

BAYISA ASEFA BIKILA

**ESTIMATION OF GENETIC PARAMETERS AND SNPs BASED MOLECULAR  
DIVERSITY OF *Coffea canephora***

Thesis submitted to the Federal University of Viçosa, as part of the requirements of the Post-Graduate Program in Plant Sciences, to obtain the title of Doctor Scientiae.

VIÇOSA  
MINAS GERAIS-BRASIL  
2015

**Ficha catalográfica preparada pela Biblioteca Central da Universidade  
Federal de Viçosa - Câmpus Viçosa**

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B594e  
2015

Bikila, Bayisa Asefa, 1978-  
Estimation of genetic parameters and Anps Based  
Molecular Diversity of Coffea Canephora / Bayisa Asefa Bikila.  
– Viçosa, MG, 2015.  
xi, 57f. : il. ; 29 cm.

Inclui apêndice.

Orientador: Ney Sussumu Sakiyama.

Tese (doutorado) - Universidade Federal de Viçosa.

Inclui bibliografia.

1. Café. 2. Parâmetros genéticos. 3. Diversidade genética.  
4. Variabilidade fenotípica. 5. Marcadores moleculares.  
I. Universidade Federal de Viçosa. Departamento de Fitotecnia.  
Programa de Pós-graduação em Fitotecnia. II. Título.

CDD 22. ed. 633.73

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APPROVED: August 13, 2015.

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**Dedicated to my wife Etaferahu Lencho and my children Eyob and Arsema Bayisa**

## ACKNOWLEDGMENTS

I would like to thank the Postgraduate Program of Plant Sciences at the Universidade Federal de Viçosa, for giving the opportunity to study my doctor science.

My deepest thank goes to my advisor Prof. Ney Sussumu Sakiyama who has helped in all aspects of achieving my degree. Especial thanks also for Eveline Teixeira Caixeta, Marcos Deon Vilela de Resende and Felipe Lopes da Silva for their technical assistance and their advice.

I would also sincerely like to thank Tesfahun Alemu for his guidance and support for the whole times of my stay here in Brazil.

I extend my special appreciation to my wife Etaferahu Lencho, my children: Eyob and Arsema Bayisa, my brother Mosisa Asefa, Gemechu Lencho, Nedhi Sori, my mother Alemi Debela and also Gete Feyissa for their unlimited support for me through my life and for their passion. I thank Fekadu Gebertentsaye, Wogayehu Worku, Sebastiao Carlos Peluzio Gomes, Melaku Degafu and Sintayehu Debebe for their friendship.

Special thanks also to Fabricio Sobreira and Gustavo Fialho who helped me when I first came to Viçosa, Brazil.

My special thanks also go to TWAS/CNPq for the financial support.

Finally, I also thanks others who I didn't mentioned their name here but they contributed for my achievement.

## **BIOGRAPHY**

The author was born in Arsi zone of Oromia region, 1978. He attended his junior and secondary education at Kersa High School. Upon his successful completion of Ethiopian School Leaving Examination (ELSCE), he joined the Alemaya Agricultural University and graduated with B.Sc. degree in Plant Sciences. Right after his graduation, he was employed by Ethiopian Coffee Plantation Enterprise where he served as production head.

After one-year service, he was recruited by Oromia Agricultural Development Bureau and assigned to Bako Agricultural Research Center. He served as a Junior Researcher in the Horticulture Research Section. Finally, the author was employed by Ethiopian Agricultural Research Institute (EARI) and assigned to Kulumsa Agricultural Research Center. At this center, he served as Junior Researcher-II in Horticulture Research Division and as Assistant Researcher-II in the Highland Maize Section. He joined the School of Graduate Studies at Alemaya University in October 2002 for the degree of Masters of Sciences and graduated in July, 2004. After graduated, he returned back to EARI to work as a researcher.

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## ABSTRACT

**BIKILA**, Bayisa Asefa, D.Sc. Universidade Federal de Viçosa, August, 2015. **Estimation of genetic parameters and SNPs based molecular diversity of Coffea canephora**. Adviser: Ney Sussumu Sakiyama; Co-advisers: Marcos Deon Vilela de Resende, Felipe Lopes da Silva and Eveline Teixeira Caixeta.

The objective of this work was to assess the genetic parameters in *Coffea canephora* (Robusta and Conilon groups) and to analyze the genetic diversity of *C. canephora* accessions, which were genotyped with SNPs molecular markers. The phenotypic data were analyzed using the mixed model methodology (REML/BLUP) through the Selegen software for estimation of genetic parameters. The results showed a low genetic variability (CVg%) among the clones of Robusta and Conilon for all the evaluated traits. On the other hand, relatively high residual coefficients of variation (CV%) for most of the traits were recorded implying that these traits seem to be highly influenced by the environmental variation. The estimated repeatability for most of the traits was lowest indicating the irregularity of the superiority of the individuals among the measurements for these characters in the case of both clones showing high irregularity of the performance across measurement, which demonstrate that genotype selection based on those traits is not reliable strategy. Generally, for both groups of clones, there was low interaction with year, as observed by the genotypic correlation across measurement ( $r_{gmed}$ ) for most of the characters evaluated demonstrating that selection can be performed at any of the development stages used for measurement. Genetic diversity was investigated using 46074 polymorphic SNP markers covering the entire genome of 50 *C. canephora* clones (24 Conilon and 26 Robusta). The genetic similarity between each pair of clones was calculated by the Jaccard coefficient and information about diversity among clones was inferred by means of the dendrogram built using UPGMA method (Unweighted Pair-Group Method with Arithmetic Mean) with the program NTSYS pc2.1. Hence, the dendrogram divided the clones into

six groups. Generally, the analysis showed that the *C. canephora* genotypes were clearly divided into diversity groups that can be used for breeding programs.

## RESUMO

**BIKILA**, Bayisa Asefa, D.Sc. Universidade Federal de Viçosa, Agosto, 2015. **Estimativas de parâmetros genéticos, diversidade molecular baseada em SNPs em Coffea canephora.** Orientador: Ney Sussumu Sakiyama. Coorientadores: Marcos Deon Vilela de Resende, Felipe Lopes da Silva e Eveline Teixeira Caixeta.

O objetivo deste trabalho foi avaliar os parâmetros genéticos e diversidade genética de *Coffea canephora* (grupo Robusta e Conilon) genotipados com marcadores moleculares SNPs. Os dados fenotípicos foram analisados utilizando a metodologia de modelos mistos (REML/BLUP) por meio do programa Selegen. Os resultados mostraram uma baixa variabilidade genética entre os clones de Robusta e Conilon para todas as características avaliadas. Por outro lado, coeficiente de variação relativamente elevