (HS, Fig. 2B) and cold stress (CS, Fig. 3).

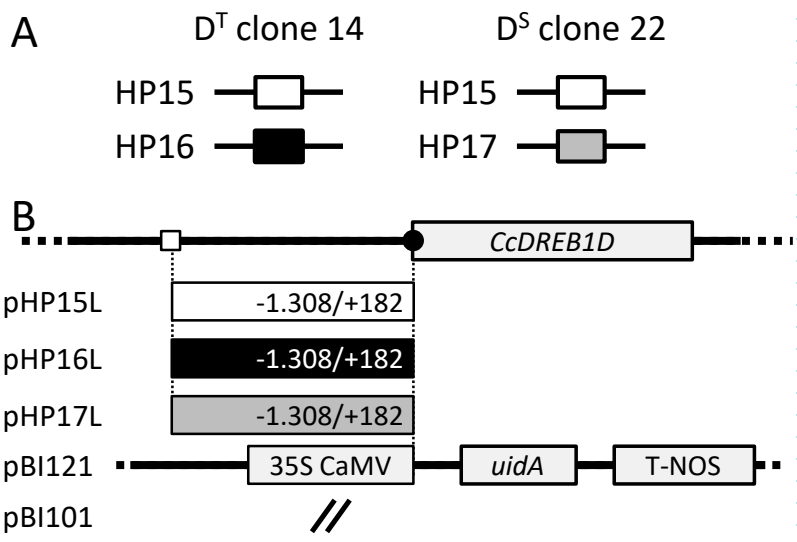

Figure 1. A: *CcDREB1D* haplotypes found in D^T clone 14 and D^S clone 22 of *C. canephora*. Color code for haplotypes: HP15 (white), HP16 (black) and HP17 (gray). **B:** Schematic representation of constructions analyzed in transgenic plants of *N. tabacum*. In all assays, pBI121 (p35S:*uidA*) and pBI101 (*uidA* promoter-less gene) were used as positive and negative controls, respectively.

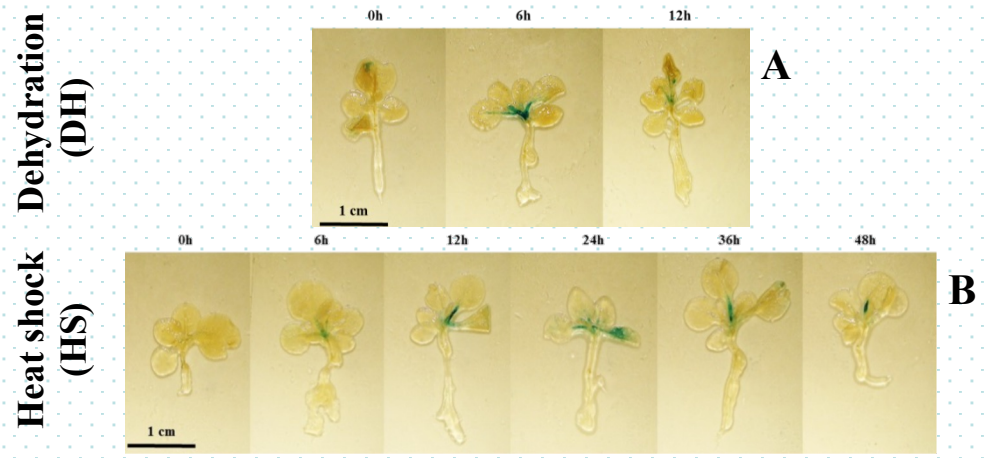

Figure 2. Histochemical localization of GUS activity in T1 transgenic tobacco plantlets transformed with pHP17L and subjected (A) to 6 and 12h of dehydration (DH) and (B) to 6, 12, 24, 36 and 48h of heat-shock (HS).

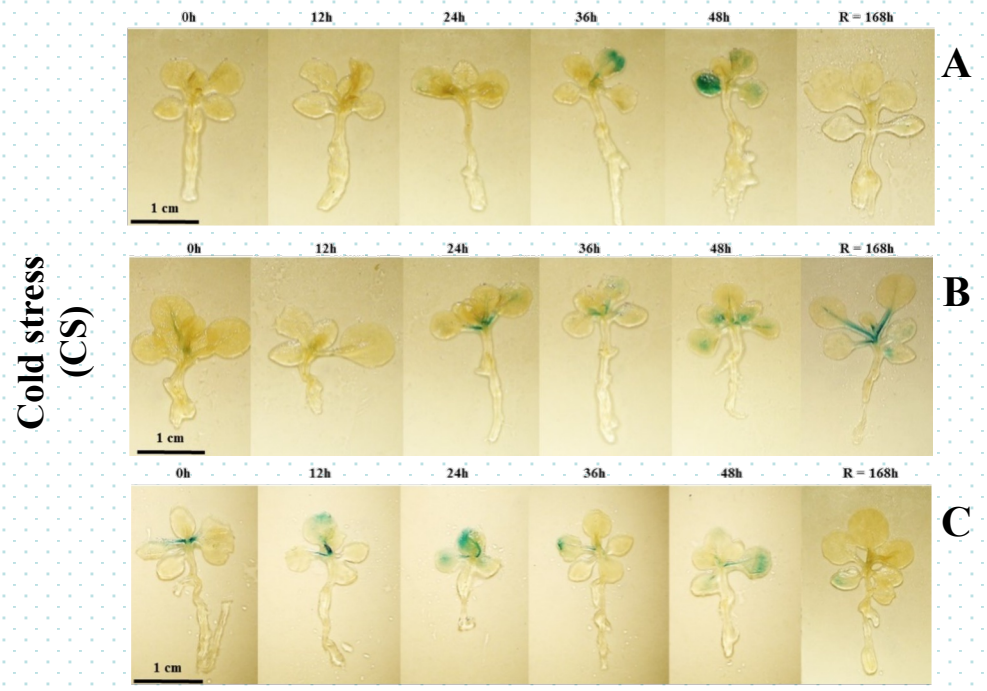

Figure 3. Histochemical localization of GUS activity in T1 transgenic tobacco plantlets transformed with the pHP15L (A), pHP16L (B) and pHP17L (C) and subjected to 12, 24, 36 and 48 of cold stress (CS). R: recovered plants grown without CS for 7 days.

Results and Discussion

- Under DH and HS conditions, GUS activity was detected at 6h in petioles and young leaves of pHP17L-transformed tobacco (Fig. 2A and B).
- Under CS, GUS staining was detected mainly in leaf lamina, petioles and vascular tissues after 36h, 24h and 12h in tobacco plantlets transformed by pHP15L (Fig. 3A), pHP16L (Fig. 3B) and pHP17L (Fig. 3C), respectively.
- For all these experiments, no GUS staining was detected in roots.

Conclusion

- The three haplotypes of the *CcDREB1D* promoter of *C. canephora* were inducible in tobacco by dehydration (DH), heat-shock (HS) and cold stress (CS), indicating that the molecular mechanisms implicated in the transcriptional control of *DREB* gene expression by abiotic stress are highly conserved between tobacco and coffee plants.
- For these three haplotypes, GUS staining was detected only in aerials tissues but not in roots, suggesting a function of *CcDREB1D* gene mainly in coffee leaves.
- GUS staining was always detected earlier in HP17-transformed tobacco plantlets than in those transformed by HP15 and HP16 coffee haplotypes, suggesting a better activation of HP17 by abiotic stress than compared to HP15 and HP16 haplotypes.
- Expression analyses by qPCR of *uidA* reporter gene are on-going to confirm the GUS staining presented here.

References:

1- Alves G.S.C., *et al*. *Coffea canephora DREB1D* promoter regulates gene expression in mesophyll and stomatal guard cells under drought stress, submitted for publication to *Plant Physiology*.

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PB232

DIFFERENTIAL EXPRESSION OF CANDIDATE GENES TO RESISTANCE TO *MELOIDOGYNE PARANAENSIS* IN CLONES OF *COFFEA CANEPHORA*

LIMA, Edriana A. de*, RÊGO, Erica C.S., COTTA, Michelle G.**, ***, COSTA, Tatiana S.**,***, CARNEIRO, Fernanda A.**, ***, CARNEIRO, Regina M.D.G.***, MARRACCINI, Pierre***,****, ANDRADE. Alan C.***,*****

*Universidade de Brasilia, Campus Universitário Darcy Ribeiro, Brasilia, DF, Brazil 70910-900, **Federal University of Lavras, MG, Lavras, BRAZIL ***Embrapa Genetic Resources and Biotechnology, Brasilia, DF, BRAZIL, ****CIRAD, UMR AGAP, Montpellier, FRANCE, *****EMBRAPA Coffee-INOVACAFÉ, Lavras, BRAZIL.

Abstract

Candidate genes for resistance to *Meloidogyne paranaensis* in roots of clones 14 and 22 of *Coffea canephora,* respectively resistant and susceptible to this nematode, were proposed.

Introduction

Root-knot nematode-plant interactions, as well the physiological processes of parasitism and coffee genes involved in the resistance, are still poorly understood. It was previously reported that the drought-tolerant clone 14 of *Coffea canephora* Conilon was resistant to six different populations of root-knot nematodes, while the drought-sensitive clone 22 was susceptible to these nematodes (1). The aims of this study were to identify candidate genes putatively involved in nematode resistance and to analyze their expression profiles in roots inoculated with *Meloidogyne paranaensis*.

Methodology

Total RNA was extracted from roots of clones 14 and 22 of *C. canephora* Conilon at different days (4, 8, 12, 20, 32 and 45) after inoculation with *M. paranaensis,* converted into cDNAs and used in real time qPCR experiments to check the expression of *Cc03_g09540* (*CcCPI1*, JF950585) coding a cysteine protease inhibitor, *Cc01_g13400* coding a protein phosphatase, and *Cc00_g16260* and *Cc10_g14530* of unknown function.

Results

- For the *Cc00_g16260*, *Cc10_g14530* and *Cc03_g09540* genes, expression profiles were up-regulated upon nematode inoculation mainly in roots of clone 14 (Fig. 1).
- Compared to clone 14, expression of *Cc10_g14530* and *Cc03_g09540* remained very low in inoculated clone 22.
- Expression of *Cc10_g14530* gene in clone 14 was high soon after nematode inoculation and decreased hereafter. In the same clone, expression of *Cc00_g16260* and *Cc03_g09540* increased gradually over the time.
- On the other hand, expression of *Cc01_g13400* decreased in roots of clone 22 inoculated with *M. paranaensis.*

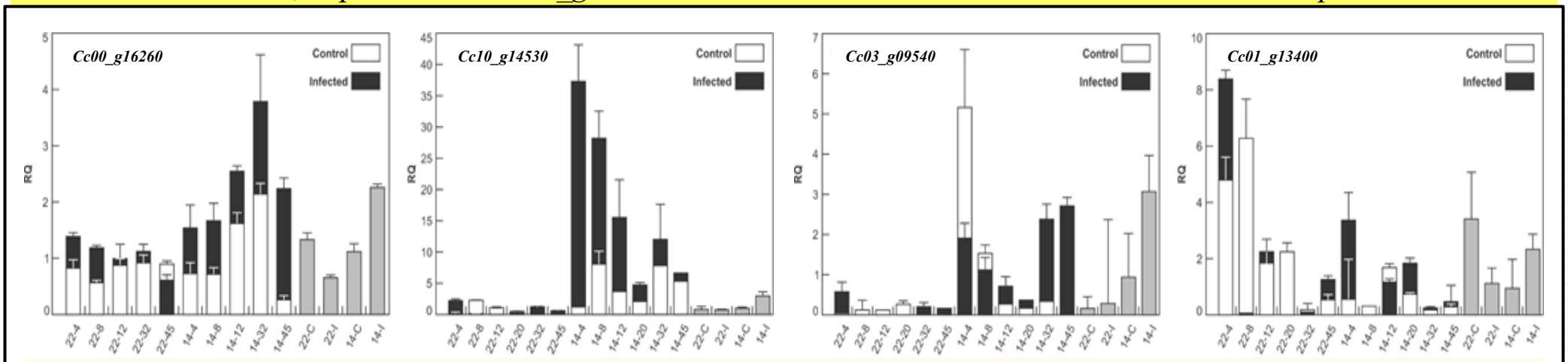

Figure 1: Relative expression of *Cc00_g16260, Cc10_14530*, *Cc03_g09540* (*CcCPI1*) and *Cc01_g13400 (*PP2C-type) genes on clones 22 and 14 of *C. canephora* Conilon at different days (4, 8, 12, 20, 32 and 45) after inoculation with *M. paranaensis*. White and black isobars represent control (non-inoculated) and inoculated plants, respectively. Grey isobars represent the mean of expression in inoculated (I) and non-inoculated (C) clones. The *x* axis mentions the days after inoculation. RQ: relative quantification in arbitrary units. The *CcUBQ10* gene was used as a reference gene.

Discussion

- Protease inhibitors are important proteins in plant defense processes as providers of natural resistance, and also excellent candidates for defense construction, being active against nematodes and other pathogens (2).
- The up-regulated expression of *Cc01_g13400* (coding a PP2C protein putatively involved in the abscisic acid signalization pathway) in inoculated clone 14 is worth noting. It suggests that "cross-talks" between biotic and abiotic signaling pathways (3,4) occurred specifically in the clone 14 of *C. canephora.*

Conclusion

Our results suggest that the four genes analyzed in this study are involved (directly or indirectly) in the resistance process of the clone 14 of *C. canephora* Conilon against infection by *M. paranaensis*.

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PB233

ANALYSIS OF THE EXPRESSION OF MANNOSE-6-PHOSPHATE REDUCTASE GENE IN ROOTS OF DIFFERENT CLONES OF *C. canephora* SUBMITTED TO WATER DEFICIT

COSTA, Tatiana S.[*,], CARNEIRO, Fernanda A.[*,**], COTTA, Michelle G.[*,**], LIMA, Edriana A.[**], RÊGO, Erica C.S.[**], MARRACCINI, Pierre[**,***], ANDRADE, Alan C.[**,****]**

*Federal University of Lavras, MG, Lavras, BRAZIL, **Embrapa Genetic Resources and Biotechnology, Brasilia, DF, BRAZIL, ***CIRAD, UMR AGAP, Montpellier, FRANCE,****EMBRAPA Coffee-INOVACAFÉ, Lavras, BRAZIL

Abstract:

This work studied the expression of the *CcM6PR* gene coding the mannose-6-phosphate reductase in roots of drought-tolerant and drought-susceptible clones of *C. canephora* grown in greenhouse and submitted or not to water limitation. Highest expression levels were observed in roots of the drought-tolerant clone 14 suggesting a key role of this enzyme in response of coffee plants to drought.

Introduction

The mannose-6-phosphate reductase is responsible for the dephosphorylation of mannitol-phosphate giving mannitol (1, 2), an important osmoprotector of plant cells in response to oxidative and osmotic stresses occuring during drought (3). Previous studies already reported differential expression of the *M6PR* gene in leaves of *C. arabica* (4, 5) and *C. canephora* (6) under drought. This study aims to evaluate the expression of the *CcM6PR* gene in roots of drought-tolerant (D^T) and drought-susceptible (D^S) clones of *C. canephora* Conilon grown under controlled drought conditions.

Methods

D^T (14, 73 and 120) and D^S (22) clones of *C. canephora* were grown in greenhouse (UFV, Viçosa-Brazil) and submitted (NI: non-irrigated, Ψ_{pd}= -3.0 MPa) or not (I: irrigated, Ψ_{pd}= -0.2 MPa) to drought.

For each clone and water condition, total RNA was extracted from roots and transcriptome profiles (RNAseq.) were studied after 454 sequencing (Fig. 1). Expression of *CcM6PR* was checked by real-time quantitative PCR (RT-qPCR) using the primer pair 11142 and the *CcUBQ10* gene as reference (Table 1).

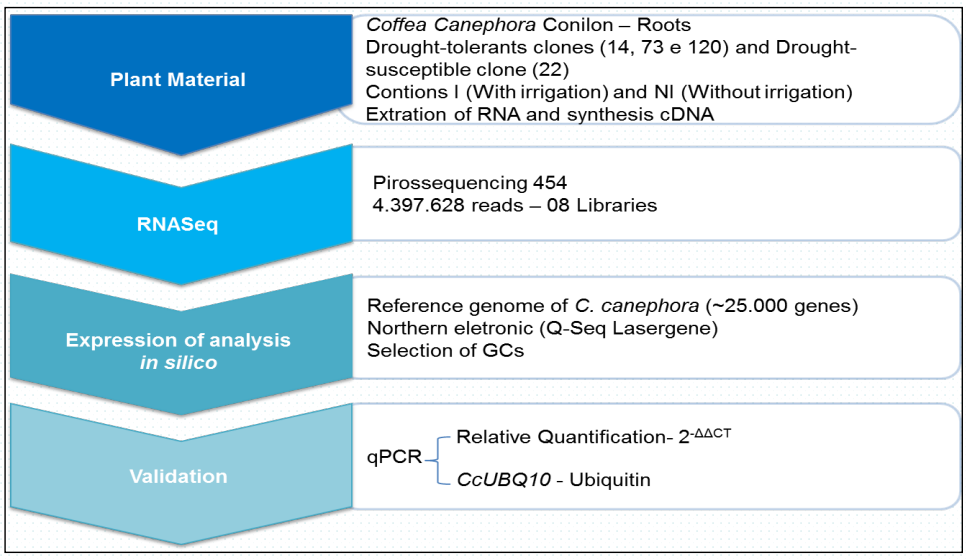

Figure 1. Flowchart of experiments realized during this work

Gene Name	GB numbers	Primer names	Primer sequences
CcM6PR	GT649707	11142-F	CGTTCTCGAGGCTTGCAAAG3
		11142-R	ATGCCTTGTGGGTACTGGAAAAT
CcUBQ10	GW488515	BUBI-F	AAGACAGCTTCAACAGAGTACAGCAT
		BUBI-R	GGCAGGACCTTGGCTGACTATA

Table 1. Primer pairs used for RT-qPCR assays. GB numbers corresponded to coffee ESTs available at NCBI (http://www.ncbi.nlm.nih.gov)

Results and Discussion

In silico analyses clearly highlighted up-regulated expression of *CcM6PR* gene under drought in roots of both D^T and D^T clones of *C. canephora* (Fig. 2A). These results were confirmed by qPCR assays, mainly in roots of the D^T clone 14, and to a lesser extent, in those of D^T (73 and 120) and D^S (22) clones of *C. canephora*.

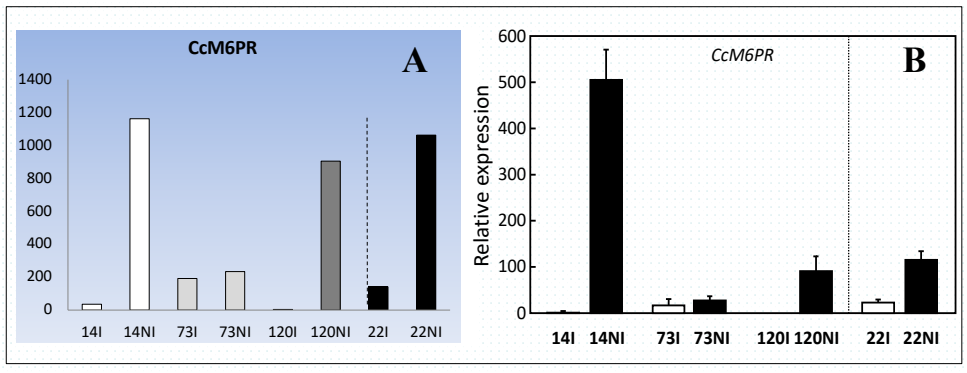

Figure 2. Gene expression profiles of *CcM6PR* in roots of D^T (14, 73 and 120) and D^S (22) clones of *C. canephora* grown with (I) or without (NI) irrigation, deduced from *in silico analyses* (A) and obtained by qPCR experiments (B). For qPCR, expression of the *CcUBQ10* gene was used as a reference and sample 14I as internal calibrator.

Conclusion:

Drought-induced expression of *CcM6PR* gene in roots of D^T clone 14 suggests that mannitol metabolic pathway plays important roles in protecting coffee roots against water limitation in this clone. This gene could be used as a molecular marker to assess the level of stress of the coffee plants subjected to drought.

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PB234

FIRST STEP OF ABA PERCEPTION AND SIGNAL TRANSDUCTION IN COFFEE: EVOLUTIONARY AND EXPRESSION OF *PYR/PYL/RCARs*, *PP2Cs* AND *SNRK2s* GENES IN *C. canephora* UNDER DROUGHT

COTTA, Michelle G.[*,], RÊGO, Erica C.S.[**], BOCS, Stéphanie S.[***], COSTA, Tatiana S.[*,**], DUFAYARD, Jean-François[***], THIS, Dominique[***], MARRACCINI, Pierre[**,***], ANDRADE, Alan A.[**,****]**

[*]Federal University of Lavras, MG, Lavras, BRAZIL, [**]Embrapa Genetic Resources and Biotechnology, Brasilia, DF, BRAZIL, [***]CIRAD, UMR AGAP, Montpellier, FRANCE, [****]EMBRAPA Coffee-INOVACAFÉ, Lavras, BRAZIL.

Abstract:

Orthologous genes of *C. canephora* coding for the PYR/PYL/RCAR receptors, PP2C phosphatases and SnRK2 protein kinases of the tripartite system involved in abscisic acid (ABA) perception and transduction pathway were characterized. For several genes, differential expression profiles were observed between drought-tolerant (D^T) and drought-susceptible (D^S) clones of Conilon, indicating a key role of ABA in the genetic determinism of drought tolerance in coffee.

Introduction

ABA is a phytohormone coordinating plant responses to drought. The mechanisms of ABA perception and transduction involves receptors (PYR/PYL/RCARs) interacting with SnRK2 kinases and PP2C phosphatases (Fig.1). Genes coding these proteins were characterized in the genome of *C. canephora* (Fig. 2) and their expression was studied in roots and leaves of drought-tolerant (D^T: 14, 73 and 120) and drought-susceptible (D^S: 22) clones of *C. canephora* Conilon grown under drought conditions (M&M: see PB233).

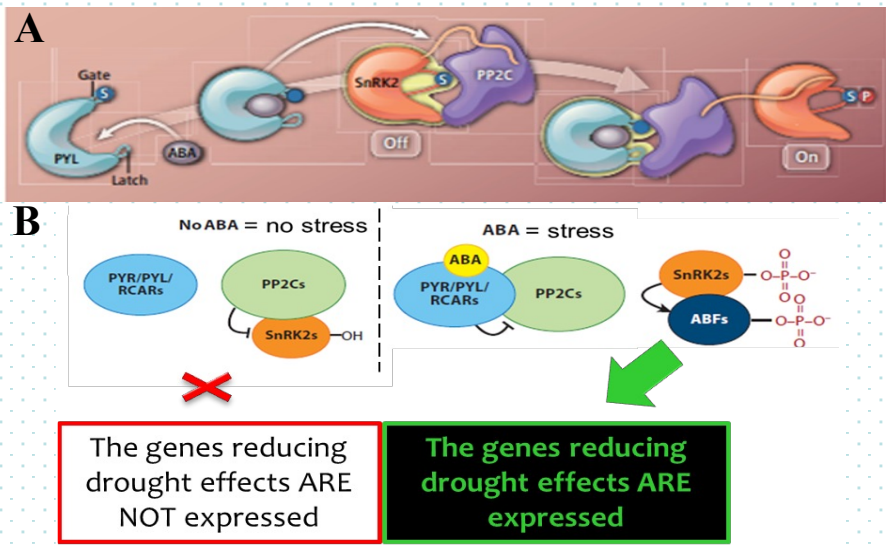


Figure 1: Tripartite system of ABA perception and signal transduction. (A) Schematic representation of the tripartite system. (B) Without ABA, SnRK2 kinases dephosphorylated by PP2Cs are inactive (left: system OFF). Under drought, ABA is fixed to PYR/PYL/RCAR receptors that interact with PP2Cs. Phosphotylated-SnRK2s then active are able to active genes in responses to drought (right: system ON).

Figure 2: Workflow of bioinformatics analyses performed to identify *PYR/PYL/RCAR*, *SnRK2* and *PP2C* orthologous genes of *C. canephora*.

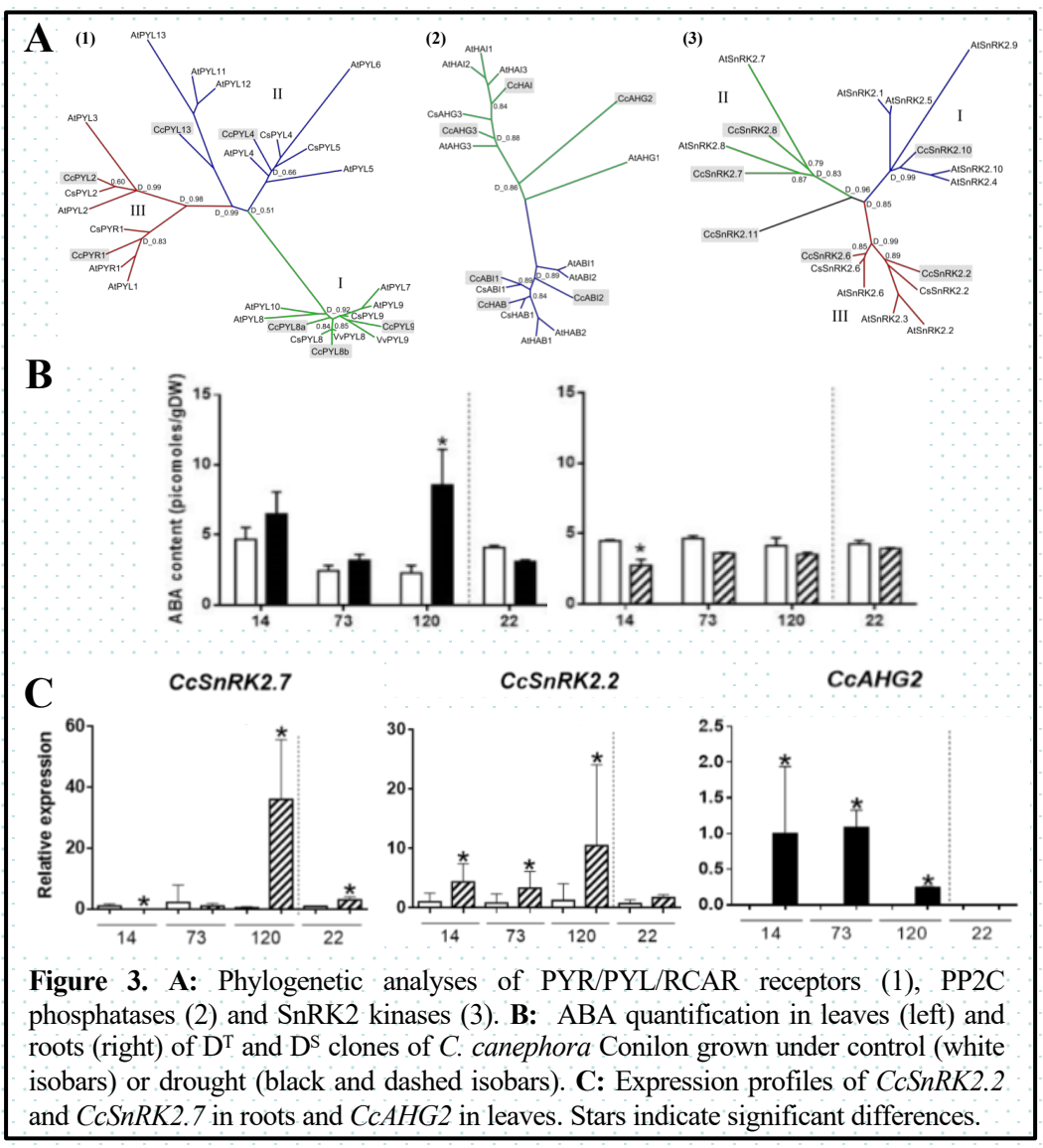


Figure 3. A: Phylogenetic analyses of PYR/PYL/RCAR receptors (1), PP2C phosphatases (2) and SnRK2 kinases (3). **B:** ABA quantification in leaves (left) and roots (right) of D^T and D^S clones of *C. canephora* Conilon grown under control (white isobars) or drought (black and dashed isobars). **C:** Expression profiles of *CcSnRK2.2* and *CcSnRK2.7* in roots and *CcAHG2* in leaves. Stars indicate significant differences.

Conclusions

- Twenty-four genes (9 *PYR/PYL/RCARs*, 6 *PP2Cs* and 9 *SnRK2s*) of the ABA tripartite system were functionally annotated in the genome of *C. canephora*.
- No significant differences of ABA contents were observed in roots and leaves (except clone 120), suggesting that D^T and D^S phenotypes of Conilon clones were probably due to altered ABA signalling pathway rather than deficiencies of ABA synthesis.
- Gene expression profiles clearly indicated the involvement of the ABA-dependent signalling pathway in the response of D^T and D^S clones of *C. canephora* Conilon subjected to drought.
- Different gene expression profiles were observed between D^T clones confirming the fact that different mechanisms are involved in the drought tolerance phenotypes in *C. canephora.*

Development and evaluation of a 8K SNP genotyping array for *Coffea canephora* and *Coffea arabica*

PB235

MEROT-L'ANTHOENE Virginie[1], DARRACQ Olivier[1], RATTINA Vimel[1], RIGOREAU Michel[1], LEPELLEY Maud[1], BELLANGER Laurence[1], TOURNEBIZE Rémi[2], TRANCHANT Christine[2], METAIRON Sylviane[3], FOURNIER Coralie[3], MUSOLI Pascal[4], SUMIRAT Ucu[5], LEGNATE Hyacinthe[6], DE KOCHKO Alexandre[2], DESCOMBES Patrick[3], CROUZILLAT Dominique[1], PONCET Valérie[2]

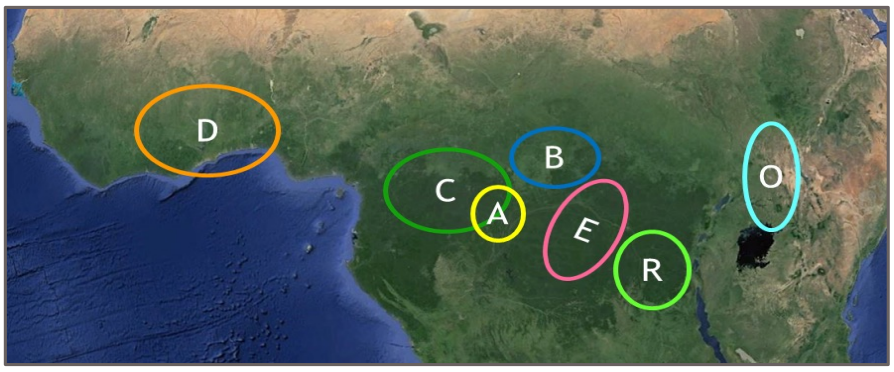
[1] Nestlé R&D Center, Tours, France, [2] IRD, UMR DIADE, Montpellier, France, [3] NIHS, Lausanne, Switzerland, [4] NaCoRi, Kituuza, Uganda, [5] ICCRI, Jember, Indonesia, [6] CNRA, Divo, Ivory Coast

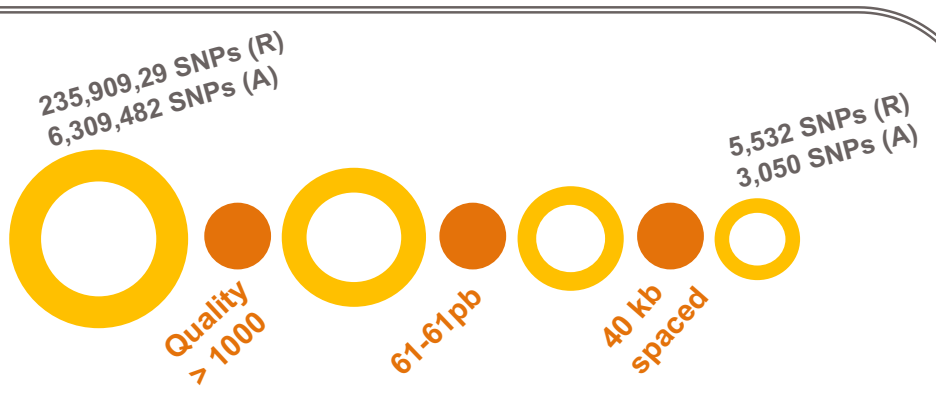
Introduction

Genotyping arrays are now becoming an usual tool for plant genetic and breeding. Over the last few years, the sequencing of various species by next-generation sequencing technologies (NGS) has led to the discovery of numerous genome-wide SNPs. This approach has allowed the design of a Coffee8K genotyping array for the two main cultivated species: *Coffea canephora* and *Coffea arabica*.

Coffee8K SNP Array Design

12 *Coffea canephora* genotypes representing the seven genetic groups [1,2] (Guinean **D**, Congolese **E**, Conilon **A**, Democratic Republic of Congo **R**, Uganda **O**, Cameroun **C**, Central African Republic **B**) were re-sequenced with Illumina technology (>30X).



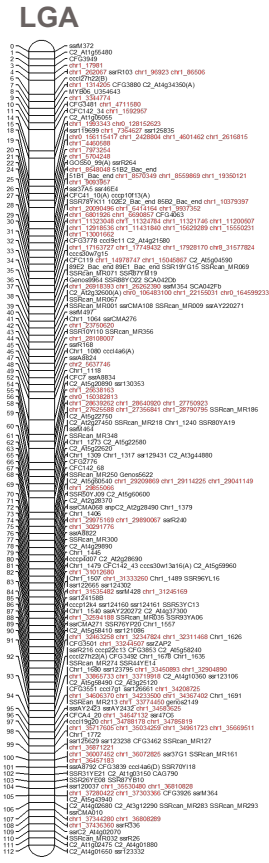


Following the mapping of the Illumina sequences to the Robusta reference genome [3], a sub-set of 8582 SNPs were selected for the design of an Illumina Infinium array, including 65% and 35% of SNPs from Robusta (R) and Arabica (A), respectively. The discovery and screening of the SNPs was performed using TOOGLE (TOolbox for Generic nGs anaLysEs) [4]. The SNPs were inserted in the MoccaDB database [5].

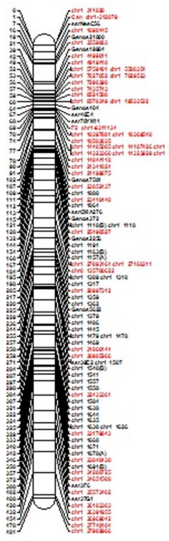
High-Density Genetic Maps

The Coffee8K SNP array was used to generate high-density genetic maps for the two species *Coffea canephora* and *Coffea arabica*, consisting of 3048 and 1842 markers, respectively. Within the international consortium ACGC, these two high-resolution maps (1370 and 7317 cM) have already been used to improve the Robusta and Arabica reference genome anchoring and QTL detection.

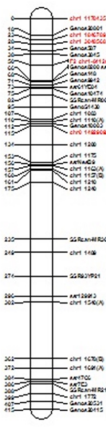
ROBUSTA LGA



ARABICA LGA
Sub-genome Robusta



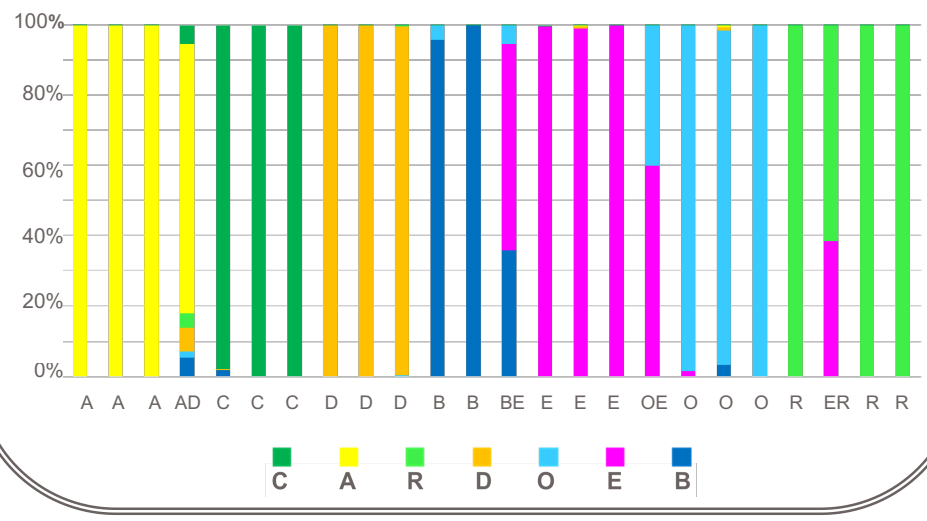
ARABICA LGA
Sub-genome Eugenoides



SNPs from Coffee8K SNP array
Previous markers (RFLP, SSR)

Genetic Diversity

The Coffee8K SNP array allowed the characterization of the seven genetic groups for *Coffea canephora* in accordance with previous results obtained with a set of 19 SSR markers. Moreover, individuals known as admixed were detected according to their hybrid status.



Conclusion

The Coffee8K SNP array will definitely provide a valuable additional resource for coffee research, which significantly accelerate various genomic studies as well as breeding programs.

Acknowledgments

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Nestlé Research

PB236

Using hand-held NIR for green coffee breeding and quality control

Jwanro Husson, Stéphane Michaux, Phuong Thong Hong, Charles Lambot, Pierre Broun
Nestlé R&D center Tours, 101 av. Gustave Eiffel, 37097 Tours, France

Introduction/Abstract

Coffee is a chemically very diverse raw material, terroir and variety influence this chemical composition which is needed to create a diversity in taste in the coffee range. Nowadays some consumers are avoiding caffeinated beverages. Caffeine is a bitter and stimulant compound, and naturally present in coffee. The caffeine content varies according to the species and the environmental conditions. The challenge is therefore to find caffeine-free varieties. Near Infrared Spectroscopy (NIR) has grown and been used as an analytical method for phenotyping, raw material testing, product quality control and process monitoring. In the current study, we built predictive models using hand-held NIR to quantify quality traits such as caffeine in Robusta and Arabica green coffee beans and leaves samples. The current models can be used in breeding programs to identify coffee varieties which naturally contain no or less caffeine.

NIR Hand-held Analyzer
taking readings in coffee leaves

Materials & methods

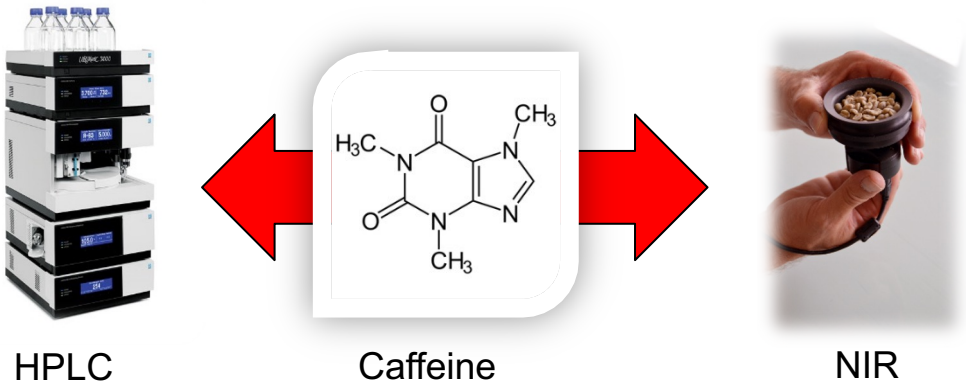
CHEMICAL ANALYSES

The caffeine content in green coffee beans and leaves was determined using HPLC The results are expressed as g/100g of dry matter.

NIR ANALYSIS

The green coffee samples were maintained for 15 days at 11°C and 65% RH. This storage phase is necessary to standardize their humidity and allow near Infrared analyses. Spectral data in reflectance mode for each coffee sample is recorded using the VIAVI MicroNIR 1700 spectrometer. The spectrometer measures reflected energy between 950 – 1650 nm, measurements were made at ambient temperature. Spectrum were collected using a cup of 6.5 cm diameter, at a resolution of 6,5nm and 100 scans per spectra. For each sample, the predictive values were calculated as an average of 2 replications.

The coffee leaves were measured fresh on both sides (face & back) at ambient temperature then the predictive values are calculated as an average of the two measurements.



Results

The caffeine predictive models have a good performance in terms of coefficient of determination (R^2) and standard error of prediction (SEP). Partial least squares (PLS) regression was used to construct the models. The table below shows the calibration and validation phases for Arabica-Robusta bean caffeine predictive model as well as Arabica-Robusta coffee leaves predictive model:

Predicted caffeine (Arabica-Robusta)	**Min value (g/100g of dry matter)**	**Max value (g/100g of dry matter)**	**Calibration** (beans n = 170) (leaves n = 64)		**Validation** (beans n = 30) (leaves n = 23)	
			SEC	R2	SEP	R2
Beans	**0,1**	**3,2**	**0,19**	**0,96**	**0,22**	**0,93**
Leaves	**0,1**	**2**	**0,28**	**0,83**	**0,35**	**0,78**

Conclusion & perspectives

The results obtained highlight the potential of using hand-held NIR as a fast, easy to use, non-destructive technique for field applications such as caffeine content determination, phenotyping and as a quality control/assurance method for green coffee. The technique is directly applicable to coffee leaves & beans in a non-destructive way. Hand-held NIR can be used to predict bean maturity or water potential of coffee trees in the field.

Nestlé Research

THE COFFEE TREE FACTORY

PB237

BRETON, David*, DUCOS, Jean-Paul*, DE FARIA MARASCHIN, Simone*
*NESTLE R&D Centre Tours. France.
David.breton@rdto.nestle.com

Abstract: Since 2000, the R&D Centre Tours has developped an accelerated propagation process to produce high quality planting material of *Coffea canephora* var Pierre[1]. Based on plant tissue culture methods, and specifically on somatic embryogenesis (SE), true-to-type copies of selected elite coffee trees can be produced. This process was validated at a large scale by the set up of a pilot unit for somatic embryo production, fully operational since 2010. Accelerated propagation has been implemented successfully to support strategic markets for Nescafé such as Philippines, Thailand and Mexico. The new challenge for the Plant Cell Biology Department at R&D Centre Tours is to leverage an improved accelerated propagation technology that is cost-competitive in comparison to classical vegetative propagation methods (cutting, grafting).

Methodology: Somatic embryogenesis was identified as the best way to answer to increasing demand for planting material compared to traditional cutting or grafting. Somatic cells from adult coffee trees are placed *in vitro* to modify their fate and initiate callogenesis **(fig.1).** Embryogenic callus is selected and cultured on liquid medium which allows a rapid scale up in biomass production. Callus is then placed on expression medium and torpedo embryos differentiate. Such embryos need to be further developed and germinated before conversion into a true-to-type plantlet. To achieve this pregermination development, a technical breakthrough was implemented in R&D Tours by the design of "box-in-bag" bioreactors managed on a temporary immersion regime. This system allows the simultaneous development of 10,000 somatic embryos on average, that can be directly transferred to the greenhouse for acclimation.

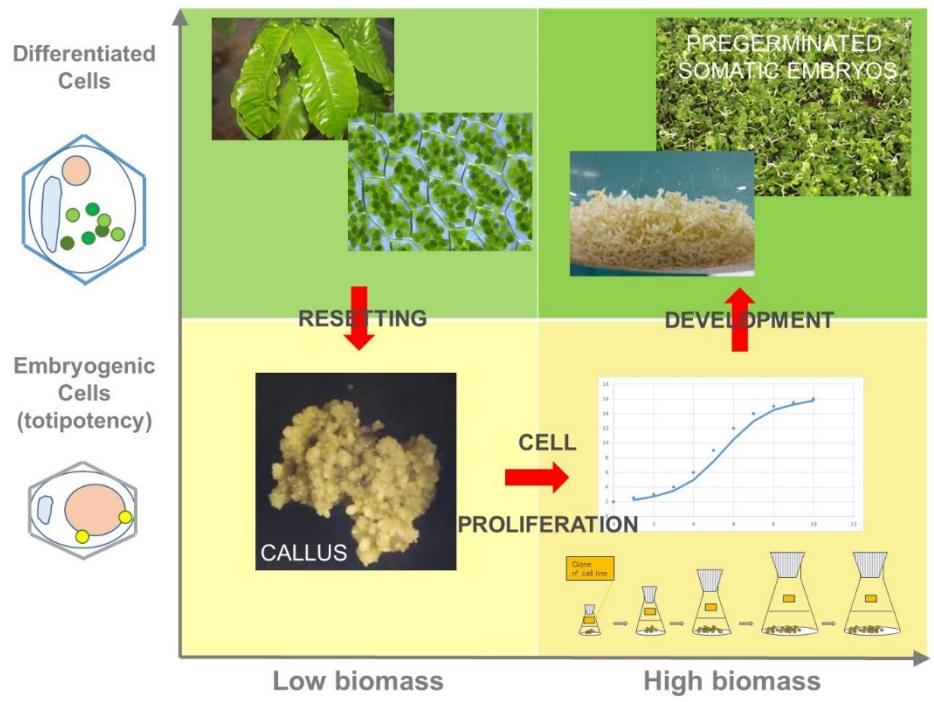


Figure 1: Schematic description of the SE process in *Coffea canephora*

Figure 2: *ex vitro* processing of somatic embryos, plantlets acclimation and farmer distribution

This technology allowed the setting up of a pilot production unit which delivered at its full capacity 10M pregerminated somatic embryos per year that were shipped by plane to coffee producing countries for acclimation and distribution to farmers **(fig.2)**. Embryos are sown into substrate for germination and initial growth. Plantlets are maintained under plastic microtunnels to promote growth and then transfered into plastic bags to be hardened under net shades. Fully acclimated plants are finally distributed to farmers and transplanted into the field..

Conclusions and perspectives

- Juvenile, **seedling-like aspect** of the plantlets compared to conventional cuttings
- **1 FTE can produce up to 1M somatic embryos** in the laboratory
- Embryo-to-plantlet conversion rate: **50%** with opportunities for improvements
- **Transfered technology** e.g. AGROMOD in Mexico (target of **5M** plants per year)
- Next steps: **automation** to reduce production costs, **mini-cutting** cycles **(fig.3)**

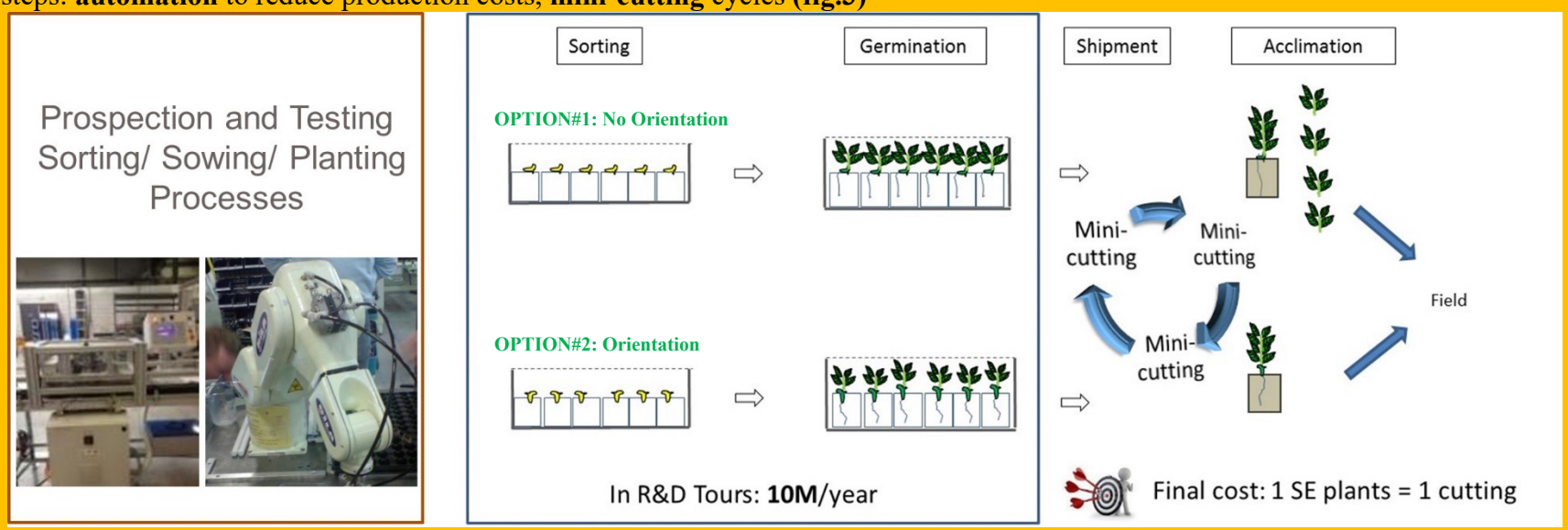


Figure 3: prospective ways of optimization for somatic embryogenesis. From left to rigth: robots for embryo handling ; *in vitro* sowing and germination ; mini cutting cycles i.e. softwood cutting from young somatic plantlets

References:
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Acknowledgements:
Talaron I., Lausanne F., Verdier D., Descourty I., Froger S., Brulard E., Bellanger L. and Broun P.

NEW PROCESS FOR IMPROVING THE FLAVOR QUALITY OF BRAZILIAN COFFEE (PART - II)

SEUNG KOOK PARK* and HYE-MIN KWON

Dept. of Food Science and Biotechnology, Kyung Hee University

*Yongin, South KOREA (*E-mail: skpark@khu.ac.kr)*

PC 400

Abstract

Two different methods of pretreatment for GCBs were developed; the first method was gentle germination and the second method was germination in presence of enzyme. GCBs were soaked in water and incubated at 55°C for 48 h. The GCBs were also germinated with the addition of enzyme to effectively produce flavor precursors, especially free amino acids and reducing sugar. The germinated green coffee beans were dried back to their initial water content, and roasted at medium degree for comparison. HPLC was used for the analysis of sugar and amino acids in GCBs and SPME-GC-MS was used for the analysis of volatile aroma compounds in the roasted coffee beans. The levels of sucrose in both pretreated beans decreased. Glucose also decreased in the germinated beans, but increased in the germination/enzyme-treated beans. SPME-GC-MS analysis showed that desirable aroma compounds, such as 2-methyl-butanal, 3-methyl-butanal, 2,3-dimethylpyrazine, 2,3,5-trimethylpyrazine increased, and undesirable compounds, such as 2-furanmethanol, 5-ethyl-2,3-dimethylpyrazine, phenol, methyl-phenol, and pyridine decreased. Interestingly, 2,3-dimethylpyrazine, tetramethylpyrazine, and several other unidentified aroma compounds, which were identified as desirable aromas by GCO, were found in both treated beans, but not in the control. Preliminary sensory evaluation showed that roasted coffees from the pretreated coffee beans had better flavor quality than the control. From this study, we demonstrate the possibility to improve the flavor quality of Brazilian coffee by simple and easy pretreatment of GCBs before roasting.

Introduction

Brazil is the largest global coffee producer, accounting for one third of the world's production, but Brazilian coffee quality has often been considered a inferior to that of coffees produced in other countries, such as Colombia, Ethiopia, and Guatemala. The aim of this study was to improve the flavor quality of Brazilian coffee by pretreatment of green coffee beans (GCBs), which resulted in the reduction of off-flavors and enhancement of desirable aroma compounds after roasting.

Materials and Methods

1. Green coffee beans: Brazil (*Coffea arabica*)
2. Germination: incubated for 48 h at 55℃ under darkness
3. Drying: dried for 5 h at 55℃ to its initial water content
4. Roasting: medium degree at 230℃ using Probat RE1
5. Analysis: weight loss (%), volume increase (%), SEM, Roasting degree (L*, a*, b*)
   - Seed viability: Tetrazolium test
   - Carbohydrates, chlorogenic acid, caffeine: HPLC
   - Free amino acids: UPLC
   - Volatile aroma compounds: SPME-GC-FID, SPME-GC-MS, SPME-GC-GCO
   - Pore structure in roasted bean: Scanning Electron Microscopy
6. Sensory evaluation: conducted by trained panels in the Food Science Department at the University

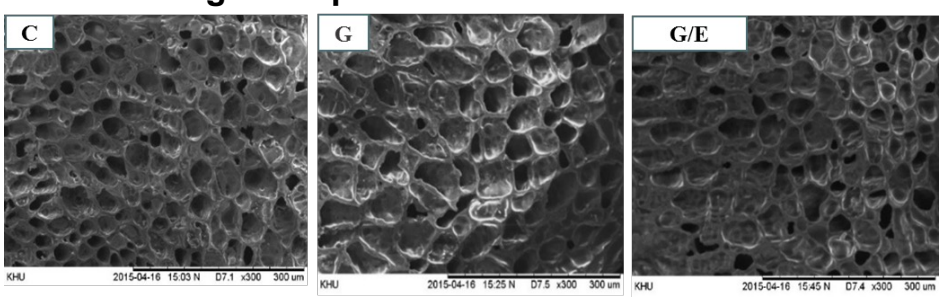
Results

1. Roasting degree

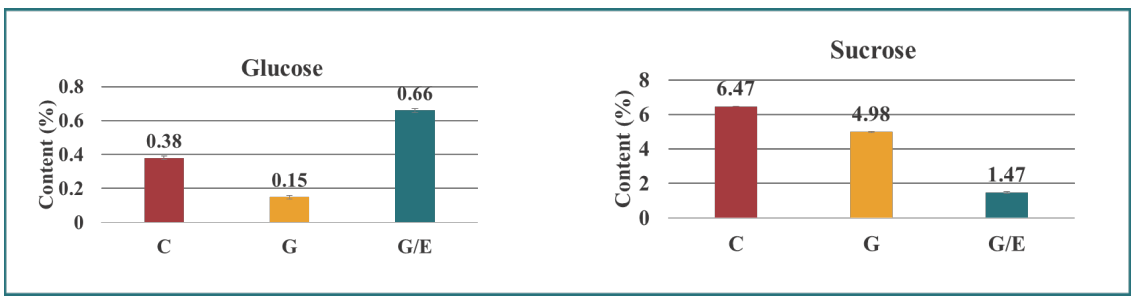
Sample	Color meter		
	L	a	b
C	24.07 ± 0.08[a]	3.48 ± 0.22[a]	10.33 ± 0.16[a]
G	24.84 ± 0.00[b]	4.22 ± 0.46[b]	11.07 ± 0.00[b]
G/E	25.85 ± 0.11[c]	4.68 ± 0.11[b]	10.85 ± 0.12[b]

All results are expressed as Mean±SD (n=3). Values with different small letters within the row are significantly different by Duncan's multiples range test (p<0.05).

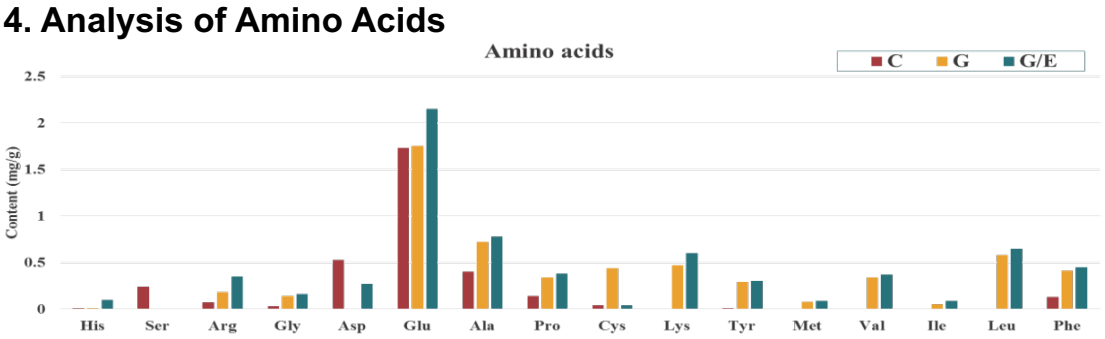
2. SEM images for pore structures of the roasted coffee beans (X300)



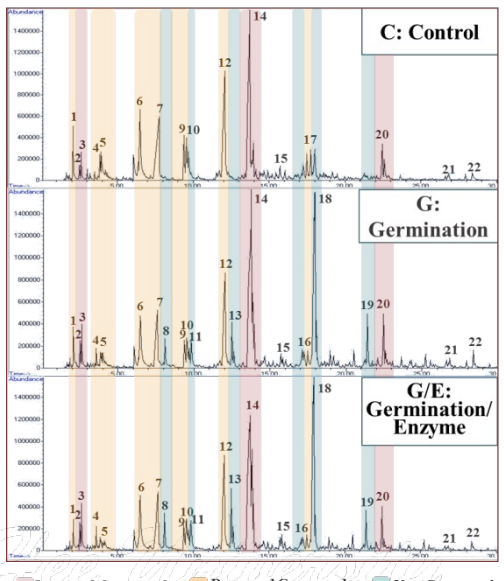
3. Analysis of glucose and sucrose



4. Analysis of Amino Acids

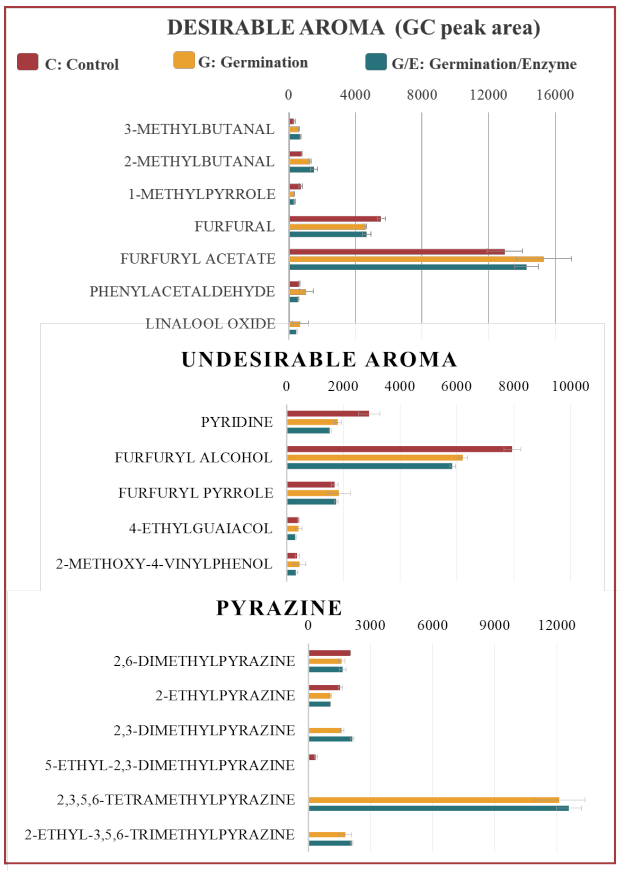


5. Analysis of Volatile Aroma Compounds



Increased Compounds | Decreased Compounds | New Compounds

No.	Compounds	No.	Compounds
1	2-methylfuran	12	5-methylfurfural
2	3-methylbutanal	13	Unknown
3	2-methylbutanal	14	Furfuryl acetate
4	1-methylpyrrole	15	Phenylacetaldehyde
5	Pyridine	16	Linalool oxide
6	Furfural	17	5-ethyl-2,3-dimethylpyrzine
7	Furfuryl alcohol	18	2,3,5,6-tetramethylpyrazine
8	5-methyl-2-oxazolidinone	19	2-ethyl-3,5,6-trimethylpyrazine
9	2,6-dimethylpyrazine	20	Furfuryl pyrrole
10	2-ethylpyrazine	21	4-ethylguaiacol
11	2,3-dimethylpyrazine	22	2-methoxy-4-vinylphenol



6. Sensory Evaluation

Sample	C: Control	G: Germination	G/E: Germination/Enzyme
	- Strongly spicy - Earthy - Off-flavor like wet newspaper	- Less spicy - Medium sweet aroma like sweet potato - Slightly acidic	- No spiciness - Strongly sweet aroma like sweet potato - Weakly acidic

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Food Flavor Laboratories
Kyung Hee University

NEW PROCESS FOR IMPROVING THE FLAVOR QUALITY OF ROBUSTA COFFEE (PART - I)

PC 401

SEUNG KOOK PARK* and SEUNG MIN OH

Dept. of Food Science and Biotechnology, Kyung Hee University

*Yongin, South KOREA (*E-mail: skpark@khu.ac.kr)*

Abstract

Robusta green coffee beans (RGCBs) were pretreated by soaking/germination (G) and soaking/germination/enzyme addition (GE) to naturally enhance the levels of flavor precursors such as amino acids and glucose, thereby improving the flavor quality of the roasted coffee. From the treatments, glucose level increased for GE, but both sucrose and glucose levels decreased for G coffee beans and sucrose level further decreased for green coffee with GE treatment. Caffeine and chlorogenic acid showed little differences between the control and the treated green beans. Most of the free amino acids increased for both G and GE-treated coffee beans. Upon roasting, undesirable flavor compounds that include 1-methylpyrrole, pyridine, 4-ethyl-2-methoxyphenol, and 2-methoxy-4-vinylphenol significantly decreased in the treated and roasted coffees, whereas desirable aroma compounds and unidentified desirable compounds significantly increased.

Introduction

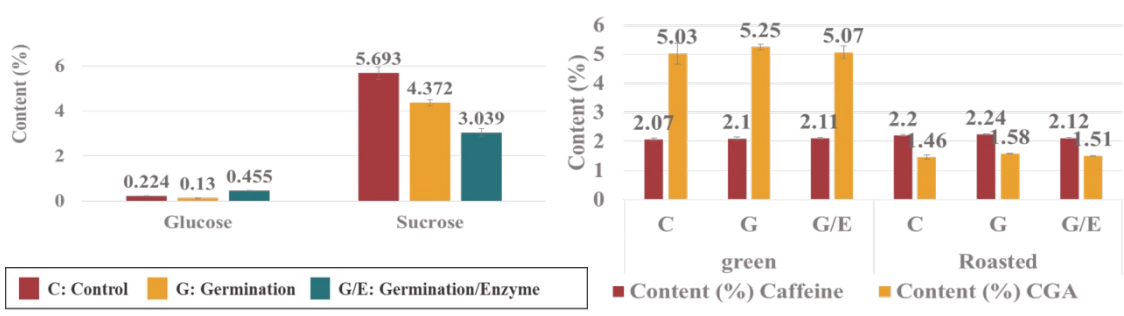
Robusta coffee accounts for 25% of world coffee production and it is primarily used for instant coffee and blending with Arabica coffees primarily due to the low flavor quality. Some Robusta coffees are of high quality and valued especially in espressos for their deep flavor and good crema, but the majority of Robusta coffee flavors are described as neutral, harsh, earthy, rubbery, grainy, strong, and/or bitter. These are all negative characters with regards to coffee quality. The aim of this study was to develop a new process for improving the flavor quality of Robusta coffee by gentle and natural germination of Robusta green coffee beans (RGCBs).

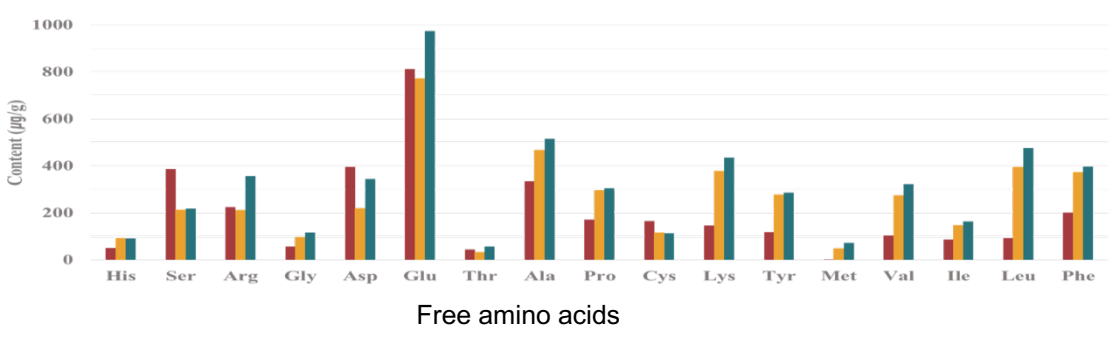
Materials and Methods

1. Green coffee beans: Robusta (*Coffea canephora*) from Vietnam
2. Germination: incubated for 48 h at 55℃ under darkness
3. Drying: dried for 5 h at 55℃ to its initial water content
4. Roasting: medium degree at 230℃ using Probat RE1
5. Analysis: weight loss (%), volume increase (%), SEM, Roasting degree (L*, a*, b*)
   - Seed viability: Tetrazolium test
   - Carbohydrates, chlorogenic acid, caffeine: HPLC
   - Free amino acids: UPLC
   - Volatile aroma compounds: SPME-GC-FID, SPME-GC-MS, SPME-GC-GCO
   - Pore structure in roasted bean: Scanning Electron Microscopy
6. Sensory evaluation: conducted by trained panels in the Food Science Department at the University

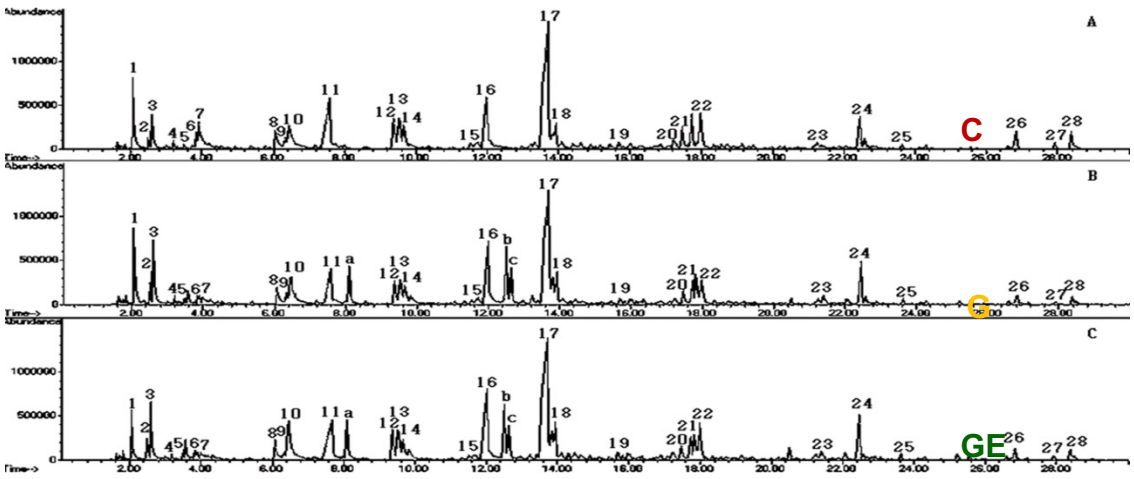
Results

1. Changes of chemical constituents in green coffee beans after germination and germination/enzyme treatment



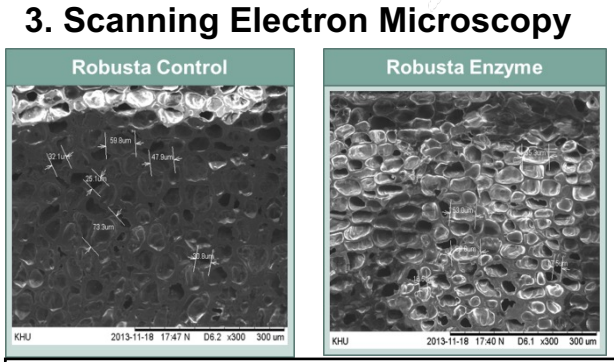


2. Analysis of Volatile Aroma Compounds

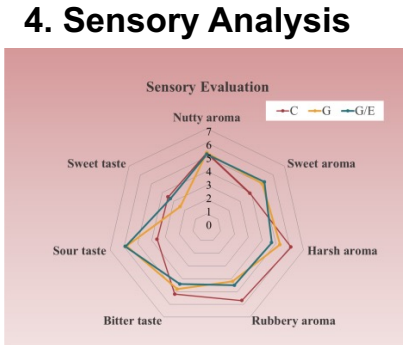


Peak No.	Retention time	Compounds	Peak area		
			C	G	G/E
1	2.069	2-Methylfuran	252.68 ± 4.52	221.53 ± 21.61	189.07 ± 16.43
2	2.503	3-Methylbutanal	35.60 ± 1.21	52.74 ± 10.01	58.86 ± 11.18
3	2.606	2-Methylbutanal	137.00 ± 2.60	221.15 ± 16.69	219.19 ± 1.58
4	3.194	2,5-Dimethyl furan	26.27 ± 2.27	26.09 ± 5.20	49.76 ± 22.92
5	3.490	3-Methylpyrazine	25.96 ± 0.84	21.01 ± 2.00	17.91 ± 4.78
6	3.839	1-Methylpyrrole	70.04 ± 23.56	21.95 ± 3.58	30.64 ± 2.70
7	3.913	Pyridine	199.10 ± 7.78	50.63 ± 12.58	65.99 ± 11.52
8	6.102	2-Methylpyrazine	134.22 ± 2.27	92.98 ± 7.77	108.95 ± 1.25
9	6.358	2-Methyl phenol	64.87 ± 3.19	52.60 ± 8.14	50.21 ± 4.52
10	6.466	Furfural	246.84 ± 21.01	324.46 ± 7.86	375.23 ± 6.07
11	7.790	Furfuryl alcohol	558.53 ± 45.47	311.66 ± 26.13	489.49 ± 14.43
12	9.361	2-Furfuryl formate	172.97 ± 2.27	120.95 ± 15.68	168.59 ± 1.39
13	9.541	2,6-Dimethylpyrazine	197.52 ± 9.08	149.62 ± 10.63	190.48 ± 2.77
14	9.668	2-Ethylpyrazine	154.86 ± 2.70	116.61 ± 7.01	157.97 ± 37.94
15	11.537	1-(2-FURYL)-2-PROPANONE	36.29 ± 4.57	26.47 ± 2.85	31.78 ± 1.28
16	11.998	5-Methyl-2-furancarboxaldehyde	448.52 ± 17.11	530.31 ± 13.67	623.88 ± 9.03
17	13.678	2-Furanmethanol acetate	1328.37 ± 70.23	1128.98 ± 78.76	1428.89 ± 15.85
18	13.915	1-Methyl-2-pyrrolecarboxaldehyde	134.55 ± 2.63	148.47 ± 7.02	175.10 ± 2.59
19	15.691	Benzenacetaldehyde	34.39 ± 1.60	34.19 ± 1.21	45.03 ± 3.14
20	17.231	2-Acetyl-1-methylpyrrole	67.69 ± 8.19	54.24 ± 1.77	71.12 ± 2.96
21	17.467	3-Ethyl-2,5-dimethylpyrazine	141.56 ± 13.65	128.18 ± 18.14	110.55 ± 23.86
22	17.988	2-Methoxyphenol	257.48 ± 13.23	174.93 ± 14.55	231.66 ± 14.40
23	21.258	3,5-Diethyl-2-methylpyrazine	37.73 ± 6.06	49.24 ± 3.94	52.69 ± 3.46
24	22.458	N-Furfuryl pyrrole	194.47 ± 25.75	224.66 ± 3.00	251.38 ± 9.99
25	23.634	Furfurylmethyldisulfide	29.03 ± 8.52	33.82 ± 1.50	26.94 ± 17.72
26	26.827	4-Ethyl-2-methoxyphenol	98.99 ± 18.34	57.32 ± 4.17	67.20 ± 6.27
27	27.916	2-Furfurylfuran	36.60 ± 7.21	12.46 ± 1.17	20.56 ± 1.91
28	28.377	2-Methoxy-4-vinylphenol	125.58 ± 12.62	50.81 ± 7.54	54.80 ± 1.08

3. Scanning Electron Microscopy



4. Sensory Analysis



Conclusion: From this study, we demonstrate the improvement of flavor quality of Robusta coffee by gentle germination or germination/enzyme treatment for RGCBs.

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Food Flavor Laboratories
Kyung Hee University

BIOCHEMICAL DIVERSITY OF UGANDAN CULTIVATED *Coffea canephora* SENSORY ORGANOLEPTIC CUP ATTRIBUTES

PC402

ALUKA, Pauline*, DAVRIEUX, Fabrice**, NGUGI, Kahiu***, BAKOMESA, Fidel****, NEUMBE, Beatrice****, KYAMUHANGIRE, Rita****, GERARD, Fourny**, NGABIRANO, Henry****

*NARO, Coffee Research Institute, Mukono, Uganda, **UMR AGAP, CIRAD, 34398 Montpellier, France, ***Depart of Plant Sciences & Crop Protection, Faculty of Agriculture, College of Agriculture and Veterinary Sciences, University of Nairobi, Kenya, ****Uganda Coffee Development Authority, Kampala.
Contact: p.aluka@yahoo.com

Abstract

Rationale: *Coffea canephora* contributes 80% annual Ugandan coffee production and 60% of US $ 4 million earned. Robusta organoleptic cup attributes affect health and define price while its variability determines market diversity.

Methods: Liquor variability of 204 cultivated *C. canephora* genotypes from 21 districts and 2 research institutes was evaluated by a panel of 3 UCDA experts using a 10 point descriptive scale. The Robusta coffee cupping protocol was developed by The Coffee Quality Institute of America (CQIA), UCDA, Specialty Coffee Association of America (SCAA) and others. (Table 1). Bar and line graphs in Fig 1 compared genotype and altitude mean liquor trait values. Regressions (Fig 2) determined most important traits to overall cup evaluation. PCA aggregated genotype organoleptic cup attributes using genetic distances estimated using Euclidean straight line method for altitude and tree age with varimax rotation (Fig 3a). Factorial step discriminant analyses spatially distributed the K means analysis organoleptic cup groups on the PC separated by Mahalanobis inter-group distances at 95% probability calculated using factorial step discriminant analyses to ascertain significant populations (Fig 3b). Two way parametric multivariate analysis of variance (Table 2) revealed significantly different cup traits among groups.

Results: A variety of fine and commercial flavours were detected in green roasted beans. The very good accession rating of 75% and above for cup balance, flavour, mouth feel, aftertaste, fragrance and aroma revealed Ugandan Robusta coffees were of high quality with a mild taste. Cup balance contributed the highest regression coefficient of 0.90 to overall cup assessment while fragrance/aroma had the least (0.22). The higher cup acidity among 'nganda' and 'erecta' genotypes coupled with about 50% sweet accessions revealed genotypes with high sugars and cup acidity can be identified from land races. Four multivariate groups that were significantly different for fragrance, aroma and flavour were formed offering opportunity to diverse cup tests to different markets. Coffee types and environmental factors such as soil texture, altitude and location influenced organoleptic cup attributes.

Conclusion & perspectives: A diversity of flavours existing among Uganda Robusta coffee can be exploited to provide different markets, enhance quality and crop husbandry.

Keywords: Robusta coffee, inherent traits, environment factors, cup quality profile.

Introduction: *Coffea canephora* in Uganda is grown by 1,200 households contributing to 80% of annual production and US$ 2.4 million earnings (UCDA, 2012). Ugandan wild and cultivated Robusta revealed enormous genetic and biochemical compound diversity (Musoli et al., 2009; Aluka, 2013) with high fat content (Aluka et al., 2016). However, limited information on Ugandan organoleptic cup variability is known, although markets regard it mild (UCDA, 2012). Genotype and environment influence quality precursors (Leroy et al., 2011) . This study therefore aimed at determining organoleptic cup variability of Ugandan cultivated Robusta coffee for strategic conservation, informed marketing and use in quality improvement.

Table 1: % liquor score rating for 204 genotypes (Scale: 1-10)

Cup brew traits	Fair 50-60	Average 61-70	V. good 71-80	Fine 81-90	control
Frag/aroma	0	32.7	64.9	2.4	71-80
Flavour	0	14	83.1	2.9	71-80
Aftertaste	0	20.7	74.5	4.8	61-70
Salt/acid	0	81.3	18.7	0	61-70
Bitter sweet	0	51.4	48.6	0	71-80
Mouth feel	0	23.1	75	1.9	71-80
Balance	0	9.1	89.5	1.4	71-80
Overall	0	13	84.1	2.9	71-80

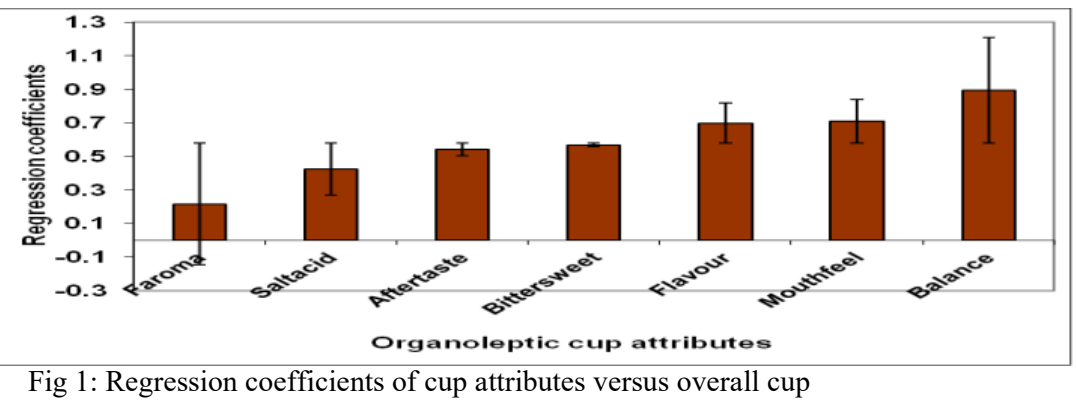

Fig 1: Regression coefficients of cup attributes versus overall cup

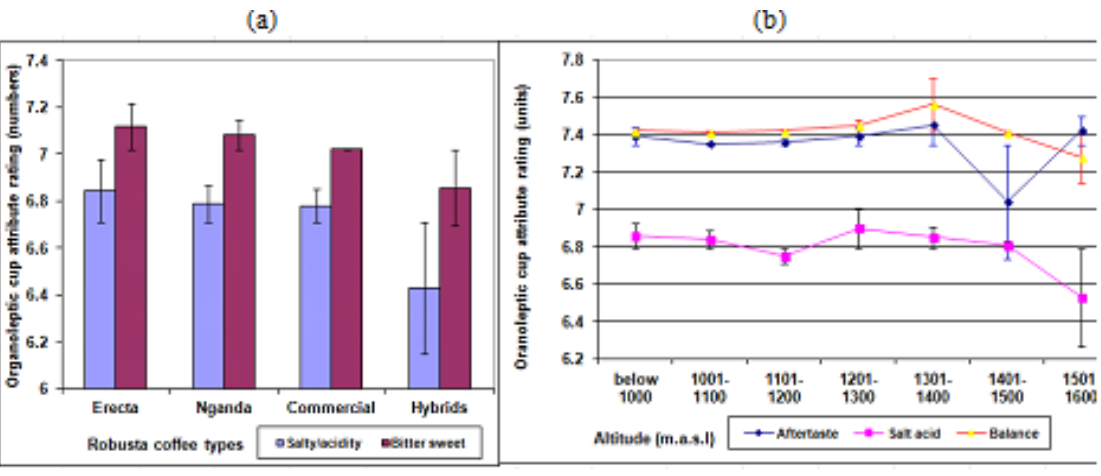

Fig 2: Comparison of cup attributes among: a) coffee types b) altitude ranges

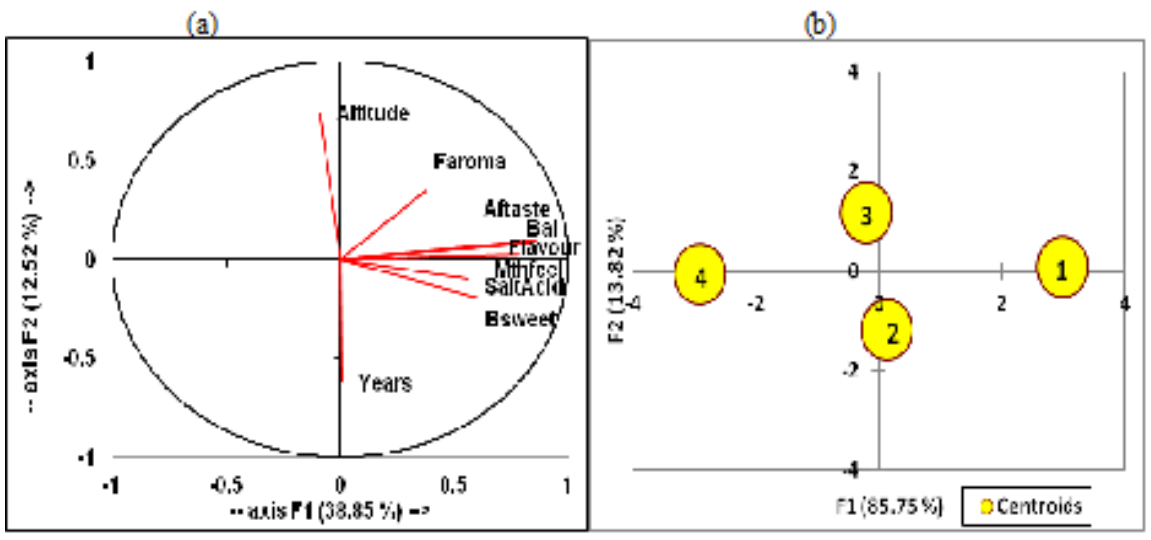

Fig 3: a) PCA plot of organoleptic attributes, altitude, tree age, b) groups separated by Mahalanobis distance.

Table 2: Two way parametric multivariate analysis of variance for four organoleptic cup groups

Source	Sum of squares	df	Mean square	F	p
Fragrance/aroma	0.0038022	2	0.0019011	1.1298	0.0003
Flavour	0.015388	2	0.007694	4.5726	0.0001
Interaction	-0.27075	4	-0.067686	-40.226	0.8791
Residual	0.33485	199	0.0016826		
Total	0.083291	207			

Results and discussion: All genotypes were rated above fair grade for cup brew traits (Table 1). Of 204 genotypes, 39 had very good acidity, 101 were sweet. 75% genotypes had very good flavour, aftertaste, mouth feel, balance and overall cup implying, Robusta genotypes with as good cup quality as Arabica can be identified from landraces for promotion, conservation and quality improvement.. Control cup traits were fair to very good. Lack of genotypes with fine grade acidity and sweet cup validated the characteristic reduced cup acidity and sweetness in Robusta coffees.

Erecta and Nganda landraces in Fig 2 had more acidity and sweeter than commercials and hybrids. 1301-1400masl produced better aftertaste while 1401-1500 masl had the least. 1201-1300 masl had highest cup acidity and 1501-1600 masl, the lowest.

Comparison of R^2 derived from other cup attributes versus overall cup in Fig 1 revealed balance was most important, fragrance and aroma were least in overall cup assessment.

In Fig 3, group 1 had genotypes that were superior in all cup traits, group 4 were inferior. Group 2 had more acidity, sweet cup and mouth feel. Group 3 had better fragrance and aroma, aftertaste, balance and flavor

The four organoleptic cup groups in Table 2 were significantly different for fragrance/aroma and flavour without interaction, hence can be improved independently.

Conclusion: Uganda trades on high quality organoleptic cup Robusta coffee with diverse flavours to serve different markets influenced by landrace variabilty and environment such as elevation.

Acknowledgement: UCDA and NaCORI for facilities used for research and facilitation to attend ASIC 2016, China. Thanks to CIRAD for technical guidance.

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Quantitative Study of Oxygen Impact on Liquid Coffee Flavor

PC 403

Xiaoping Fu and Carol Borland, Nestlé Development Center, Marysville, Ohio, USA;
Xiaoping.Fu@RD.Nestle.com , Carol.Borland@RD.Nestle.com

Abstract

Chemical and sensory characteristics of oxygenated liquid coffee were investigated. Sensory data showed a clear relationship between the amount of oxygen consumed by the liquid coffee and development of cardboard, pruney and other negative flavor attributes. These flavor attributes were linked to sulfur and nitrogen containing aroma compounds.

Introduction

Aroma in liquid coffee is not stable and rapidly reacts with oxygen. Off-flavors such as cardboard, pruney etc. from aroma oxidation affects beverage quality. Most of research is focused on oxidation of coffee that has been in contact with oxygen. Impact of oxygen on a oxygen-free liquid coffee is not known, especially the oxidation kinetics.

Results and Discussion

- *Oxygen-free liquid coffee and dosing of oxygen*.

Oxygen-free liquid coffee was produced as in figure 1, then different amounts of oxygen were added.

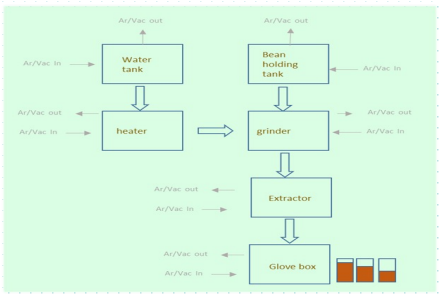

Figure 1: Preparation of oxygen free liquid coffee where different amounts of oxygen were added by varying the headspace. The oxidation was accelerated by a thermal treatment at $F_0$10

- *Rate of oxygen absorption by coffee*

Coffee reacts rapidly with oxygen. During our study, all oxygen added to the coffee beverage has been consumed.

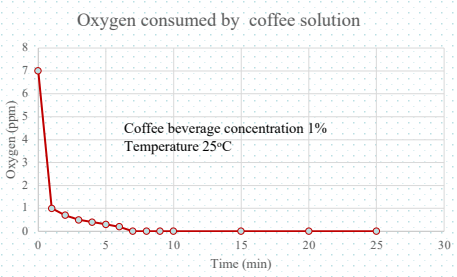

Figure 2: Kinetics of oxygen absorption by coffee

- *Impact of sensorial attributes by oxidation*

The more oxygen consumed by coffee or oxidation results in more cardboard and woody and less roasty aroma (figure 3).

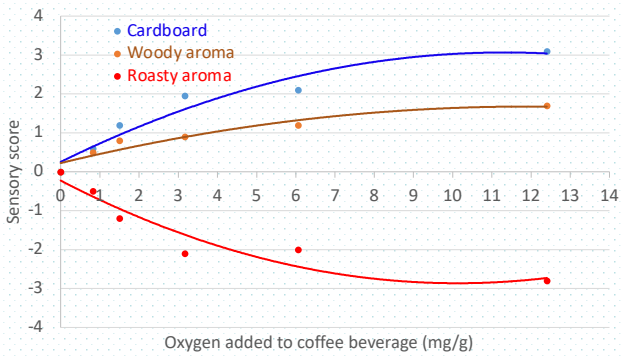

Figure 3: Impact on some sensorial attributes by oxidation in liquid coffee

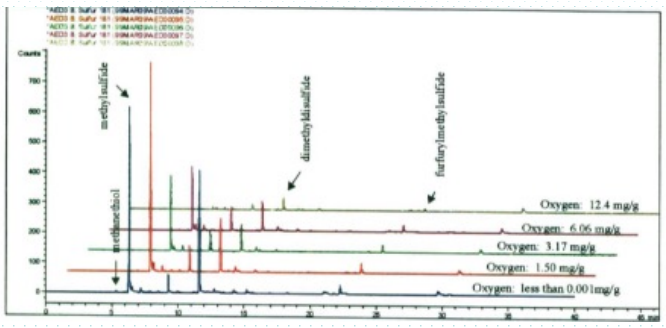

Figure 4. Impact on Sulfur components by oxidation

- *Impact of Sulfur/Nitrogen aroma by oxidation*

Methanethiol was only observed in the oxygen-free sample. Concentration of different sulfur compounds could be used as an indicator of oxidation degree of the beverage. Different sulfur components and their concentration were altered as the different amount of oxygen was consumed, and the oxidation kinetics to these sulfur compounds (figure 4 and table 1) and to nitrogen compounds (figure 5) was obtained.

Table 1:Suflur compounds as indicators for oxidation degree

Name	Sensitivity to oxygen	Disappearing in coffee as an indication of
Mehtanethiol	High	Little oxidation
Dimethyl Disulfide	Moderate	Moderate oxidation
Methyl Sulfide	Low	High oxidation
Methyl Furfuryl Sulfide	Low	Extensive oxidation

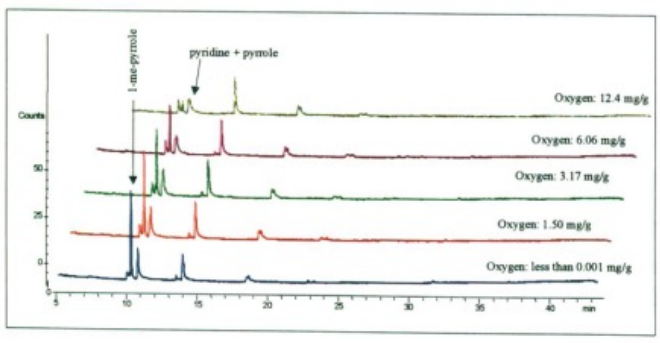

Figure 5. Impact on Nitrogen components by oxidation

Conclusion and future work

Liquid coffee reacts with oxygen rapidly. Sulfur compounds could be used as oxidation indicators. Relationship between oxygen concentration in coffee and cardboard, woody, roasty flavor attributes was obtained. Sulfur and nitrogen aroma compounds were altered by oxidation.

The non-volatiles were also impacted by oxidation (figure 6) and will be further studied.

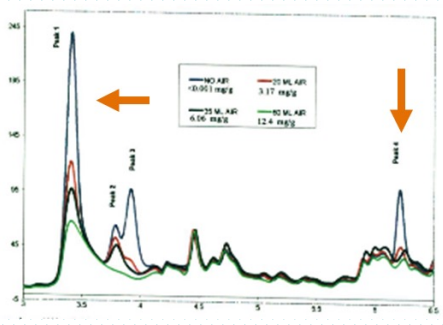

Figure 6. HPLC (r-Phase) showed some peaks were sensitive to the amount of oxygen added to the beverage

What are these peaks, how does oxygen impact them and what is their flavor contribution? –future work

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Nestlé Research

PC404

Chemical Composition of Coffee Shaded by *Grevillea robusta*

SOUZA, Antonio J.J.[1], LEITE, Jakson.[2], MATSUMOTO, Sylvana N.[3], MALTA, Marcelo R.[4]

[1,2] Universidade do Estado da Bahia, BRA, [3] Universidade do Sudoeste da Bahia, BRA, [4]Empresa de Pesquisa Agropecuária de Minas Gerais, BRA.

Abstract

The arboreal component in coffee growing favors greater relationship between leaf area and number of fruits per plant.

Introduction

This aspect combined with the most fruit development period allows the formation of larger fruits (Guyot 1996). There are published reports that coffee intercropped with trees showed fruits with greater weight compared to those where plants are far from the trees component. So it becomes necessary to evaluate the chemical quality of the fruits of coffee on this system.

METHODS

To access the quality of coffee fruits from plant growing intercropped with different densities of *Grevillea robusta* A. Cunn (31, 62, 69, 123, 139, 278 plants ha^{-1}). The plots consisted of coffee samples benefited with four replications. We determined the total acidity and total phenolic compounds of coffee fruits.

RESULTS

Increase in total acid titratable was observed when *G. robusta* density was increasing. The effect of shade on coffee quality was reported by Muschler (2001). According to the these author, wooded coffee showed high rates of acidity and body sharp. To Guyot et. al. (1996), coffee subjected to cultivation with woody has an increase of 16% in total titratable acidity.

The phenolic compounds also where inscrised with the *G. robusta* density. Similarly, Avelino et al (2005) evaluated the effect of shading on the chemical characteristics of coffee in Costa Rica and noted that woody cafes hade higher quality and phenolic compounds with increased *G. robusta* density.

Conclusion

The higher density of the tree component favors higher titratable acidity and total phenolic compounds in the coffee fruits.

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AVELINO, J. et al. Effects of slope exposure, altitude and yield on coffee quality in two altitude *terroirs* of Costa Rica, Orosi and Santa Maria de Dota **Journal of the Science of Food and Agriculture** 85, pg. 1869–1876, 2005

GUYOT, B. et al. Influence de l'altitude et de l'ombrage sur la qualité des cafes Arabica . **Plantations Recherche Developpement**, v.3, n.4, p. 272- 283, 1996

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PC405

Quantitation of mangiferin, caffeine and chlorogenic acid in two cultivars of *Coffee Arabica*: MGS Travessia and Catiguá MG3

TREVISAN, Maria T, S*, PAIVA, José R*, ALMEIDA, Ricardo F.*, OWEN, Robert W., DA CUNHA, Rodrigo L.*****
*Universidade Federal do Ceará, Fortaleza, CE, BR. ** German Cancer Research Center (DKFZ), Heidelberg, DE, ***Epamig, Lavras, Mg, Br.

Abstract

Recently mangiferin and other bioactive compounds were detected in *Coffea arabica* leaves, inspiring the initiation of this study, which involved a quantitative analysis of mangiferin (Figure 1a), chlorogenic acid (Figure 1b) and caffeine (Figure 1c) contents in the leaves of the cultivars MGS Travessia and Catiguá MG3 collected in Brazil, The latter one contains resistance factors to coffee-rust.

Introduction

Coffee (Figure 1d) is the third most consumed beverage in the world after water and tea. Coffee consumption is related to the potential prevention of diseases, which may be due to its content of polyphenols [1] such as mangiferin, chlorogenic acid and also the alkaloid caffeine. Mangiferin is a polyphenolic compound of the xanthone class with various biologic activities, such as antioxidant, anti-inflammatory, hypoglycemic, immunomodulatory, among others [2]. Chlorogenic acid is an ester formed by quinic acid and caffeic acid and has many biologic activities such as hypotensive effects, prevention of type 2 diabetes, antioxidant activity, antimutagenic, antiviral, and anticarcinogenic activity among others. [1,3,4]. Caffeine is probably the most frequently consumed substance in the world, and is a psychoactive stimulant of the nervous system [5]. In this study, these metabolites were quantitated in the leaves of two cultivars of Arabica coffee: "MGS Travessia" and "Catiguá MG3“ grown in the shade or exposed to full sunlight. The leaf samples were collected in Brazil, São Sebastião do Paraíso city (Mg).

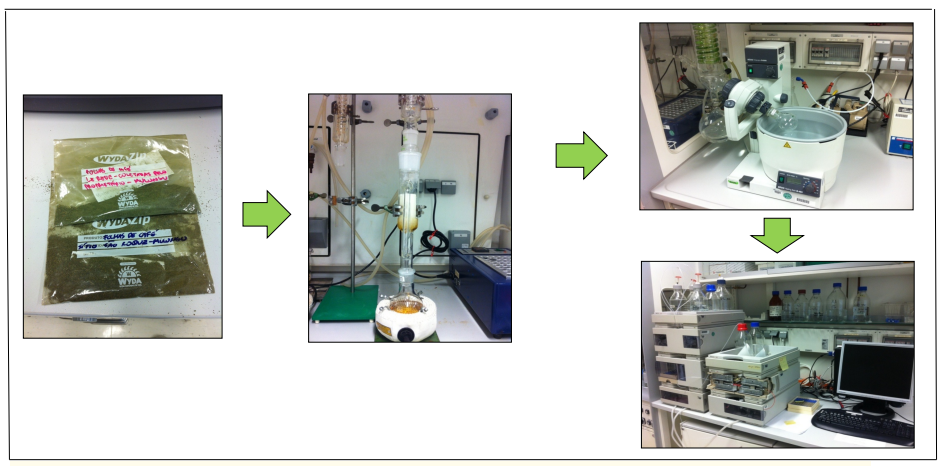


Figure 2: Methodology fluxogram.

Conclusion

The higher production of the phenolic compounds mangiferin and chlorogenic acid in the leaves of the cultivar Catiguá MG3 provides a possible natural and promising source of these phenolics, beyond their possible relationship with the biochemical mechanism of plant defence against pathogens and sun exposition. The higher values of polyphenols in leaves of trees grown in the shade, can be associated with coffee-rust, because a shady environment favors the growth of this pathogen.

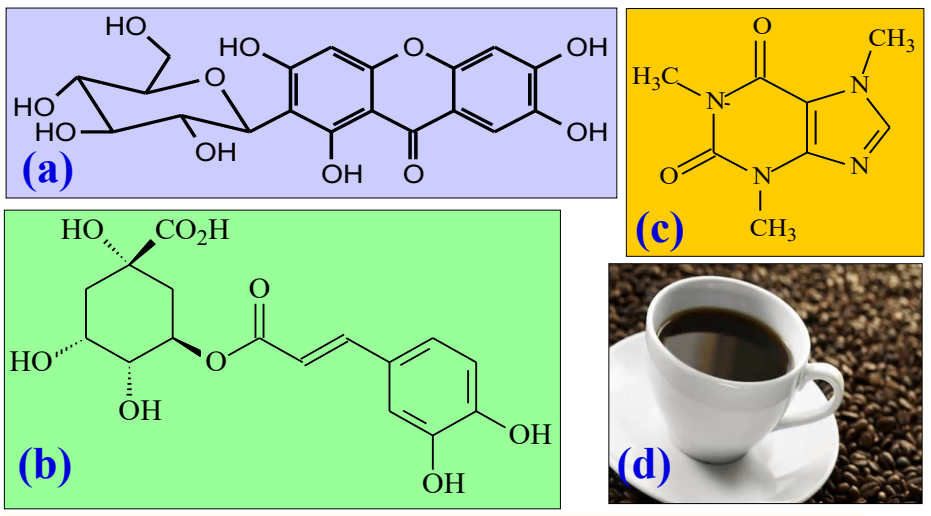
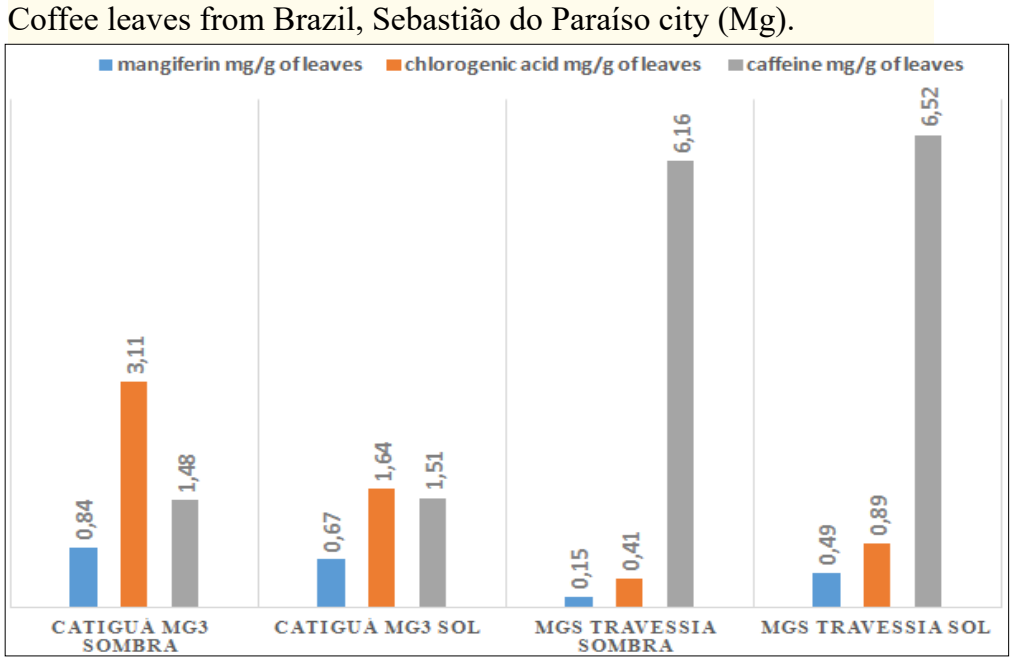


Figure 1: Structure of mangiferin (a), chlorogenic acid (b) and caffeine (c). Coffee beverage (d).

Table 1: Content of mangiferin, chlorogenic acid and caffeine in Coffee leaves from Brazil, Sebastião do Paraíso city (Mg).



Main Text

The leaf extracts were obtained by hot extraction with hexane followed by methanol (x 3) in a Soxhlet apparatus and the extracts were analysed by HPLC-DAD (Figure 2). Quantitations were performed using calibration curves of pure standards by linear regression.

The cultivar Catiguá MG3 which has coffee-rust resistance factors, had by far the highest concentration of mangiferin (2.3 times higher) and chlorogenic acid (3.6 times higher) than the cultivar without coffee-rust resistance factors (MGS Travessia). However, the cultivar MGS Travessia showed 4.2 times more caffeine than Catiguá MG3 (Table 1).

The leaves of coffee trees exposed to the sun of the cultivar MGS Travessia showed higher concentrations of mangiferin (3.2 times) and chlorogenic acid (2.2 times higher) compared to the leaves of coffee trees of the same cultivar grown in the shade. However, the result was inverse for the Catiguá MG3 cultivar: the leaves from trees exposed to the sun showed concentrations of mangiferin and chlorogenic acid 1.2 and 1.9 times lower respectively than leaves of coffee trees grown in the shade. With regard to caffeine there was no difference (Table 1).

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EFFECT OF FERMENTATION ON THE 'MOCHA' FLAVOUR PROFILE AND VOLATILE ORGANIC COMPOUNDS OF NATURAL COFFEE

PC-407

FERNANDEZ ALDUENDA, Mario R., BIRCH, E. John, SILCOCK, Patrick, LUSK, Karen A.
University of Otago, Department of Food Science, PO Box 56, Dunedin 9054, New Zealand.

Abstract

The 'Mocha' flavour profile is characteristic of some natural coffees and is defined by the presence of fruity or winey flavours (Fernandez Alduenda et al., 2014). The relationship between drying conditions and the 'Mocha' profile has not been established. In the present study, the links between drying rate, volatile organic compounds (VOCs) and flavour profile of natural coffees were investigated.

Natural coffees were prepared from Arabica coffee cherries using seven drying treatments that varied in their time to pass through different water activity ranges. The headspace VOCs of the green beans were characterised using PTR-MS. The beans were roasted and characterised using Descriptive Cupping (Fernandez Alduenda et al., 2014), headspace solid-phase extraction GC-O/MS and static headspace GC-MS. The 'Mocha'flavour profile was found to be correlated with 3-methylbutan-1-ol, ethyl 2-methylbutanoate and ethyl 3-methylbutanoate. These and other compounds found to be relevant are linked to the catabolism of valine, isoleucine and leucine. Analysis of VOCs from the green bean found that an ethanol/methanol ratio > 1 in green beans was obtained when the fresh cherries were held for two days prior to drying. This ratio was correlated to the formation of the fruity esters, which were linked to the fruitiness of the 'Mocha' profile.

Introduction

The term 'Mocha' originated in the Yemen port of Mocha and historically referred to the coffees exported from that port. Nowadays, the term 'Mocha' is used to describe a the presence of fruity or winey flavour characteristic that is present in some natural coffees. It is considered a desirable characteristic, provided it is not accompanied by undesirable "overfermented" characters (Fernandez Alduenda et al., 2014). Flavour differences within the natural coffee category have been recently attributed to the different microorganisms that grow under different drying conditions (Evangelista et al., 2014). However, the relationship between drying condition, fermentations and the 'Mocha' profile has not been established. In the present study, the links between drying rate, volatile organic compounds (VOCs) and flavour profile of natural coffees were investigated.

Materials and methods

To control raw material heterogeneity, the different treatments were drawn from one batch of coffee cherries. Natural coffees were prepared from Arabica coffee cherries using 7 drying treatments that varied in their time to pass through the water activity ranges 0.99 to 0.91, 0.91 to 0.85 and below 0.85 aW. The different drying profiles were selected to either promote or limit growth of bacteria, yeast or mould as the drying cherries passed through each drying stage. Treatment 3 had a particularly low drying rate during the first two days (high a_W range). Drying rates were controlled using techniques available to the typical coffee producer.

Dry cherries were hulled and the headspace VOCs of the green beans were characterised using PTR-MS. The beans were roasted and characterised using Descriptive Cupping (Fernandez Alduenda et al., 2014), headspace solid-phase extraction GC-O/MS and static headspace GC-MS. Data were analysed using Multiple Factor Analysis (MFA).

Figure 1: Multiple factor analysis (MFA) map representing the projection on F1 and F2 of descriptor subgroups (circle) and significant odour-active compounds (square) as active tables, and number of drying days, water activity (on day 2 and day 9) and pH (on day 6 and day 10) as supplementary tables (triangle).

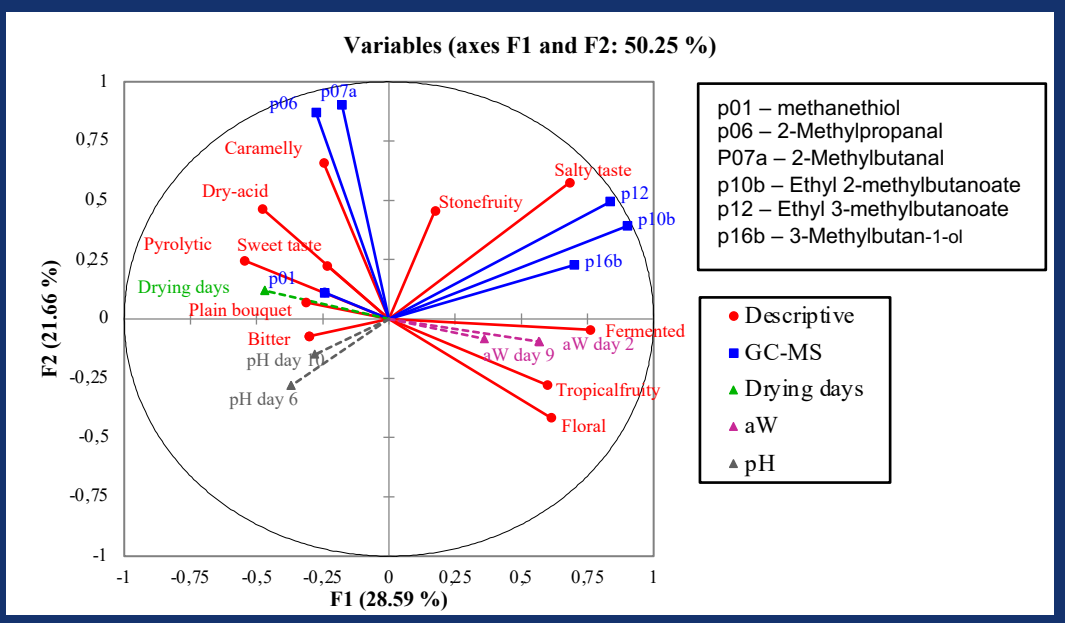

Table 2: Volatile compounds derived from the catabolism of branched-chain amino acids and their odour character. Adapted (Roze et al., 2010; Thonning Olesen & Stahnke, 2004). *(a) Aroma descriptors in italic taken from Flament and Bessière-Thomas (2002). (b) Underlined descriptors taken from GC-MS/O study.*

Chemical class	From the catabolism of valine	From the catabolism of isoleucine	From the catabolism of leucine
Alcohol	2-methylpropan-1ol	2-methyl-1-butanol Ethereal-fruity [a] Stinky [b]	3-methyl-1-butanol Fruity, winey [a]
Aldehyde	2-methylpropanal Overripe fruit [a] Peanuty/Fruity/Chocolate [b]	2-methylbutanal Fruity, fermented [a] Toasted bread/Peanut/Fruit [b]	3-methylbutanal Fruity [a] Toasted bread/Peanut/Fruit [b]
Carboxylic acid	2-methylpropanoic acid Acid odour, fruity [a] Tropical fruit [b]	2-methylbutanoic acid Fruity [a] Fermented/Tropical fruit [b]	3-methylbutanoic acid Fruity, cheesy [a] Fermented/Tropical fruit [b]
Ethyl ester	Ethyl 2-methylpropanoate Ethereal, fruity, sweet, pine-cone [a]	Ethyl 2-methylbutanoate Apricot, apple, strawberry [a] Strawberry [b]	Ethyl 3-methylbutanoate Blueberry, fruity [a] Blueberry [b]

Peak No	GC-O Character	Identified compound name	CAS No
1	Flatulence	Methanethiol	74-93-1
6	Peanuty/Fruity/Chocolate	2-Methylpropanal	78-84-2
7a	Toasted bread/Peanut/Fruit	2-Methylbutanal	96-17-3
10b	Strawberry	Ethyl 2-methylbutanoate	7452-79-1
12	Blueberry	Ethyl 3-methylbutanoate	108-64-5
16b	Stinky	3-Methylbutan-1-ol	123-51-3

Table 1: Significant odour-active compounds from GC-MS data - peak number, olfactory character, compound name and CAS number.

Results and discussion

The significant odour-active compounds are listed in Table 1. The 'Mocha' flavour profile was found to be correlated (Figure 1) with 3-methylbutan-1-ol, ethyl 2-methylbutanoate and ethyl 3-methylbutanoate, which were found to have a fruity character by GCO. These and other related compounds found to be relevant to the development of fruity and winey notes are linked to the catabolism of valine, isoleucine and leucine (Table 2).

Analysis of VOCs from the green bean found that an ethanol/methanol ratio > 1 in green beans was obtained when the fresh cherries were held for two days prior to drying (Treatment 3). This ratio was correlated to the formation of the fruity ethyl esters, which were linked to the fruitiness of the 'Mocha' profile.

The 'Mocha' profile was correlated with a longer drying time (number of days), higher water activity and lower pH, which suggests the specific fermentation created by these drying conditions are important in the development of the 'Mocha' profile.

Fruitiness was not pronounced in treatments with the highest drying rate, whereas the treatment with suspended drying for 48 hours (Treatment 3) showed a distinct fermented character. However, over-fermentation may deplete the levels of important precursors for sugar pyrolysis and Maillard reactions creating defects. Suspending drying during week 2 of drying (when a_W was ≤0.800 and batch weight was about 50% the initial weight) also resulted in a distinctive fruit character. This appeared a safe, controllable way to develop the 'Mocha' character. As the understanding of these flavour formation pathways evolves in the future, technologies will become available for producers to deliberately create and maintain their own natural coffee 'style'.

Conclusion

The fruity 'Mocha' profile of certain natural coffees is linked to the catabolism of branched-chain amino acids during processing, and appears to require ethanol to be produced in greater quantities than methanol to produce the required ethyl esters that contribute fruity flavours.

Application of this knowledge should allow to "dial up" the natural coffees flavour by manipulating drying conditions. It should also allow producers to consistently produce enough fruity esters for the coffee to be noticeably fruity with minimal risk of over-fermentation

Lastly, the desired coffee drying process can be controlled using low-level technology tools that are available to any coffee farmer.

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Bioactive polyphenols from *Coffea arabica* leaves: methodological aspects

PC 409

M. CORTESE, G. SAGRATINI, F. SUGGI LIVERANI*, S. VITTORI, L. NAVARINI*
School of Pharmacy, University of Camerino, Via Sant'Agostino 1 - Camerino 62032 – Italy
*illycaffè S.p.A., Via Flavia 110 – 34147 Trieste, Italy

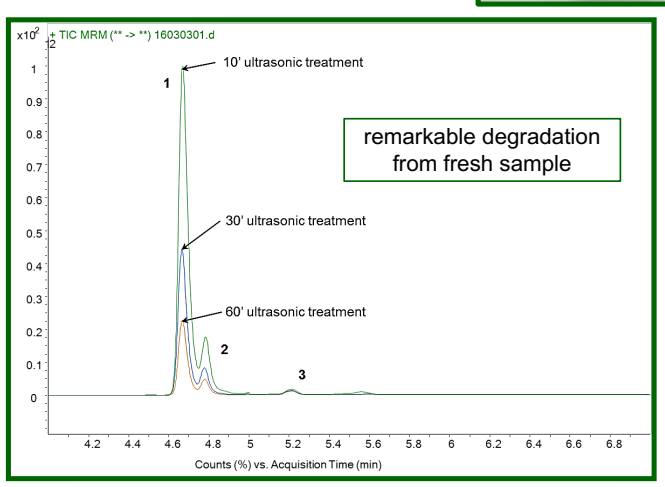
INTRODUCTION

The phenolic composition of *Coffea arabica* leaves has not yet been investigated in detail and a very limited number of studies have been reported in the literature so far. However, in view of the presence of mangiferin [1], a bioactive polyphenol with several pharmacological properties ranging from anti-inflammatory to antidiabetic and neuroprotective activities, and of the possible role exerted by polyphenols in the response to biotic and abiotic stresses, more attention should be paid in investigating these compounds in coffee leaves. It has been suggested that coffee resistance to coffee leaf rust, may be related to the oxidative potential of the leaf tissue regarding the phenolic composition [2]. In the present preliminary study we focused our attention on phenolic compounds different from chlorogenic acids and the effect of different sample preparation on the quantitative determination of these compounds.

MATERIALS & METHODS

Coffee leaves were collected from a single plant (*C. arabica* var. Caturra) growing under a natural photoperiod in a greenhouse at illycaffè. Two different sizes were selected: approx. 17 – 18 cm (big) and 10 – 11 cm (small), respectively. Fresh leaves were extracted and immediately analyzed or they were dried at 40°C, extracted and analyzed (see Scheme 1). Aqueous extracts were stored at -20°C for 3 weeks and re-analyzed.
HPLC analysis was performed by a HPLC 1290 Agilent Technologies, equipped with an Agilent Technologies 6420-Triple Quadrupole. The column was a Kinetex C18, 2.6 mm, 4.6x100 mm. The mobile phase consisted of an isocratic step of formic acid 1% watery solution:CH3CN 90:10 (V/V) maintained for 2 minutes, followed by a gradient step reaching 60% of the organic modifier in 5 minutes. This mobile phase composition is maintained constant for 3 minutes before restoring the initial conditions.

Scheme 1: sample preparation & analyses
Bar = 1.35 cm



RESULTS & DISCUSSION

Aqueous extracts from coffee leaves prepared as described in Scheme 1 contained mangiferin (**1**), isomangiferin (**2**) and rutin (**3**), however the content of these phenolics is strongly affected by sample preparation. Fresh leaves prove to be an unsuitable starting material to get reliable quantitative data due to possible enzymatic and/or hydrolytic activity during both the extraction step and the subsequent storage of the frozen aqueous extracts. On the contrary, dried material let to reproducible quantitative data on aqueous extracts after preparation. Aqueous extract from dried material, after three weeks storage at -20°C showed a 17% decrease of the mangiferin peak area and a very small decrease, if any, of isomangiferin and rutin. According to sample preparation as described in Scheme 1 (10' ultrasonic bath treatment) from dried material, two different extractions were performed on both big and small leaves. Good reproducibility can be observed (see Figure 1). Quantitatively, extraction by MeOH/H_2O 8:2 is by far more efficient (~ 36%) than by H_2O. Mangiferin is the dominant phenolic and together with rutin are more abundant in small leaves (see Table). Isomangiferin was not quantified.

Extraction Time (min.)	Mangiferin (%)	Rutin (%)
10	100,0	100,0
30	42,8	74,1
60	21,7	63,8

Sample	Mangiferin (mg/Kg)	Rutin (mg/Kg)
Big leaves	3071.5	26.9
Small leaves	11996.4	177.9

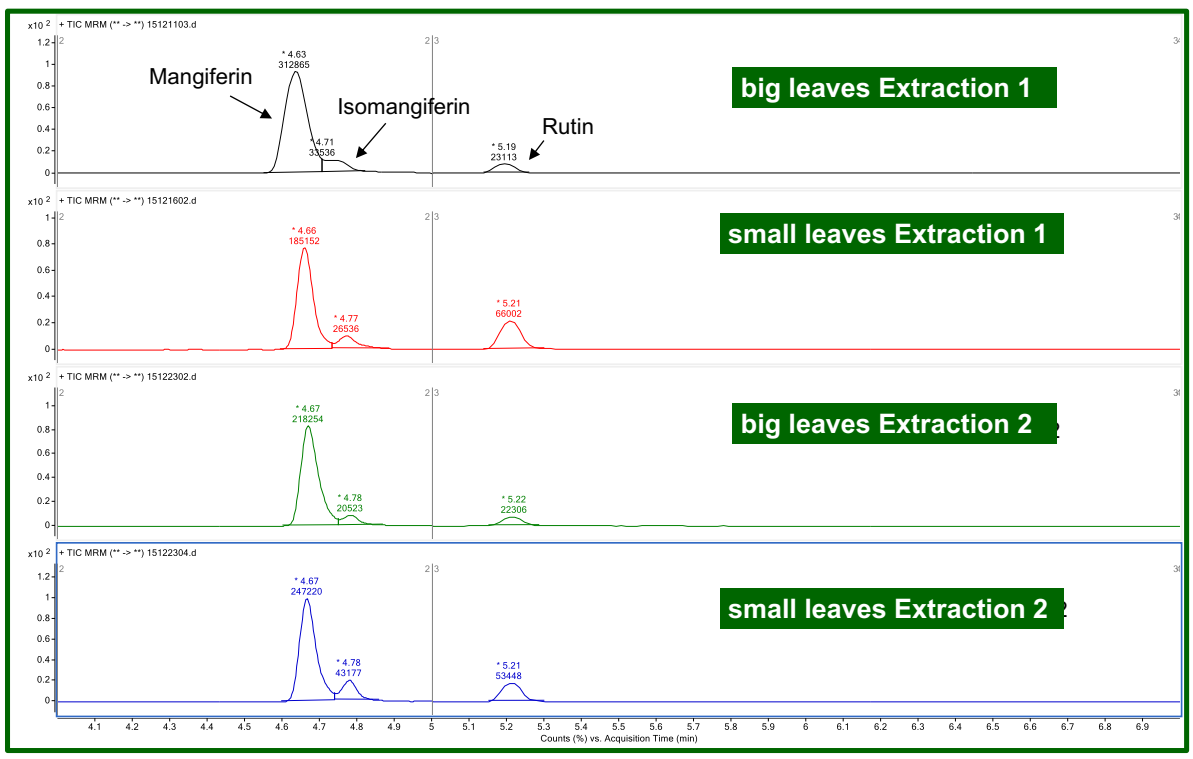


Figure 1: Chromatograms from two different extractions from dried material on big and small coffee leaves

CONCLUSIONS & PERSPECTIVES

In order to obtain reliable quantitative data on some selected polyphenols from *Coffea arabica* leaves, special care must be taken as far as both the sample preparation and extraction are concerned. Chemical characterization of coffee leaves is the first step towards the possible exploitation of this material for commercial purposes

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Influence of the extraction process on the aroma profile of roasted Arabica coffee oil

L. NAVARINI[1], V. LONZARICH[2], D. MUTARUTWA[2], E. DE ANGELIS[2], L. GUERRINI[3], G. ANGELONI[3], A. PARENTI[3]

[1]illycaffè S.p.A., via Flavia, 110 - Trieste 34147 – Italy
[2]Aromalab, illycaffè S.p.A., Padriciano 99 - Trieste 34012 – Italy
[3]Università degli Studi di Firenze, GESAAF - Piazzale Cascine 16, Firenze 50144 - Italy

PC 410

INTRODUCTION

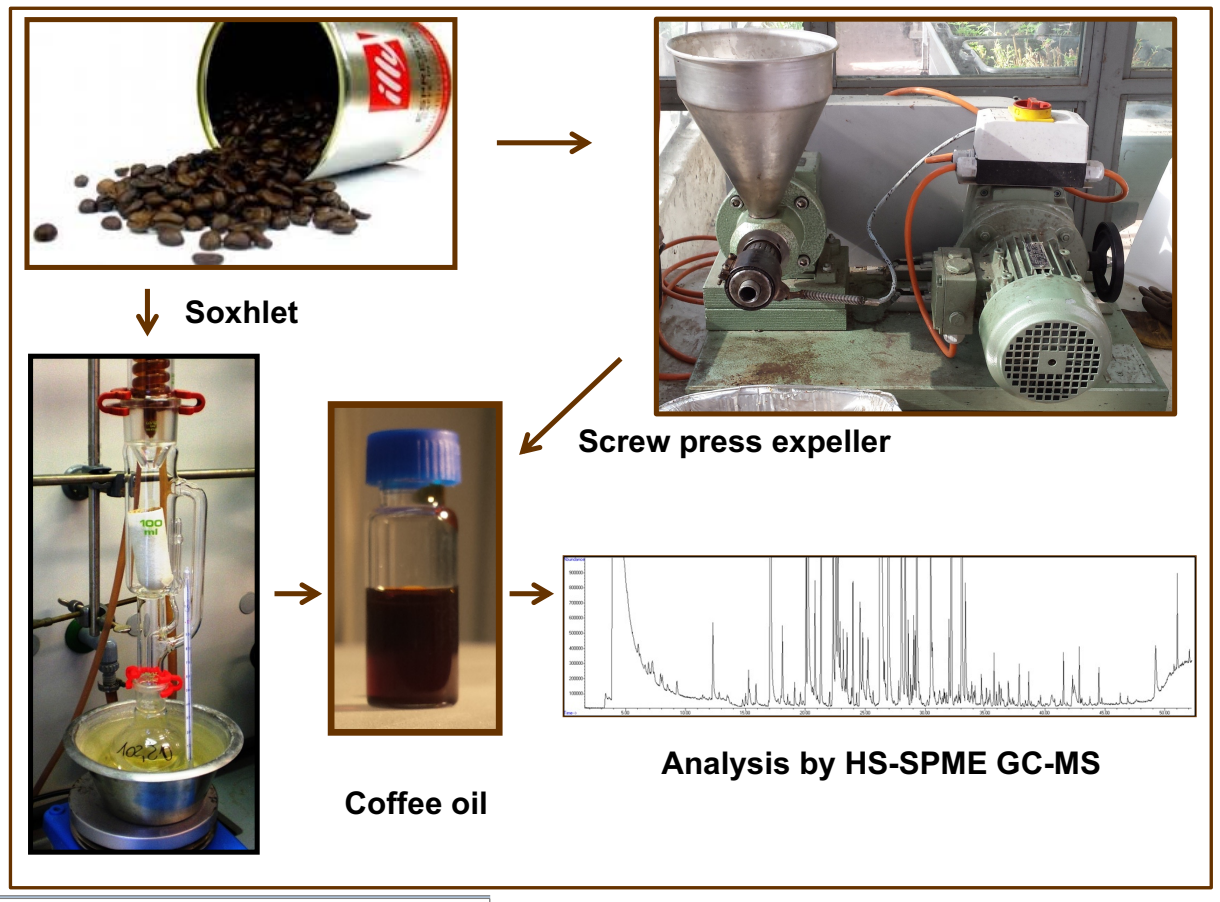
The coffee lipid fraction, also known as coffee oil, is mainly constituted by triacylglycerols with a fatty acids profile similar to that of common edible vegetable oils. The unsaponifiable fraction is rich in diterpenes, contains sterols, tocopherols, phosphatides and waxes (tryptamine derivatives) and is very important, together with fatty acid composition, as a chemical marker for authentication and traceability purposes. Another extremely important role played by coffee oil, particularly in coffee brews like *espresso*, is to act as an aroma carrier and then to render lipophilic aroma compounds available for interactions with our oral cavity. Aroma profile of roasted Arabica coffee oil has not been the subject of detailed studies, so far. In the present preliminary study, the influence of the extraction process on the roasted coffee oil aroma profile has been assessed by gas-chromatographic method.

MATERIALS & METHODS

Medium roasted 100% Arabica coffee oils extracted by two different processes (screw press expeller and Soxhlet) under different conditions have been characterized by HS-SPME GC-MS. In order to perform the extraction by screw press expeller (bench Komet screw press model CA59G, pressing capacity 3-5 Kg seed per hour, power of drive motor of 1.1 kW), the moisture content of the roasted coffee beans (initially 1.2 %) was adjusted to desired levels by addition of the calculated amount of water (two different samples at nominal moisture of 30% and 15% have been prepared). Soxhlet extraction (60-80 g coffee powder particle size according to DIN 10779 and 650 mL solvent volume) has been performed by using tert-butyl methyl ether and n-pentane. By solvent extraction, the coffee oil yield was slightly higher in the case of tert-butyl methyl ether (TBME) (17.4 ± 0.6 %) than n-pentane (15.6 ± 0.4%)

RESULTS & DISCUSSION

The isolation of coffee oil by Soxhlet apparatus with both n-pentane and TBME as tested solvents, remarkably influence the aroma profile. In spite of the solvents removal, not negligible solvent traces are present in the coffee oil headspace resulting in a strong signal in the region of the chromatograms where highly volatiles aroma compounds are expected (Figure 1, major volatile compounds are reported). The latter was confirmed when coffee oil isolated by screw press expeller was used for comparison. The expelled oil shows an aroma profile more rich of components than coffee oil extracted with solvents. By comparing the two Soxhlet oils (in Figure 2 major volatile compounds are reported), the sample extracted by TBME appears to be richer than the other, however this could be related to the post-extraction process necessary to remove the solvents. Residual TBME accounts for ca. 54 % of total peaks area while residual n-pentane is even higher (ca. 62 %).



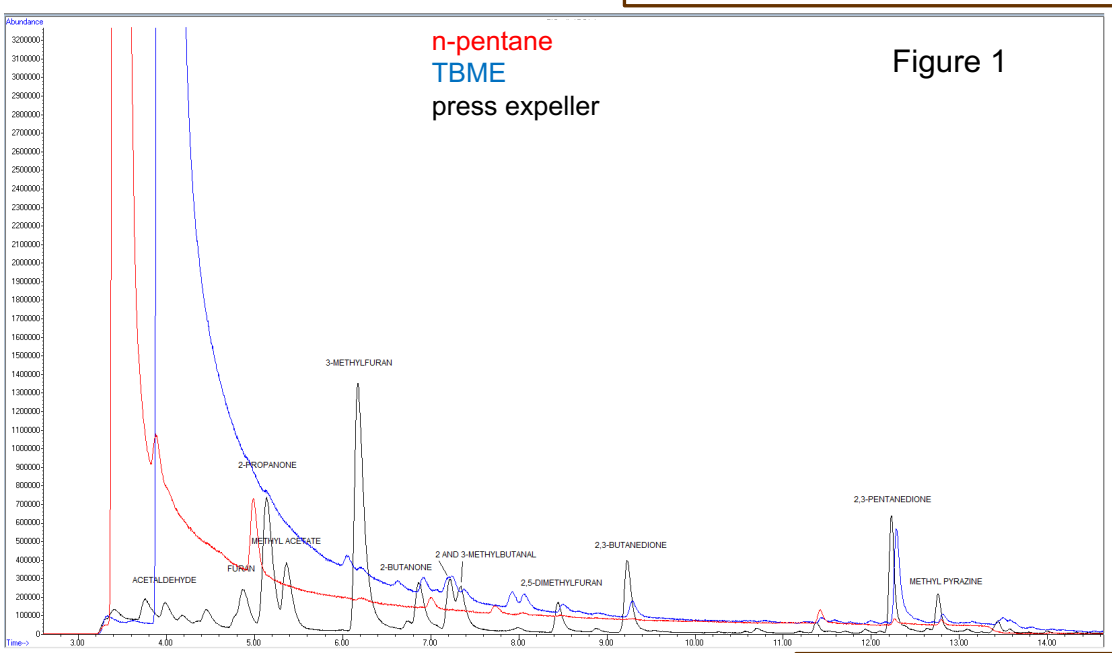


Figure 1

The chromatogram of the expelled oil in the region with RT > 14 min is very close, if not overlapped, to that of the oil extracted by TBME (see Figure 2) apart from a remarkable decrease of the acetic acid peak (not shown).

In addition to the screw press parameters (nozzle diameter: 5 mm, speed: 9.5 rpm and temperature: 60/68°C) moisture content of roasted coffee beans resulted to be very important in obtaining coffee oil by screw press expeller. Even though the extraction by screw press expeller has been performed to obtain few tens of mL for analytical purposes and has not been optimized in terms of process yield, the sample at the nominal moisture of 15 % led to higher amount of oil, in comparison with the sample at 30 % in the same operational time window. In the case of dry sample no oil has been obtained.

CONCLUSIONS & PERSPECTIVES

Both the necessity to exhaustively remove the solvent and the persisting presence of traces of residual solvent makes Soxhlet extraction not appropriate to isolate coffee oil for further aroma profile characterization. This is particularly evident as far as the highly volatiles aroma compounds are concerned. Major volatile compounds of expelled oil in addition to pyrazines and furans already reported to play an important role in the aroma of Arabica roasted coffee oil extracted by supercritical fluid [1], include aldehydes and ketones, very important coffee aroma components. Further studies are necessary to draw an exhaustive picture.

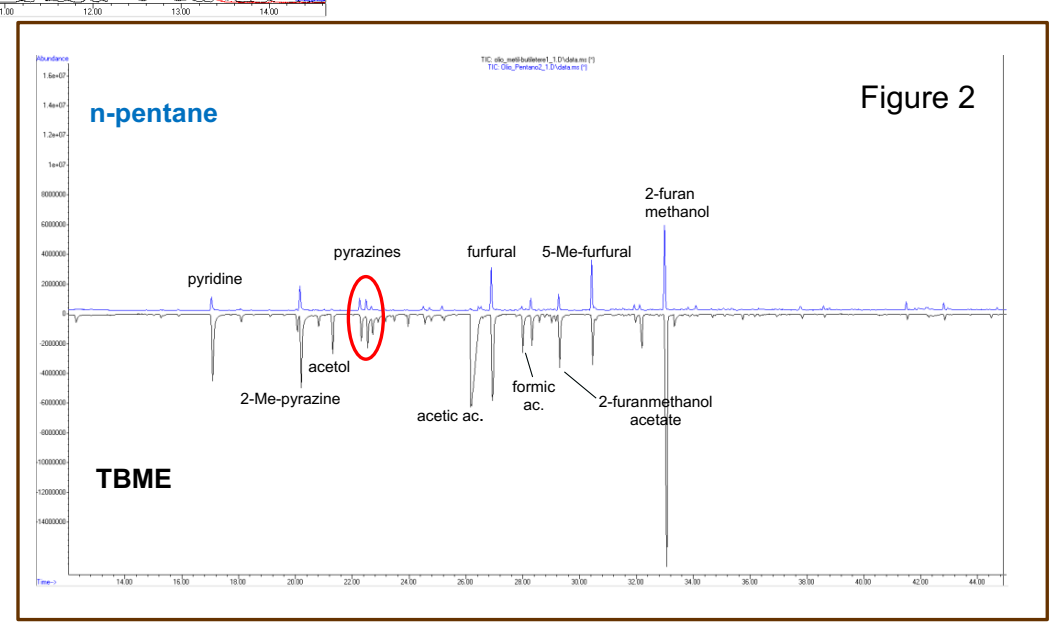


Figure 2

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CHANGES IN CHEMICAL COMPOSITION AND QUALITY OF COFFEE IN FUNCTION OF DIFFERENT POST-HARVEST OPERATIONS[1]

[1]Support: Fapemig and Consórcio Pesquisa Café

PC5

MALTA, Marcelo Ribeiro*, ALCÂNTARA, Elifas Nunes*, ROSA, Sttela Dellyzete Veiga Franco, ABREU, Giselle Figueiredo***, LISKA, Gilberto Rodrigues***, FASSIO, Larissa de Oliveira***, LIMA, Priscilla Magalhães*****

***Agricultural Research Company of Minas Gerais, Lavras, Minas Gerais, Brazil, **Brazilian Agricultural Research Corporation, Lavras, Minas Gerais, Brazil, ***Federal University of Lavras, Lavras – MG, Brazil.**

ABSTRACT

The storage is one of the most important steps of the post-harvest coffee, aiming to maintain the quality. However, several factors can promote changes in quality of coffee along the storage, such as air conditions of storage and the form of storage of grains. Thus, this study aimed to verify the effect of processing, storage temperature and coffee processing on the quality of coffee along the storage. Coffee fruits were harvested at the stage of ripening cherries and either processed dry (natural coffee) or wet (pulped coffee) being dried in mechanical driers until they reached 11% water content. After drying, part of the coffee was processed either manually or mechanically and another part was held without processing along the storage. The storage conditions were: storage under controlled conditions of air conditioning (10° C and 50% relative humidity) and in ambient conditions at 25° C without relative humidity control. The coffees were evaluated at 0, 4, 8 and 12 months of storage, through sensory evaluation and tests of electric conductivity (EC), leaching of potassium (LK), total titratable acidity (ATT), total sugars (AT), soluble solids (SS) and enzyme activity of poliphenoloxidase (PPO). To analyze the effect of processing, the storage temperature, and coffee processing on the quality of coffee along the storage was performed the Principal Component Analysis (PCA) using the computational software R (2013). The sensory attributes and total sensory note were determinant in characterizing the first main component and the chemical compounds to characterize the second main component. The coffees not processed, stored at temperature of 10ºC, were the ones who presented the highest values of all the sensory attributes and final sensory note. On the other hand, smaller values of the sensory attributes of coffee were observed in natural coffees manually processed, stored at a temperature of 25° C at the end of the storage season. The analyses of ATT, CE and LK allowed discrimination of mechanically processed coffees, that is, higher values of these parameters were observed in mechanically processed coffees, especially in storage temperature of 25° C at the end of the storage time. The reduction in air temperature of storage as well as storage of the grains without processing favors the maintenance of the quality of coffee along the storage.

INTRODUCTION

The storage is one of the most important steps of the post-harvest coffee, aiming to maintain the quality.

However, several factors can promote changes in quality of coffee along the storage, such as air conditions of storage and the form of storage of grains.

Thus, this study aimed to verify the effect of processing, storage temperature and coffee processing on the quality of coffee along the storage.

MATERIALS AND METHODS

Coffee fruits were harvested at the stage of ripening cherries (*Coffea arabica* L.)

Processed dry (natural coffee) or wet (pulped coffee)

Dried in mechanical driers until they reached 11% water content

After drying, part of the coffee was processed either manually or mechanically and another part was held without processing along the storage.

The storage conditions were: storage under controlled conditions of air conditioning (10° C and 50% relative humidity) and in ambient conditions at 25° C without relative humidity control.

The coffees were evaluated at 0, 4, 8 and 12 months of storage, through sensory evaluation and tests of electric conductivity (EC), potassium leaching (LK), total titratable acidity (ATT), total sugars (AT), soluble solids (SS) and enzyme activity of poliphenoloxidase (PFO).

To analyze the effect of processing, the storage temperature, and coffee processing on the quality of coffee along the storage was performed the Principal Component Analysis (PCA) using the computational software R (2013).

RESULTS

The sensory attributes and total sensory note were determinant in characterizing the first main component and the chemical compounds to characterize the second main component.

The coffees not processed, stored at temperature of 10ºC, were the ones who presented the highest values of all the sensory attributes and final sensory note.

On the other hand, smaller values of the sensory attributes of coffee were observed in natural coffees manually processed, stored at a temperature of 25° C at the end of the storage season.

The analyses of ATT, CE and LK allowed discrimination of mechanically processed coffees, that is, higher values of these parameters were observed in mechanically processed coffees, especially in storage temperature of 25° C at the end of the storage time.

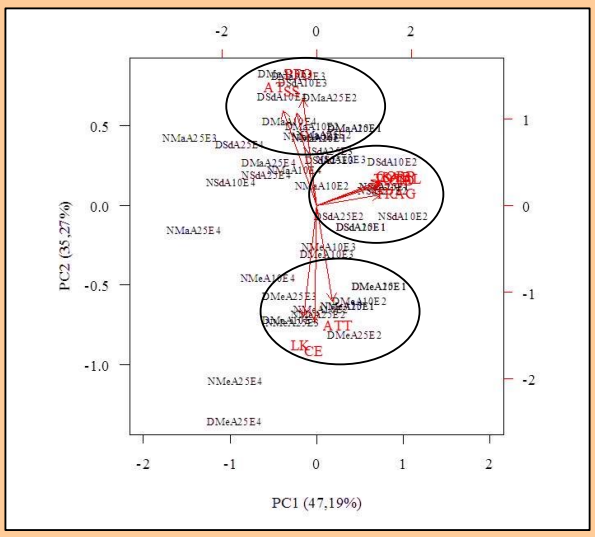


Figure 1. Biplot of the first two axes of the principal component analysis to data from two types processing (dry mehod - natural coffee (N) and wet method - pulped coffee (D)), three processing forms (Manually = Ma, mechanically = Me and another part was held without processing = Sd), two storage temperatures (10 ° C = A10 and 25 ° C = A25) and four storage times (E1 = 0 month, E2 = 4 months, E3 = 8 months, E4 = 12 months), depending of the sensory attributes, the final sensory note and tests of PFO, IC, AT, SS, ATT, CE and LK.

Table 1. Correlations between the parameters evaluated the first two principal components

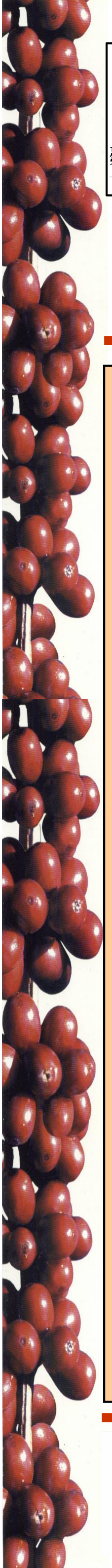
Parameters	PC1	PC2
Total titratable acidity (ATT)	0,2494	**-0,7344**
Electric conductivity (CE)	-0,0280	**-0,8884**
Soluble solids (SS)	-0,2976	**0,7074**
Poliphenoloxidase Activity (PFO)	-0,2106	**0,8154**
Total sugars (AT)	-0,5075	**0,7275**
Potassium Leaching (LK)	-0,1909	**-0,8545**
Fragance	**0,9733**	0,0782
Flavor	**0,9660**	0,1760
Acidity	**0,9674**	0,1592
Body	**0,9626**	0,1847
Finally	**0,9787**	0,1724

CONCLUSIONS

The reduction in air temperature of storage as well as storage of the grains without processing favors the maintenance of the quality of coffee along the storage.

REFERENCES

R Development Core Team. **R:** a language and environment for statistical computing. Foundation for Statistical Computing, Viena (2013).



CHEMICAL COMPOSITION AND QUALITY OF COFFEE STORED UNDER DIFFERENT FORMS OF PROCESSING AND DRYING[1]

[1]Support: Fapemig and Consórcio Pesquisa Café

PC413

MALTA, Marcelo Ribeiro*, ALCÂNTARA, Elifas Nunes*, ROSA, Sttela Dellyzete Veiga Franco, LISKA, Gilberto Rodrigues***, FASSIO, Larissa de Oliveira***, LIMA, Priscilla Magalhães*****

***Agricultural Research Company of Minas Gerais, Lavras, Minas Gerais, Brazil, **Brazilian Agricultural Research Corporation, Lavras, Minas Gerais, Brazil, ***Federal University of Lavras, Lavras – MG, Brazil.**

ABSTRACT

Due to the high humidity content in which is harvested, coffee requires adequate drying in order to preserve its quality. This work aimed to check the effects of drying and processing on chemical composition and quality of coffee along the storage. Fruits of *Coffea arabica* L. were collected at the stage of ripening cherries and underwent three forms of processing: natural coffee, cherry with mucilage removed and cherry pulped. Then were submitted to either slow drying, being dried in suspended screenhouse in the shade or fast drying, in fixed layer dryer with temperature control of 35ºC, until they reach approximately 11% of water content (b.u.). After the drying process, the coffees were processed and stored at a temperature of 20ºC, being evaluated at 0, 4, 8 and 12 months of storage through sensory evaluation and testing of electric conductivity (EC), leaching of potassium (LK), total titratable acidity (ATT), total sugars (AT), colour index (CI) and enzyme activity of poliphenoloxidase (PPO). To analyze the effect of the processing and the way of drying on the quality of coffee along the storage was performed the Principal Component Analysis (PCA) using the computational software R (2013). The coffees submitted to slow drying at the beginning of storage were the ones that presented the highest sensory notes and higher values of PPO and IC. On the other hand, natural coffees submitted to fast drying at the end of storage presented the lowest sensory notes and lower IC and PPO. In addition, natural coffees submitted to fast drying presented higher values of EC and LK. It is concluded that the natural coffees are more sensitive to drying process than the coffees processed wet, especially when they are submitted to fast drying.

INTRODUCTION

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This work aimed to check the effects of processing and drying on chemical composition and quality of coffee along the storage.

MATERIALS AND METHODS

Fruits of *Coffea arabica* L. were collected at the stage of ripening cherries.

Three forms of processing: natural coffee (N), cherry with mucilage removed (Dm) and cherry pulped (Dp).

Submitted to either **slow drying**, being dried in suspended screenhouse in the shade or **fast drying**, in fixed layer dryer with temperature control of 35ºC, until they reach approximately 11% of water content (b.u.).

After the drying process, the coffees were processed and stored at a temperature of 20ºC, being evaluated at 0, 4, 8 and 12 months of storage.

Sensory evaluation and testing of electric conductivity (CE), potassium leaching (LK), total titratable acidity (ATT), total sugars (AT), colour index (IC) and enzyme activity of poliphenoloxidase (PFO).

To analyze the effect of the processing and the way of drying on the quality of coffee along the storage was performed the Principal Component Analysis (PCA) using the computational software R (2013).

RESULTS

The coffees submitted to slow drying at the beginning of storage were the ones that presented the highest sensory notes and higher values of PFO and IC.

On the other hand, natural coffees submitted to fast drying at the end of storage presented the lowest sensory notes and lower IC and PFO.

In addition, natural coffees submitted to fast drying presented higher values of CE and LK.

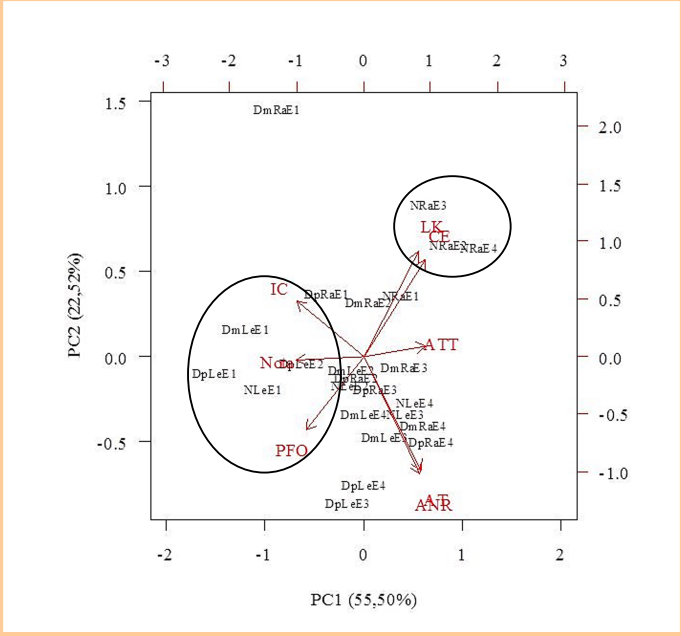

Figure 1. Biplot of the first two axes of the principal component analysis to data from three types processing (dry method - natural coffee (N) and wet method - pulped (Dp) and desmucilled (Dm) coffee), two types drying (slow - Le and fast – Ra drying) and four storage times (E1 = 0 month, E2 = 4 months, E3 = 8 months, E4 = 12 months), depending of the final sensory note and testing of PFO, AT, ANR, ATT, CE and LK.

Table 1. Correlations between the parameters evaluated the first two principal components

Parameters	PC1	PC2
Total titratable acidity (ATT)	0,7597	0,0593
Colour index (IC)	**-0,8248**	**0,3135**
Sugar not reducing (ANR)	0,6869	**-0,6708**
Total sugars (AT)	0,7040	**-0,6469**
Activity of poliphenoloxidase (PFO)	**-0,7063**	**-0,4190**
Electric conductivity (CE)	0,7581	**0,5488**
Potassium Leaching (LK)	0,6708	**0,5954**
Sensory note	**-0,8319**	-0,0220

CONCLUSIONS

It is concluded that the natural coffees are more sensitive to drying process than the coffees processed wet, especially when they are submitted to fast drying

REFERENCES

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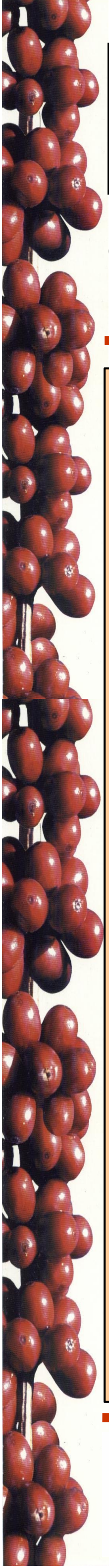

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Sharing Advance in Coffee Science & Technology
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CHANGES IN THE QUALITY OF COFFEE STORED SUBJECTED TO DIFFERENT FORMS OF PROCESSING AND DRYING[1]

[1]Support: Fapemig and Consórcio Pesquisa Café

PC414

MALTA, Marcelo Ribeiro*, ALCÂNTARA, Elifas Nunes*, ROSA, Sttela Dellyzete Veiga Franco, LISKA, Gilberto Rodrigues***, FASSIO, Larissa de Oliveira***, LIMA, Priscilla Magalhães*****

***Agricultural Research Company of Minas Gerais, Lavras, Minas Gerais, Brazil, **Brazilian Agricultural Research Corporation, Lavras, Minas Gerais, Brazil, ***Federal University of Lavras, Lavras – MG, Brazil.**

ABSTRACT

Coffee quality is influenced by a number of factors and the post-harvest handling is one of the most important. Several studies indicate that the chemical composition of the coffee beans is dependent on the form of processing and drying, contributing to different characteristics in coffee quality. This work aimed to check the effects of processing and drying on quality of coffee along the storage. Fruits of *Coffea arabica* L. were collected at the stage of ripening cherries and underwent the following forms of processing: dry (natural coffee) and, wet (cherry with mucilage removed and cherry pulped). Then underwent three forms of drying: in suspended screenhouse in the shade, under the Sun or in fixed layer dryers with temperature control of 35ºC, until they reach approximately 11% of water content (b.u.). After the drying process, the coffees were processed and stored at a temperature of 10ºC, being evaluated at 0, 4, 8 and 12 months of storage through sensory evaluation under the protocol of the Specialty Coffee Association of America (SCAA). To analyze the effect of the processing and the way of drying on the quality of coffee along the storage was performed the Principal Component Analysis (PCA) using the computational software R (2013). Coffees with mucilage removed dried in mechanical dryers at the end of storage, along with Sun-dried coffees at the beginning of storage, regardless of the type of processing adopted, presented the highest values of all the sensory attributes and final note. On the other hand, natural coffees dried in mechanical dryers presented the lowest notes of all sensory attributes and final note. According to the second main component, processed coffee under the wet method, when dried in the Sun, at the beginning of storage, presented the highest values of the sensory attributes, fragrance and acidity and lower values of the attributes, flavor and body. On the other hand, the coffees with mucilage removed, dried in mechanical dryers, by the end of the storage featured the highest values of the sensory notes of flavor and body and lowest fragrance and acidity. It can be seen an increased tolerance to drying of wet processed coffees relative to the dry processed coffees, mainly when drying is performed in mechanical dryers.

INTRODUCTION

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This work aimed to check the effects of processing and drying on quality of coffee along the storage.

MATERIALS AND METHODS

Fruits of *Coffea arabica* L. were collected at the stage of ripening cherries

Processing: dry (natural coffee) and, wet (cherry with mucilage removed and cherry pulped)

Three forms of drying: in suspended screenhouse in the shade, under the Sun or in fixed layer dryers with temperature control of 35ºC, until they reach approximately 11% of water content (wb)

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To analyze the effect of the processing and drying on the quality of coffee along the storage was performed the Principal Component Analysis (PCA) using the computational software R (2013)

RESULTS

Coffees with mucilage removed dried in mechanical dryers at the end of storage, along with Sun-dried coffees at the beginning of storage, regardless of the type of processing adopted, presented the highest values of all the sensory attributes and final note.

Natural coffees dried in mechanical dryers presented the lowest notes of all sensory attributes and final note.

Coffee processed under the wet method, when dried in the Sun, at the beginning of storage, presented the highest values of the sensory attributes, fragrance and acidity and lower values of the attributes, flavor and body.

The coffees with mucilage removed, dried in mechanical dryers, by the end of the storage featured the highest values of the sensory notes of flavor and body and lowest fragrance and acidity.

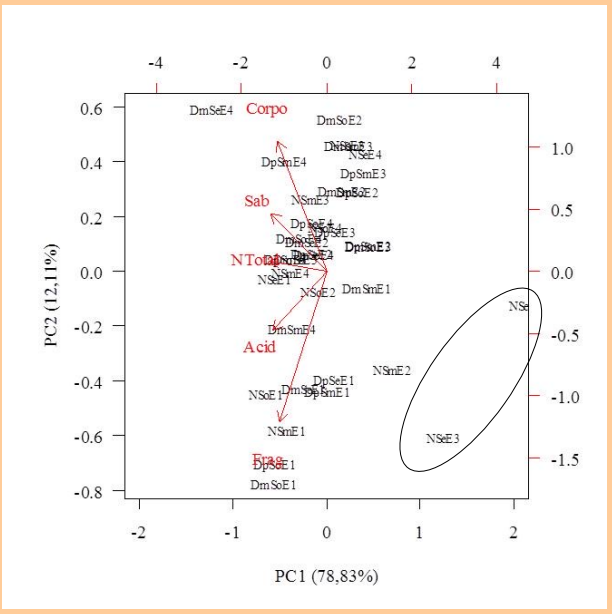

Figure 1. Biplot of the first two axes of the principal component analysis to data from three types processing (dry method - natural coffee (N) and wet method - pulped (Dp) and desmucilled (Dm) coffee), three types drying (Shade, sun and dryers) and four storage times (E1 = 0 month, E2 = 4 months, E3 = 8 months, E4 = 12 months), depending of the sensory evaluation.

Table 1. Correlations between the parameters evaluated the first two principal components

Parameters	PC1	PC2
Fragance (Frag)	**-0,7900**	**-0,5422**
Flavour (Sab)	**-0,9411**	0,2096
Acidity (Acid)	**-0,9084**	-0,2135
Body (Corpo)	**-0,8254**	**0,4697**
Finally (NTotal)	**-0,9620**	0,0388

CONCLUSIONS

It can be seen an increased tolerance to drying of wet processed coffees relative to the dry processed coffees, mainly when drying is performed in mechanical dryers.

REFERENCES

R Development Core Team. **R:** a language and environment for statistical computing. Foundation for Statistical Computing, Viena (2013).

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TRIPLE-OPTIMIZATION SYSTEM TO IMPROVE COST-EFFECTIVENESS OF ROASTED AND GROUND COFFEE PRODUCTS.

PC415

ISHIWAKI, Tomohiro*, YAMAISHI, Eriko, OSAMURA, Mayuko, SENDA, Takuya
Research and Development Office, S. ISHIMITSU & CO., LTD., KOBE, JAPAN.
***t-ishiwaki@ishimitsu.co.jp**

Abstract

TRIPLE-OPTIMIZATION SYSTEM TO IMPROVE COST-EFFECTIVENESS OF ROASTED AND GROUND COFFEE PRODUCTS.

ISHIWAKI, Tomohiro*, YAMAISHI, Eriko, OSAMURA, Mayuko, SENDA, Takuya
Research and Development Office, S. ISHIMITSU & CO., LTD., KOBE, JAPAN.
*t-ishiwaki@ishimitsu.co.jp

INTRODUCTION S. ISHIMITSU & CO., LTD. is one of the biggest green coffee importer in Japan, which has big roasting factories in the group. Research and development office supports product developments of our customers by scientific approaches.
The Japanese coffee consumption market seems to become polarized between low-cost market and high-quality market. The "triple-optimization system" was developed to provide cost-effective products for both markets. The system was constructed by three steps, which was optimization of "maturity of green coffee beans", "blending", and "grinding".
The purpose of this presentation is to share our approach with a lot of coffee persons for the better coffee products.

METHODS
1) Optimization of maturity Near infrared (NIR) analyzer Spectra Star2500RTW (Unity Scientific) was applied to measure maturity. About 300 sets of NIR spectra and the contents of cholorogenic acids were used to make a calibration curve. Maturity was calculated by the ratio of concentration of mono-chlorogenic acids to that of di-chlorogenic acids[1].
2) Optimization of blending TS-5000Z (Intelligent Sensor Technology, Inc.) ,which is a taste-sensing system with artificial lipid-based membrane sensors, was applied to quantification of the taste of coffees[2]. There was a clear correlation between the outputs by the system and the sensory evaluations of our blender. The output of acidity and bitterness were mainly used for calculations. Zero point for each taste is that of Nestle Gold Blend.
3) Optimization of grinding A200 Basic (Verder Scientific) was used to measure the size distribution of ground coffee. The Rosin-Rammler distribution was applied to control the granule size. Level of astringency was measured by TS-5000Z.

RESULTS Commodity grade coffee showed lower maturity and higher maturity/price. In contrast, specialty grade coffee, like "Cup of Excellence" winner, showed higher maturity and lower maturity/price. The maturity/price index won the admiration of our customers as a tool for the choice of raw materials. It is possible to improve cost-effectiveness by blending and/or grinding. The idea of optimization by blending was shown in the last ASIC[3]. Optimization by grinding is mainly used for single served coffee and coffee for restaurant. About 15 percent cost cut was achieved on average.

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1. Clifford, M. N. and Ohiokpehai, Anal. Proc., 20(2), 83-86, 1983
2. Toko, K. ed.,Biochemical Sensors: Mimicking Gustatory and Olfactory Senses; Pan Stanford Publishing : Singapore; 2013.
3. Ishiwaki, T. *et al.*, Application of Taste Sensor to Coffee Industry. In *Proceedings of the 25th ASIC Colloquium*; Colombia; ASIC: Paris, 2014.

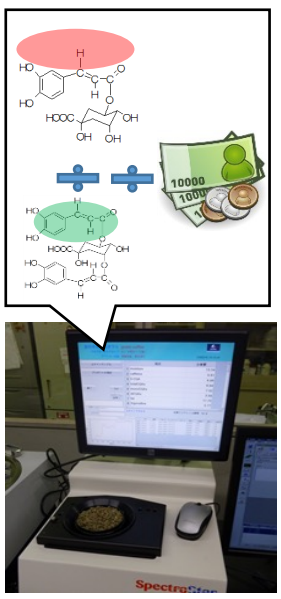

Fig.1 Process of optimization for each step. 1) The choice of green coffee beans was optimized by maturity/price. 2) The choice of roasted coffee for blending was optimized by PC calculation for the database which included taste and price. 3) The choice of granule size distribution was conducted by comparison of astringency. Total surface area was standardized by sieving test followed by calculation.

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3) Optimization of grinding A200 Basic (Verder Scientific) was used to measure the size distribution of ground coffee. The Rosin-Rammler distribution was applied to control the granule size. Level of astringency was measured by TS-5000Z.

Results

An example of cost-effectiveness of green coffee beans is shown in fig.2 Commodity grade coffee showed lower maturity and higher maturity/price. In contrast, specialty grade coffee, like C.O.E. winner, showed higher maturity and lower maturity/price. The maturity/price index won the admiration of our customers as a tool for the choice of raw materials. It is possible to improve cost-effectiveness by blending and/or grinding. Table 1 is a result of optimization by blending. Optimization by grinding is mainly used for single served coffee and coffee for restaurant. As is shown in fig.3, about 15 percent cost cut was achieved on average.

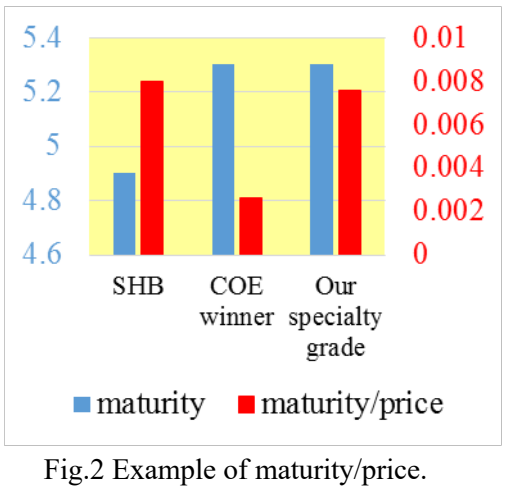

Fig.2 Example of maturity/price. This index was helpful to choose green coffee beans.

Table 1. Example of the effects of optimization of R&G product.
Taste measurement for 8 samples were conducted at first.
Taste data and price data of each sample were set.
Optimization of the price within the taste allowance was performed with a simple PC program. In this case, about 10 percent cost cut was achieved. In addition, roasting process got simpler than before.

	parts 1	parts 2	parts 3	parts 4	parts 5	parts 6	parts 7	parts 8
before optimization	27%	20%	3%		13%	20%	3%	14%
after optimization	40%			40%				20%

	Acidity	Bitterness	Yen/kg
before optimization	-2.8	0.4	768.7
after optimization	-2.1	0.2	714

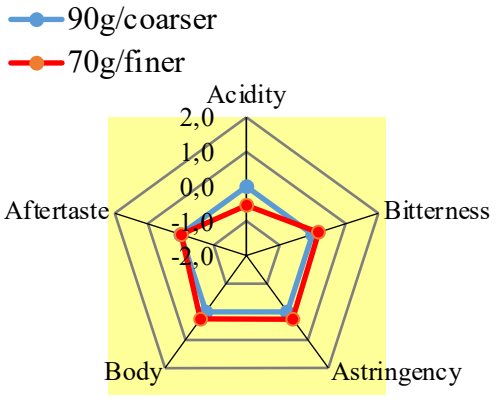

Fig.3 Example of optimization of grinding. In this case, about 15 percent cost cut was achieved without damage of taste.

Conclusion

It is difficult to raise price in Japan. Most popular way is to shift to lower grade raw materials to reduce the cost. Customers who ask us to re-design their products by the system are increasing now. The idea can be applied to any coffee product with wide price range.

The difference in the quality of specialty coffee and commercial coffee

PC416

HORIGUCHI, Toshihide* FURUSHO, Tadasu**
Dept. of Ecological Symbiotic Science,
graduate schools Tokyo University of Agriculture.
*papa@kohikobo.co.jp **tfurusho@nodai.ac.jp

<Abstract>

Specialty coffee to contribute to sustainability in the coffee industry

Currently, coffee consumption in coffee- producing countries and throughout the Asia-Pacific is beginning to exceed production. While production of Arabica has stagnated, that of Robusta is growing. The result is a mixed coffee market of specialty coffee, commercial coffee and the Robusta varietal.

The diversification of coffee has created some confusion among consumers, researchers and industry people about quality and added value. Maintaining production volume is important, but the cycle of production and consumption of specialty coffee is key to sustaining the coffee industry. Therefore, there is a need for developing metrics for determining coffee bean quality backed by scientific analysis.

We are carrying out research of specialty coffee using scientific analysis, sensory evaluation and field studies of actual conditions.

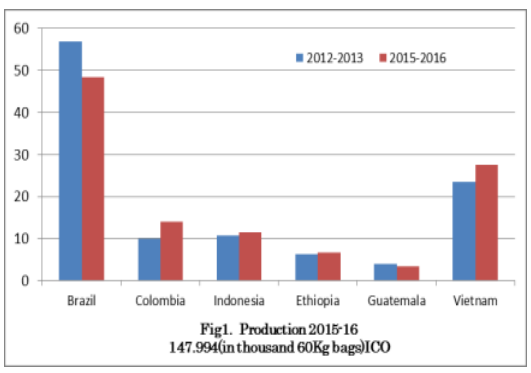

Fig1. Production 2015-16
147.994(in thousand 60Kg bags)ICO

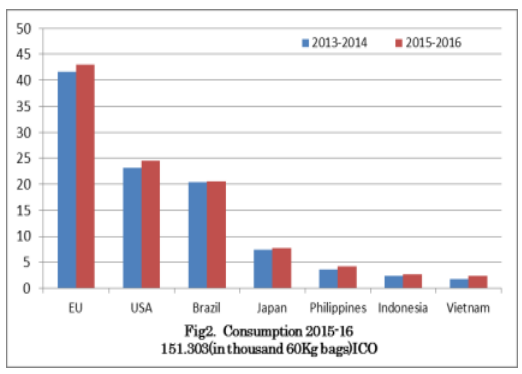

Fig2. Consumption 2015-16
151.303(in thousand 60Kg bags)ICO

<Introduction>

Coffee of various quality circulates, but it is difficult to understand the difference.

There is little production of specialty coffee with around7%, but it is important to development of the coffee industry that I inspect the quality by experimental data and a sensuality evaluation.

I checked a change of the quality in the circulation process of the green coffee beans.

Three packaging methods: vacuum packs, Grain Pro bags and hemp bags will be examined, with specialty coffee shipped in refrigerated containers .(at a constant 15-degrees Celsius). Commercial coffee is shipped in dry containers and stored at room temperature.

Table1 **Comparison of specialty coffee and commercial coffee**

Item	Specialty coffee	Commercial coffee
trace	Grasp production history	Production history is vague
volume	Low, and low distribution	Large production volume
quality	No defective beans	Includes defective beans
estate	Station small farm estates	Wide area, mixed coffee
flavor	Flavor traits by production region	Average, or mean, flavor
price	Special pricing	Linked to NY market price
indication	Kenya factories	Kenya AA

<Material>

Table2 **Four production countries of specialty and commercial coffee 2015-2016**

Production countries	Specialty coffee	Commercial coffee
Ethiopia（E）	Yirgacheffe G-1	G-4
Kenya（K）	Kirinyaga AA	AA
Guatemala（G）	Antigua estate	SHB
Colombia（C）	Huila small holder	Supremo
Robusta(R)		

Table3 **inport method of Colombia and kenya coffee 2015-2016**

coffee	SP(specialty coffee)	CO(commercial coffee)	
container	R(reefer container)	D(dry container)	
packing	VP(vacuum pack)	GP(grain pro)	H(hemp)

Table4 **Experimental method**

Experiment	Content	Method
1. pH	Acid strength	Hydrogen-ion concentration
2. Acidity	Organic acid gross volume	Titratable acidity
3. Acid value	Green bean degradation rate	Titration of free fatty acid
4. Lipid	Green bean lipid content	Chloroform methanol method
5. Organic acids	Organic acid composition	HPLC
6. Sensory evaluation	SCAA cupping form	Analytical appraisal

<Result-1>

For the flavor of each country of origin, the acid is important.

Specialty coffee is that which scores 80 points or higher in sensory assessments by the SCAA and is characterized with distinctive flavors.

Acidity is what gives flavor allowing for individual differences to be clearly classified, making its research important. “Specialty” means coffee that is stronger in acidity that of commercial coffee and contains much organic acids.

This is the meaning of high quality coffee.

Moreover coffee includes about 15% lipid which impacts flavor.

Analyzing acid values of the lipid enables a grasp of quality.

Specialty coffee has less acid value that of commercial coffee with no flavor degradation.

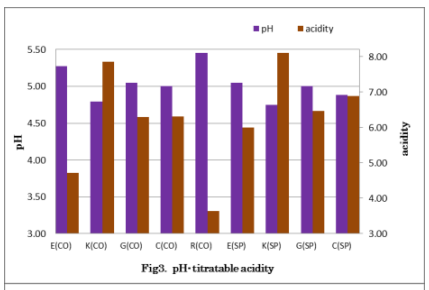

Fig3. pH･titratable acidity

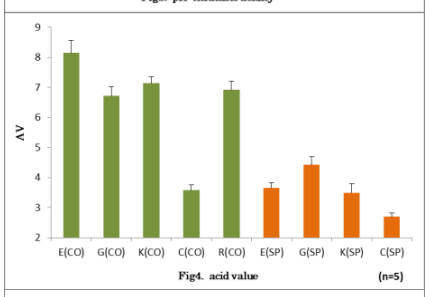

Fig4. acid value (n=5)

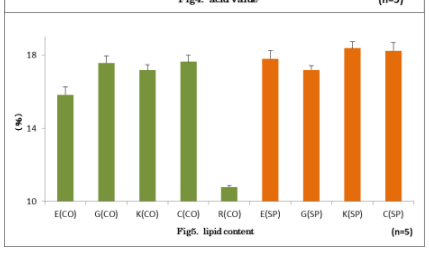

Fig5. lipid content (n=5)

<Result-2>

Freshness of green coffee beans is important to the flavor of the coffee

Data of July, 2016

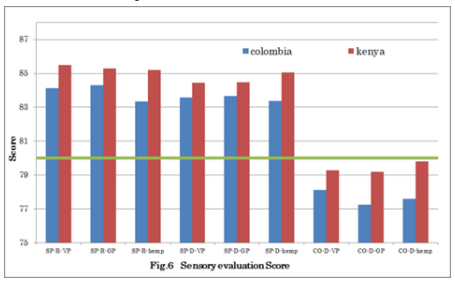

Fig.6 Sensory evaluation Score

Sensory evaluation
Specialty coffee is high evaluation

An evaluation is globally high in the Kenya coffee.

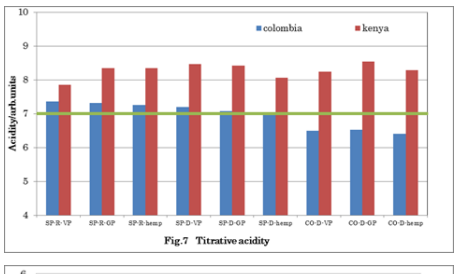

Fig.7 Titrative acidity

Titratable acidity
Specialty coffee is characterized by acid.

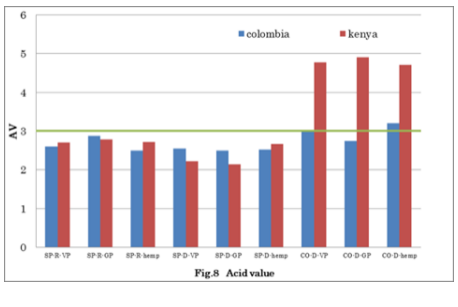

Fig.8 Acid value

Acid value
The lipid deterioration greatly influences flavor.
Specialty coffee has less degradation of the lipid .

<conclusion>

Maintaining green coffee bean quality requires effective packaging, shipping and storage methods, all of which are currently in the process of being studied and emphasized as part of the process of sustaining flavor and useful in the development of specialty coffee.

Impact of roasting degree on physical and chemical properties of coffee beans

PC420

XU, S, Linforth, RL and Fisk, ID
Food Science, Biosciences, University of Nottingham, UK

Abstract

Coffee beans (*coffea arabica*) which underwent different roasting preparations (green, light roasting (4 mins), medium roasting (4.5 mins) and dark roasting (5 mins)) were prepared. Roasting loss, moisture content, water activity and colour were determined. The total protein content of the coffee beans was determined using BCA protein assay kits and mineral content was determined using an inductively coupled plasma mass spectrometry (ICP-MS). Fatty acid and volatile compound content were determined using a gas chromatography-mass spectrometry (GC/MS). Sugar content was measured using high performance liquid chromatography-mass spectrometry (HPLC/MS). Analysis of the roasting of the coffee beans revealed that as temperature increased roasting loss significantly increased ($P<0.05$). Increasing temperature was characterized by significantly darker coffee beans which had a significantly reduced moisture content and water activity. Analysis of roasting revealed that total protein content was significantly reduced as temperature increased due to maillard reactions and degradation of protein. Analysis of sugars content revealed that sugar content in roasted coffee beans were significantly lower than it in green coffee beans due to maillard reactions and caramelisations. Analysis of fatty acids revealed that there was no significant differences in fatty acid content under different roasting conditions. There was no significant difference in mineral content between coffee beans which had undergone different roasting methods. Forty-five volatile compounds were detected and the concentrations of these compounds increased as temperature for roasting increased ($P<0.05$).

Introduction

Coffee roasting plays an essential role in the formation of organoleptic properties including flavour, aroma and colour (Hernandez, 2007). During the roasting process, moisture content decreases and many chemical reactions occur, along with crucial variations in terms of flavour, colour, volume, weight, bean pop, pH, density and presence of volatile compounds (Bottazzi, 2012). The roasting process can be divided into two main steps: drying and roasting. In the first stage, bean temperature is below 160° C and the water content descends from around 8-12% downwards (Gloess, 2014). In the second stage, pyrolytic reactions occur at 190° C leading to oxidation, reduction, hydrolysis, polymerisation, decarboxylation and many other chemical reactions, which causes the formation of coffee flavour, aroma and colour (Singh, Singh, Bhamidipati, Singh and Barone, 1997). In addition, roasting also generates CO_2, some CO_2 escapes and some is kept in the cells of the beans (Schwartzberg, 2002). After the second step, the beans have to be quickly cooled to stop the reactions, either by water or air and to avoid an excessive roast, which is perceived to damage product quality (Gloess, 2014).

During the roasting progress, a large number of volatile compounds are produced due to Maillard reactions, Strecker degradation, pyrolysis, and other chemical reactions (Schenker et al., 2002). More than 800 different volatile compounds from a wide range of chemical classes (including hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids, esters, pyrazines, pyrroles, pyridines, sulphur compounds, furanes, phenols and so on) have been identified in roasted coffee (Nijssen et al, 1996). In recent years, research has highlighted the sensory relevance of these volatile compounds and has identified the key odourants in coffee beans and brewed coffee beverages (Parliment, 1994; Czerny et al., 1999).

In order to obtain a cup of good quality coffee, the roasting process plays a very important role in generating flavour, aroma and colour. Nevertheless, it is very difficult to understand all reaction mechanisms, as the coffee beans contains many chemical substances and a large number of physical and chemical reactions occur during the roasting process. Therefore, for understanding and exploring these reaction mechanisms and further guiding coffee production, the aim of this study was to investigate the effect of roasting degrees on roasting loss, moisture content, colour, sugar, protein, fatty acid, mineral content and volatile compounds in coffee beans.

Table 1 : Roasting loss, water activity, moisture content and *L*, *a*, *b* Hunter values of ground coffee beans as a function of the roasting time

Roasting time (min)	Roasting degree	Roasting loss (g/kg)	Water activity	Moisture content (g/kg)	L Value	a Value	b Value
0	Green	0^a	0.65 ± 0.01^a	136 ± 1^a	67 ± 0^a	1.1 ± 0.2^a	14.2 ± 0.2^a
4	Light	129 ± 10^b	0.29 ± 0.02^b	44 ± 3^b	43 ± 1^b	8.2 ± 0.2^b	10.6 ± 0.5^b
4.5	Medium	161 ± 4^c	0.23 ± 0.01^c	23 ± 1^c	37 ± 1^c	5.9 ± 0.2^c	6.5 ± 0.8^c
5	Dark	206 ± 11^d	0.11 ± 0.01^d	18 ± 2^d	32 ± 1^d	2.6 ± 0.3^d	1.9 ± 0.5^d

An increase in degrees of roasting (from green to dark roasting) correlated with a significant increase in roasting loss ($P<0.05$) however, water activity and moisture content decreased significantly ($P<0.05$).

In terms of coffee colour, as degrees of roasting increased, *L* value significantly declined ($P<0.05$), a decline in the *L* value is an indicator that the colour of coffee bean becomes progressively darker under higher roasting.

Degree of greenness can be assessed where a negative *a* value denotes a green colour and positive *a* values denotes a red colour. Assessment of colour revealed that green beans had the highest degree of greenness characterised by having the lowest *a* value ($P<0.05$), while the light roasting beans had the highest degree of redness having the highest *a* value ($P<0.05$). Both medium and dark roasting beans had a lower ($P<0.05$) degree of redness than light roasting beans. Degrees of blue can be denoted with negative *b* values and degrees of yellow with a positive *b* values, analysis of blue/yellow colour formation revealed that as roasting degree increased the *b* value significantly decreased. Thus, coffee green beans had the highest degree of yellowness ($P<0.05$), while dark roasting coffee beans had lowest degree of yellowness ($P<0.05$). In addition, both light and medium roasting coffee beans had lower degrees of yellowness than green coffee beans ($P<0.05$).

Table 2 : Fatty acids content (mg/kg) by dry weight of coffee beans under different roasting degrees

Fatty acids	Roasting degree			
	Green	Light roasting	Medium roasting	Dark roasting
Palmitic	11354 ± 1084^a	11467 ± 488^a	12171 ± 659^a	13183 ± 851^a
Stearic	86 ± 12^a	93 ± 4^a	98 ± 8^a	108 ± 10^a
Linoleic	198 ± 22^a	209 ± 14^a	224 ± 19^a	236 ± 15^a
Linolenic	2.3 ± 0.3^a	2.5 ± 0.1^a	2.6 ± 0.2^a	2.9 ± 0.3^a
Arachidic	32 ± 3^a	36 ± 3^a	36 ± 3^a	39 ± 2^a
Cosenoic	78 ± 8^a	78 ± 8^a	87 ± 7^a	95 ± 7^a
Heneicosanoic acid	0.46 ± 0.04^a	0.48 ± 0.06^a	0.51 ± 0.05^a	0.57 ± 0.05^a
Behenic acid	190 ± 17^a	186 ± 14^a	189 ± 18^a	192 ± 13^a
Ligoceric	2.1 ± 0.2^a	2.1 ± 0.2^a	2.3 ± 0.2^a	2.5 ± 0.2^a

Results revealed that there was a large concentration of palmitic, stearic, linoleic, arachidic, cosenoic and behenic acid detected in the four varieties of coffee beans, whilst only small amount of linolenic, heneicosanoic acid and ligoceric were found. In terms of all fatty acids content there were no significant differences ($P<0.05$) detected between coffee beans regardless of roasting degrees.

Table 3 : Mineral content (mg/kg) by dry weight of coffee beans under different roasting degrees

Minerals	Roasting degree			
	Green	Light roasting	Medium roasting	Dark roasting
K	17593 ± 856^a	18083 ± 647^a	17974 ± 276^a	18100 ± 216^a
Mg	2267 ± 99^a	2299 ± 67^a	2318 ± 30^a	2348 ± 29^a
S	1701 ± 118^a	1707 ± 37^a	1709 ± 36^a	1639 ± 47^a
P	1558 ± 21^a	1559 ± 50^a	1518 ± 45^a	1570 ± 50^a
Ca	1501 ± 47^a	1606 ± 75^a	1592 ± 81^a	1573 ± 104^a
Fe	35 ± 2^a	40 ± 4^a	40 ± 4^a	40 ± 3^a
Mn	35 ± 2^a	33 ± 1^a	35 ± 2^a	34 ± 2^a
Sr	25 ± 1^a	27 ± 6^a	29 ± 5^a	28 ± 2^a
Cu	23 ± 1^a	23 ± 1^a	23 ± 4^a	24 ± 2^a
Ba	10 ± 0^a	12 ± 1^a	12 ± 1^a	11 ± 1^a
Zn	9 ± 1^a	9 ± 1^a	9 ± 1^a	10 ± 1^a
B	9 ± 0^a	9 ± 1^a	9 ± 0^a	9 ± 0^a
Na	8 ± 0^a	8 ± 0^a	9 ± 0^a	8 ± 1^a
Al	2 ± 0^a	2 ± 1^a	2 ± 0^a	2 ± 0^a

Twenty-eight minerals were found in different degrees of coffee bean roast; however, only minerals present at above trace concentrations (> mg/kg) in coffee beans were presented. For all minerals found in this study, no significant differences ($P<0.05$) were detected in mineral content between coffee beans which had undergone different degrees of roasting.

Table 4 : The concentration of volatile compounds (mg/Kg) by dry weight in the headspace of roasted coffee beans

Class of compounds	Volatile compounds	Roasting degree		
		Light roasting	Medium roasting	Dark roasting
Alcohols	3-Methyl-3-buten-1-ol	0.09 ± 0.01^a	0.11 ± 0.01^b	0.13 ± 0.01^b
Aldehydes	3-Methylbutanal	2.3 ± 0.1^a	2.3 ± 0.1^b	4.1 ± 0.5^b
	Hexanal	0.29 ± 0.01^a	0.79 ± 0.04^b	1.23 ± 0.19^c
	Acetaldehyde	0.24 ± 0.02^a	0.25 ± 0.01^a	0.53 ± 0.09^b
Ketones	Hydroxyacetone	4.5 ± 0.7^a	6.6 ± 0.5^b	7.9 ± 0.6^c
	Acetylpropionyl	2.0 ± 0.3^a	3.3 ± 0.4^a	2.9 ± 0.4^b
	Diacetyl	1.6 ± 0.2^a	1.5 ± 0.1^a	1.6 ± 0.3^a
	Acetoin	1.1 ± 0.2^a	1.3 ± 0.1^a	1.4 ± 0.2^a
	1-Hydroxy-2-butanone	0.8 ± 0.1^a	1.1 ± 0.1^a	0.9 ± 0.1^a
	2,3-Hexanedione	0.11 ± 0.02^a	0.17 ± 0.01^b	0.35 ± 0.04^c
	1-(Acetyloxy)-2-butanone	0.06 ± 0.01^a	0.11 ± 0.01^b	0.15 ± 0.01^c
	3-Ethyl-2-hydroxy-2-cyclopenten-1-one	0.009 ± 0.001^a	0.014 ± 0.001^b	0.036 ± 0.001^c
	3,4-Hexanedione	0.006 ± 0.001^a	0.009 ± 0.001^b	0.009 ± 0.001^b
Carboxylic acids	Acetic acid	12.8 ± 1.7^a	12.6 ± 0.7^a	10.2 ± 1.3^a
	3-Methylbutanoic acid	1.6 ± 0.3^a	1.5 ± 0.2^a	1.4 ± 0.2^a
	4-Hydroxybutanoic acid	0.7 ± 0.1^a	1.0 ± 0.1^b	2.1 ± 0.3^c
	Propionic acid	0.6 ± 0.1^a	0.9 ± 0.0^b	1.0 ± 0.1^b
Pyrazines	Methylpyrazine	6.9 ± 0.9^a	7.5 ± 0.6^a	6.0 ± 0.8^a
	2,6-Dimethylpyrazine	1.6 ± 0.2^a	1.5 ± 0.2^a	1.4 ± 0.2^a
	2,5-Dimethylpyrazine	1.3 ± 0.1^a	1.2 ± 0.1^a	1.0 ± 0.2^a
	Pyrazine	1.0 ± 0.1^a	1.0 ± 0.1^a	1.2 ± 0.1^a
	Ethylpyrazine	0.34 ± 0.04^a	0.35 ± 0.03^a	0.32 ± 0.04^a
	3-Ethyl-3,5-dimethylpyrazine	0.20 ± 0.03^a	0.22 ± 0.01^a	0.34 ± 0.01^b
	2,3-Dimethylpyrazine	0.19 ± 0.03^a	0.18 ± 0.01^a	0.20 ± 0.01^a
	2-Ethyl-6-methylpyrazine	0.18 ± 0.02^a	0.17 ± 0.02^a	0.17 ± 0.02^a
	2-Ethyl-3-methylpyrazine	0.06 ± 0.01^a	0.05 ± 0.01^a	0.06 ± 0.00^a
Pyrroles	Pyrrole	0.12 ± 0.02^a	0.13 ± 0.02^a	0.26 ± 0.02^b
	1-Methylpyrrole	0.06 ± 0.01^a	0.08 ± 0.01^a	0.17 ± 0.02^b
	2-Formyl-1-methylpyrrole	0.03 ± 0.01^a	0.06 ± 0.01^b	0.10 ± 0.01^c
	2-Acetylpyrrole	0.022 ± 0.004^a	0.038 ± 0.002^b	0.075 ± 0.002^c
	2-Carboxaldehydepyrrole	0.026 ± 0.003^a	0.050 ± 0.002^b	0.062 ± 0.007^c
Pyridines	Pyridine	1.0 ± 0.2^a	2.2 ± 0.4^b	5.8 ± 0.3^c
	N-Acetyl-4H-pyridine	0.12 ± 0.02^a	0.16 ± 0.02^b	0.22 ± 0.02^c
Sulphur compounds	Dimethyl disulfide	0.11 ± 0.01^a	0.10 ± 0.01^a	0.13 ± 0.02^a
Furanes	2-Methanolfuran	8.6 ± 1.5^a	11.7 ± 1.0^b	16.1 ± 1.0^c
	Furfural	3.3 ± 0.4^a	4.7 ± 0.9^b	6.7 ± 0.6^c
	Furfuryl acetate	0.27 ± 0.04^a	0.92 ± 0.12^b	2.11 ± 0.05^c
	5-Methyl-2-furfural	0.86 ± 0.14^a	1.88 ± 0.06^b	1.91 ± 0.22^b
	3-Methylfuran	0.18 ± 0.02^a	0.24 ± 0.01^b	0.67 ± 0.09^c
	Acetylfuran	0.32 ± 0.06^a	0.52 ± 0.04^b	0.54 ± 0.06^b
	Furfuryl formate	0.11 ± 0.04^a	0.24 ± 0.03^b	0.42 ± 0.02^c
	2(5H)-Furanone	0.06 ± 0.01^a	0.09 ± 0.01^b	0.09 ± 0.00^b
	5-Methyl-2-furanmethanol	0.030 ± 0.003^a	0.029 ± 0.002^a	0.032 ± 0.002^a
Phenols	Guaiacol	0.011 ± 0.002^a	0.026 ± 0.003^b	0.090 ± 0.005^c
	Phenol	0.008 ± 0.001^a	0.022 ± 0.001^b	0.065 ± 0.005^c

Forty-five volatile compounds were detected in coffee beans with different roasting degrees. As roasting degree increased, the concentration of aroma compounds increased. During the coffee roasting, many complex chemical reactions occur, which leads to the formation of coffee flavour (Clark and Vitzthum, 2001). Furthermore, many researchers have proved that Maillard reaction and the degradation of sugar are two main chemical reactions to form volatile compounds (Aeschbacher *et al.*, 1989, Weenen and Apeldoorn, 1996, Schieberle, 1992).

The increasing of roasting degree resulted in a longer roasting time, higher roasting temperature and lower moisture content, which enhanced Maillard and pyrolysis reactions and therefore promoted the formation of volatile compounds.

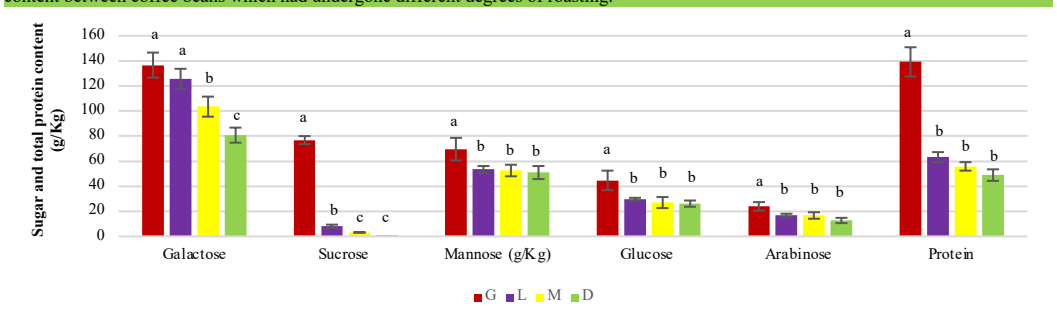

Figure 1:The sugar and total protein content (g/kg) by dry weight of coffee beans under different roasting degrees G, L M and D represent green beans, light roast beans, medium roast beans and dark roast beans

The sugar and total protein content in roasted coffee beans were significantly lower ($P<0.05$) than them in green coffee beans. During the roasting process, three main chemical reactions (Maillard, Caramelisation and Pyrolytic) are related to protein and sugar content in the coffee beans (Montavon et al., 2003). Maillard reactions are chemical reactions between amino acid and reducing sugars which leads to colour and flavour formation. It is also called non-enzymatic browning. The speed of Maillard reactions increases as temperature increases and high moisture content has been shown to be beneficial in terms of reaction occurrence. When temperatures are higher, caramelisation and subsequently pyrolysis have been shown to become more pronounced (Parliment, 1994).

Conclusion

To conclude, as degree of coffee roasting increased (green, light roasting, medium roasting and dark roasting), the roasting loss of coffee beans increased significantly ($P<0.05$) due to a loss of moisture and CO_2 release, while the moisture content and water activity decreased significantly ($P<0.05$). Moreover, the colour of coffee beans became significantly darker ($P<0.05$) due to Maillard reactions and caramelisation.

Nine different fatty acids were detected: palmitic (C16:0), stearic (C18:0), linoleic (C18:2), linolenic (C18:3), arachidic (C20:0), cosenoic (C20:1), heneicosanoic acid (C21:0), behenic acid (C22:0) and ligoceric (C24:0). In terms of all fatty acids content there were no significant differences ($P<0.05$) detected between coffee beans regardless of roasting degrees.

Twenty-eight minerals were found in different degrees of roasted coffee beans and no significant differences ($P<0.05$) were detected.

Forty-five volatile compounds were detected in roasted coffee beans. The amounts of most aroma compounds increased with an increase in degree of roasting ($P<0.05$).

In terms of individual sugars and total protein, their content in roasted coffee beans was significantly lower ($P<0.05$) than them in green coffee beans due to Maillard reactions, caramelisation and pyrolytic.

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PC422

Determination of Cr, Cd, Pb, As in coffee powder by an optimized and validated ICP-MS method with microwave digestion

Qin Qiao, Feifei He, Xian-zhi Wang, Shu-mei Hao
School of Agriculture, Yunnan University, Kunming, Yunnan, China

Abstract

In this study, we use microwave digestion and ICP-MS technology to develop an optimized and validated metal detection method in coffee. The results suggesting this method have good reproducibility and operability.

Introduction

Coffee is one of the most widely consumed beverages in the world. Recently years following the contamination of environment by industry, heavy metals widely dispersed in varying concentrations in human foods, including coffee. It is necessary to develop an optimized and validated metal detection method in coffee.

Table 1 : different microwave digestion process in green tea

Code	Time（min）	Temperature (ºC)
1	10+19+15+15+1	150+180+210+120+75
2	10+19+15+15+1	165+190+220+90+75
3	10+10+18+15+1	175+205+75+75+75
4	10+10+18+15+1	180+210+75+75+75

Table 2 : Analytical results of green tea under different process

Elements	Microwave digestion process	Detection in green tea ($\mu g\ g^{-1}$)	RSD (%)	Standard value ($\mu g\ g^{-1}$)
Cr	1	0.840	2.61	0.920
	2	0.870	7.22	
	3	1.046	6.00	
	4	0.833	5.91	
Cd	1	0.064	7.73	0.076
	2	0.063	2.94	
	3	0.072	3.67	
	4	0.066	3.38	
Pb	1	2.403	19.3	1.600
	2	1.695	7.05	
	3	1.761	3.45	
	4	1.578	2.81	
As	1	0.296	10.3	0.270
	2	0.339	17.5	
	3	0.372	15.5	
	4	0.323	9.62	

Table 3 : Analytical results of samples and recovery of standard addition and precision

Elements	Samples ($\mu g \cdot g^{-1}$)	RSD (%)	Adding standard ($\mu g \cdot g^{-1}$)	After adding standard ($\mu g \cdot g^{-1}$)	RSD (%)	Recovery rate(%)
Cr	0.484	5.33	2.000	2.146	5.02	83.1
Cd	0.023	2.42	2.000	1.716	4.35	84.6
Pb	0.262	4.77	2.000	2.132	2.17	93.5
As	0.060	6.43	2.000	2.057	2.58	99.9

Main Text

We using microwave digestion combined with ICP/MS to investigate the concentrations of four elements: chrome (Cr), cadmium (Cd), lead (Pb), arsenic (As) in coffee powder produced in Yunnan province. The concentrations of these elements in GBW10052 standard green tea were tested firstly in different processing to optimize the microwave digestion process. Then the four metal contents in the coffee sample were investigated.

Conclusion

The results shows that concentration of Cr is highest and that of Cd is lowest in Coffee producing in Yunnan. The recovery of four elements was ranged from 83% to 99%, and relative standard deviation was 2.42%-8.36%.All these results suggesting this method have good reproducibility and operability.

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Influence of pretreatment methods for the determiantion of Chlorogrenic acid content in green coffee bean by HPLC

PC423

DONG Xiang-shu, HE Fei-fei, WANG Xian-Zhi, HAO Shu-mei
School of Agriculture, Yunnan University, Kunming, 650091, China

Abstract
Green coffee beans were previously frozen by liquid nitrogen in order to minimize CGA degradation and then pre-treated with different extraction solution. The resulting suggesting that 60% ethanol was the optimal pretreatment solution for determination of CGA concentration.

Introduction
Chlorogenic acids (CGA) are interesting natural antioxidants widespread in the plant kingdom. Green coffee beans contain the largest amount of CGA found in plant. In this study, we tried to find out an optimal pre-treatment extraction solution for the determination of CGA content in coffee bean by HPLC.

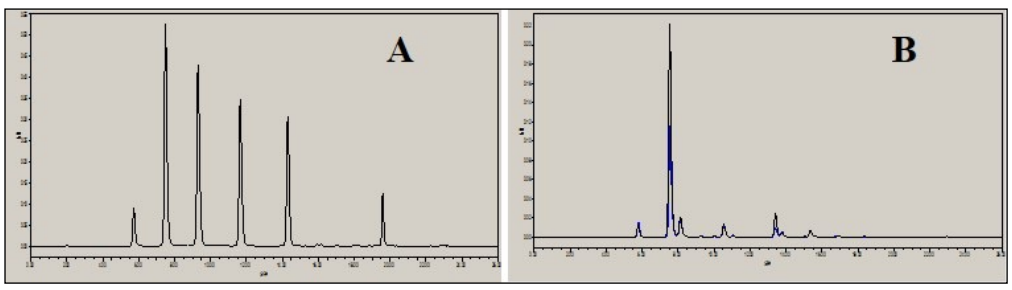

Figure 1: HPLC chromatograms of reference substances (A), sample (B). Green curve indicates sample without liquid nitrogen treatment.

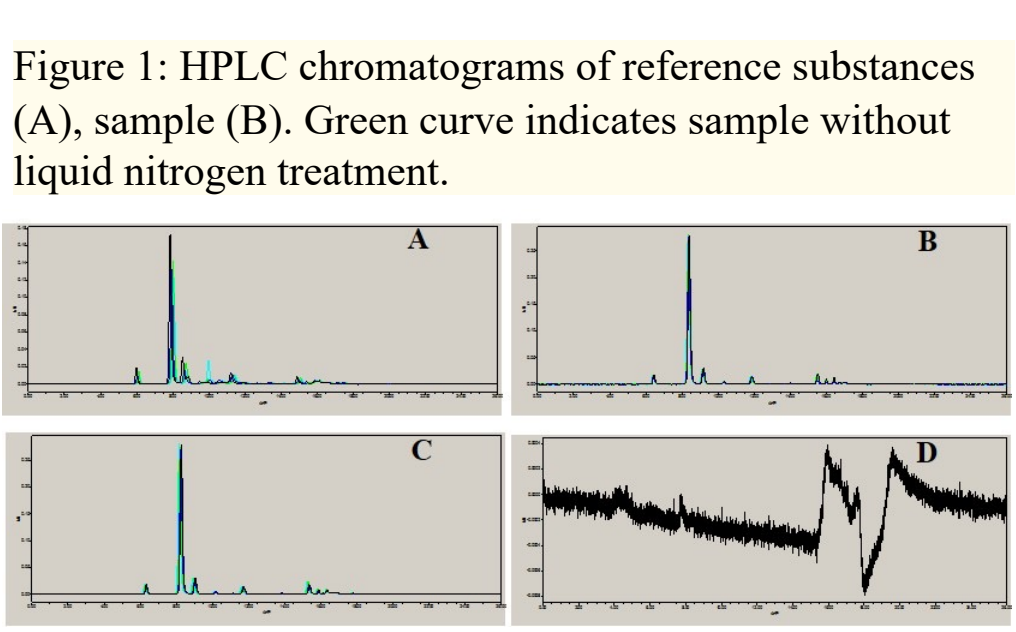

Figure 2: HPLC chromatograms of samples pre-treated with different extraction solution. A, sample pre-treated with distilled water; B, sample pre-treated with 60% ethanol; C, sample pre-treated with 40% methanol. Curves with different color indicate biological replicates. D, example of control check.

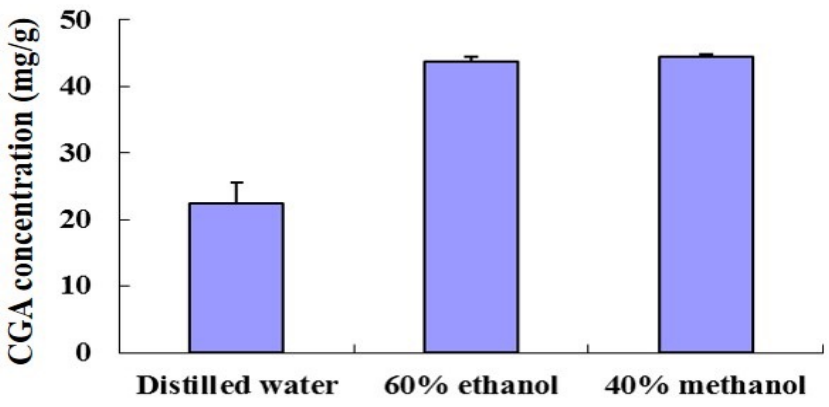

Figure 3: Comparsions of extraction efficiency among distilled water, 60% ethanol and 40% methanol.

Main Text
Green coffee beans were previously frozen by liquid nitrogen in order to minimize CGA degradation. The lyophilized coffee beans were ground with a coffee grinder to pass a 0.5 mm sieve. 0.5 g power was ultrasonic pre-treated with 25 mL extraction solution (60% ethanol, 40% methanol, or distilled water) for 30 min at 80oC under dark condition. The final CGA concentration was determined by HPLC.

Conclusion
The results indicated that 1) pre-treatment with 60% ethanol or 40% methanol had higher accuracy and lower relative error than distilled water; 2) 60% ethanol and 40% methanol pretreatment had similar extraction efficiency and showed no significant yield loss.
Considering the extraction security, 60% ethanol was the optimal pretreatment solution for determination of CGA concentration from green coffee beans using HPLC.

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PC424

Influence of different pretreatment methods for the determination of caffeine content in coffee by HPLC

Xianzhi Wang, Fei-fei He, Shu-mei Hao
School of Agriculture, Yunnan University, Kunming, Yunnan, China

Abstract

Five pretreatments were used to analysis the influence of different extraction methods for the determination of caffeine content in coffee by High Performance Liquid Chromatography (HPLC). The results indicated that 45 minutes of ultrasonic pretreatment could be used as the reference method for the determination of caffeine in coffee by HPLC.

Introduction

Caffeine is a kind of alkaloid compounds which generally determines the quality of coffee products. The accurate and rapid measuring of caffeine content is very important for the development of coffee industry. Currently, HPLC is the most widely used method for the determination of caffeine content in coffee. Traditional pretreatment methods for HPLC involves many steps and toxic chemicals, but with low yield and accuracy. The objective of this study was to set up an accurate, fast and safe pretreatment method for the determination of caffeine content in coffee by HPLC.

Table 1 : Comparison of different pretreatment methods for the extraction of caffeine in coffee by HPLC

Pretreatment methods	Caffeine content (mg/L)	RSD (%)	Relative error (%)
1, Water extraciton (GB 5009.139-2014）	3.97	3.03	2.367827
2, Ethanol extraction	-	-	-
3, Chloroform extraction	4.21	6.92	7.918459
4, Ultrasonic extraction	3.98	1.38	1.105141
5, Microwave extraction	-	-	-

Note: Method 2 did not obtain results and method 5 got extremely low extraction rate, the results were not listed in the table.

Table 2 : Detection of caffeine contents under different processing time by ultrasonic extraction method

Pretreatment methods	Caffeine contents (mg/L)	RSD (%)
A（Control, GB 5009.139-2014）	5.98b	1.32
B（Ultrasonic extraction for 30min）	5.60a	3.13
C（Ultrasonic extraction for 45min）	5.79ab	3.63
D（Ultrasonic extraction for 60min）	5.77ab	1.79

Note: Different lowercases in the same row indicated significant differences at the 0.05 level.

Table 3 : Comparison of recovery rates of different pretreatment methods

Pretreatment methods	sample content (mg/L)	Added value (mg/L)	Measured value (mg/L)	Recovery rate (%)
A（Control, GB 5009.139-2014）	5.98	5	10.3	86.55
B（Ultrasonic extraction for 30min）	5.6	5	10.31	94.08
C（Ultrasonic extraction for 45min）	5.79	5	10.38	91.76
D（Ultrasonic extraction for 60min）	5.77	5	10.76	99.66

Main Text

Five pretreatment methods (water pretreatment, ethanol pretreatment, chloroform pretreatment, ultrasonic pretreatment and microwave pretreatment) were used to analysis the influence of different extraction methods for the determination of caffeine content in coffee by HPLC.

The results indicated that ultrasonic pretreatment showed higher accuracy and lower relative error, compared with the water pretreatment method, which is the national standard for the determination of caffeine content by HPLC (Table 1 and Table 2).

The optimal processing time of ultrasonic pretreatment was also investigated. The results indicated that there was no significant yield loss between forty-five minutes of ultrasonic pretreatment method and the water pretreatment method (Table 3).

Conclusion

Considering the extraction efficiency and time, we recommend 45 minutes of ultrasonic pretreatment as the optimal pretreatment method. This method showed the advantage of less operation time and steps, but higher efficiency and accuracy, and thus could be used as the reference method for the determination of caffeine in coffee by HPLC.

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云南省高原特色农业产业研究院

PC426

The relevance of non-enzymatic transglycosylation reactions for coffee melanoidins formation

MOREIRA, Ana S.P., PASSOS, Cláudia P.*, SIMÕES, Joana*, MACIEL, Elisabete*,§, NUNES, Fernando M.**, DOMINGUES, Pedro*, DOMINGUES, M. Rosário*, COIMBRA, Manuel A.*,†*

**QOPNA, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; †mac@ua.pt*
***UTAD, Department of Chemistry, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal*
§CESAM, Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal

Abstract

Polysaccharides polymerize under roasting conditions, forming new polymers through non-enzymatic transglycosylation reactions (TGRs). TGRs can also occur between carbohydrates and aglycones such as chlorogenic acids present in coffee. Because proteins are very reactive with reducing sugars, it is possible that they play a regulatory role in TGRs extension and, consequently, modulating the composition of melanoidins, defined as end products of Maillard reaction. To investigate this hypothesis, solid state mixtures mimicking coffee beans composition were roasted and analyzed. The results show that the occurrence of TGRs catalyzed by the acids and the inhibition of their extension by amino groups are key steps for the formation of the different melanoidin populations.

Introduction

During the roasting of coffee beans, browning is the most visible physico-chemical change occurring as a result of the formation of melanoidins, the high molecular weight heterogeneous polymers that are, by definition, formed in the late stages of the Maillard reaction (MR) between carbohydrates and proteins. However, phenolic compounds have been found as components of melanoidins [1]. Despite their structures are far from being disclosed, they are known to have structural features that are related to coffee health benefits [2]. Roasting promotes the depolymerization of polysaccharides, although non-enzymatic transglycosylation reactions (TGRs) can also occur. As a consequence, non-hybrid and hybrid polysaccharide structures composed by galactomannans and arabinogalactans can be formed [3], and they can also be linked to chlorogenic acids [4].

Because proteins are very reactive with reducing sugars through MR, it is possible that their presence plays a regulatory role concerning TGRs extension, modulating the composition of melanoidins. To investigate this hypothesis, solid state mixtures containing models of coffee beans components (Figure 1) were roasted and analyzed (Scheme 1).

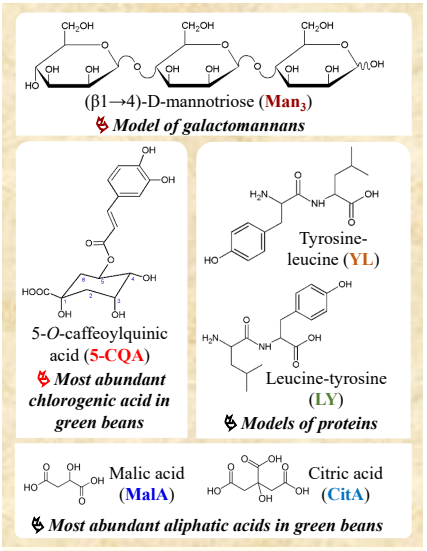


Figure 1: Commercial standards used as models of green coffee bean components.

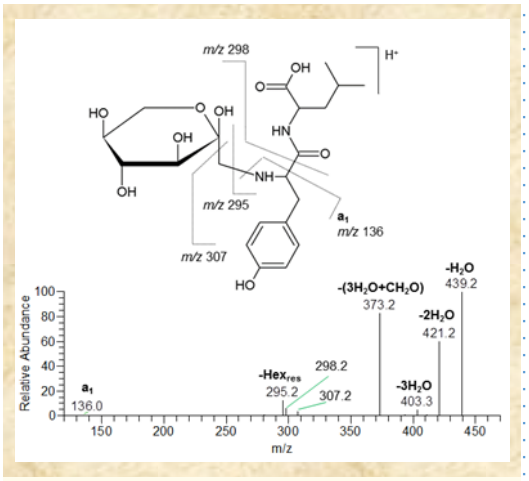
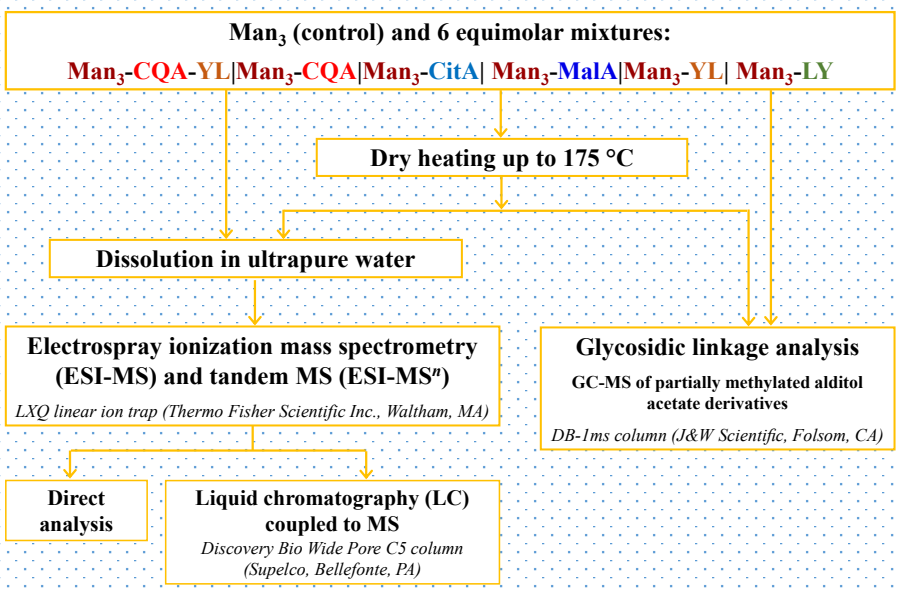


Figure 2: LC-MS^2 spectrum of $[M+H]^+$ ions of Amadori compound HexYL (*m/z* 457), acquired from the roasted Man_3-CQA-YL mixture at retention times 6.70 min.

Table 1: Glycosidic linkage composition (% area) of unroasted (T0) and roasted (T1) samples of Man_3 and mixtures.

Linkage	Man_3		Man_3-CQA-YL		Man_3-CQA		Man_3-MalA		Man_3-CitA		Man_3-YL		Man_3-LY	
	T0	T1	T0	T1	T0	T1	T0	T1	T0	T1	T0	T1	T0	T1
T-Man*p*	42.1	43.6	43.2	60.5	40.3	42.5	39.2	41.8	41.3	44.5	42.1	60.4	42.8	62.2
2-Man*p*				1.0		2.9		3.0		3.1		0.7		1.0
4-Man*p*	52.7	51.4	52.2	23.1	52.7	19.5	57.3	24.8	54.9	10.3	53.3	27.8	53.2	26.3
6-Man*p*				3.7	0.7	14.1		12.6		18.9		3.2		2.8
3,4-Man*p*		0.1		0.5	0.3	1.1		0.8		0.4		0.3		0.4
2,3-Man*p*						0.8		0.2		0.4				
2,4-Man*p*		0.2		0.9	0.3	1.0		1.4		1.1		0.9		0.6
4,6-Man*p*	1.6	1.7	1.5	2.8	2.0	6.2	0.6	7.5	1.0	7.1	1.7	1.7	1.2	1.3
2,6-Man*p*						2.6		0.3		2.4		0.6		0.6
3,6-Man*p*						0.8		0.7		2.2				
3,4,6-Man*p*						0.7		0.4		0.7				
2,3,6/2,4,6-Man*p*						2.3		0.4		1.5				
2,3,4,6-Man*p*						0.9		0.1		0.3				
T-Gal*p*	2.1	2.2	2.1	4.1	2.6	1.2	1.8	1.1	1.9	0.8	2.1	3.0	2.0	2.5
6-Gal*p*						1.8		1.7		2.8				
T-Glc*p*	0.9	0.2	0.2	0.2	0.4	0.6	0.5		0.4		0.2	0.2	0.2	0.3
4-Glc*p*	0.7	0.7	0.8	3.1	0.7	1.0	0.5	3.2	0.5	3.6	0.6	1.2	0.4	2.0



Scheme 1: Overview of the experimental workflow.

• Roasting of **mixtures containing a peptide** (Man_3-CQA-YL; Man_3-YL; Man_3-LY) led to a marked ↑ **of T-Man*p*** (Table 1). According with LC-MS, this is due to:

- **DEPOLYMERIZATION**, yielding Hex and Hex_2, either free or linked to the peptide
- Formation of **AMADORI COMPOUNDS** (Figure 2).

• Roasting of **Man_3 in mixture with an acid** (Man_3-CQA; Man_3-CitA; Man_3-MalA) led to a **higher number of new glycosidic linkages** compared with the mixtures containing a peptide (Table 1).

Hydroxy acids act as catalyst as well as reagent for TGRs and peptides suppresses this reaction.

Conclusion

- The dry environments at high temperatures, as those found when coffee is roasted, were able to promote new acetal and ketal groups between carbohydrates, phenolic compounds, organic acids, and amino acids.
- These reactions occur competitively, leading to the formation of highly diverse melanoidins. Their structures and bioactivity are defined by the composition of the matrices where they are formed, modulated by the occurrence of TGRs and the inhibition of their extension by MR.

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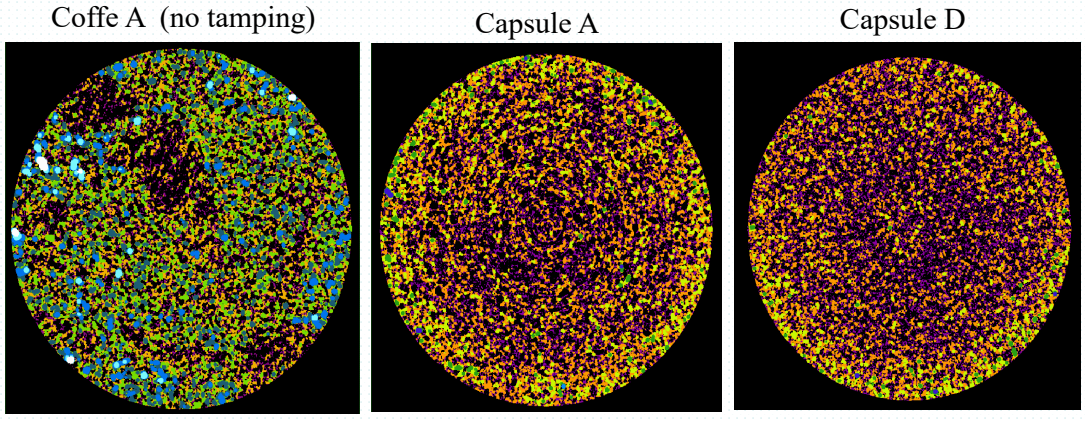
Espresso coffee from the single-dose capsule system. A comparison among different brands based on macroscopic and microscopic properties.

PC431

Derossi*, A., Severini, C., Ricci, I., Fiore, A., G., Caporizzi, R
Department of Science and Agriculture, Food and Environment, University of Foggia, Italy.
*email: antonio.derossi@unifg.it

Abstract

The single-dose capsule systems from different brands were compared in terms of physical, chemical and microstructure properties. Each brand uses coffee with very different characteristic. The microstructure inside coffee cake, particularly the pathway of water could affect extraction.



Introduction

The use of single-dose capsule systems to prepare espresso coffee (EC) is exponential increasing. Its quality is affected by chemical and physical properties (Illy, 2005; Severini et al., 2015; Severini et al., 2016). In the last 5 years the importance of food microstructure have been recognized and some papers analyzed the relationship between it and the quality of food. However, any experiment on the microstructure of coffee cake was performed in details.

Capsule (dose, g)	Porosity fraction of coffe cake (%)	Solid content (g d.w./mL)	Acidity (mL NaOH/mL)	Caffeine content (mg/mL)	Antioxidant (TEAC mg Trolox/mL)	Phenols content (mg GAE/mL)
A (7.56±0.04)	56.90±1.5	0.064±0.005	0.18±0.012	3.73±0.160	6.00±1.30	30.04±4.37
B (5.99±0.11)	55.48±1.2	0.063±0.001	0.13±0.016	3.65±0.040	7.54±0.51	23.11±1.31
C (5.53±0.11)	53.98±2.1	0.048±0.003	0.14±0.002	3.16±0.006	10.82±0.93	17.65±0.85
D (6.50±0.05)	56.20±2.5	0.055±0.004	0.12±0.001	4.01±0.250	8.92±0.34	24.21±0.67

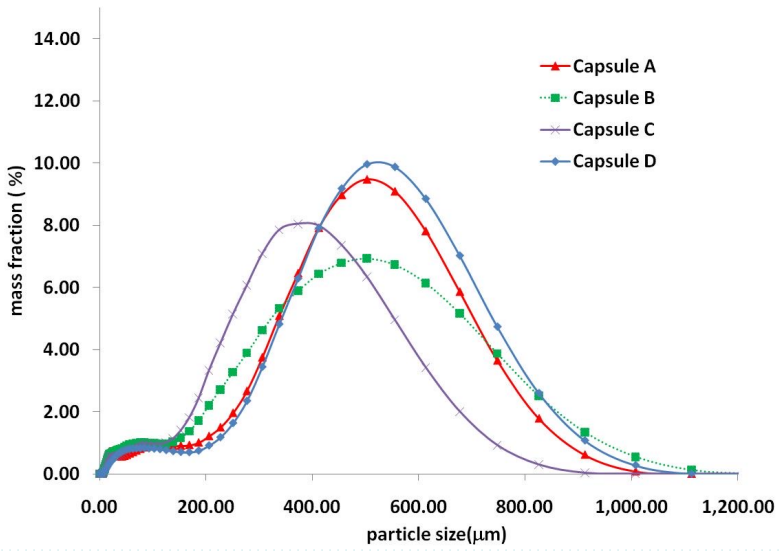


Figure 1 – Particle size distribution of coffe powder from different capsules

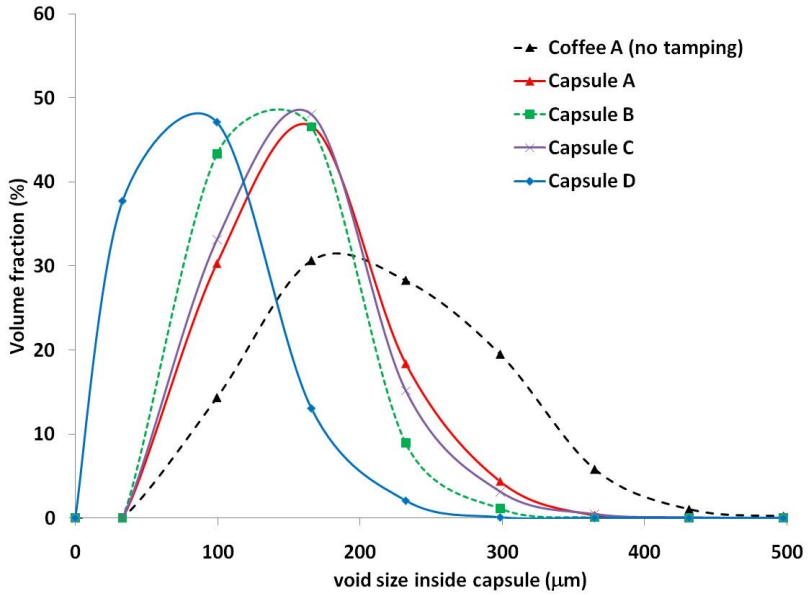


Figure 2 – Pore size distribution inside capsule

Results and Discussion

In table 1 the main properties of the coffee cakes from some single-dose systems and of the obtained EC, are reported. They exhibited several differences in dose which was between 5.53 and 7.56 g as well as in antioxidant capacity (from 6.0 to 10.82 mg Trolox/mL) and phenol contents (from 17.65 to 30.04 mg GAE/mL) while the changes in caffeine content were negligible. The differences in particle size distribution as shown in Figure 1 and the dose were not related to the microstructure properties. Porosity fractions values were very similar among the samples (from 53.98 to 56.90 %) although the doses were different. Moreover, samples having similar particle size (i.e. capsule A and D) showed different pore size distribution (Fig. 2) leading to the idea that the pathway of water through coffee capsule could play and important role in EC quality.

Conclusion

Each coffee brand use single-dose systems having specific chemical and physical characteristics. The differences are in coffee varieties, blend, roasting degree, doses, grinding level, etc. All these affect the quality of espresso coffee. However, we highlighted that microstructure of coffee cakes inside capsule should be considered as an important extraction variable. Coffee powder having different particle size distribution could be exhibit the same porosity fraction as well as different pathways through which water may move during extraction.

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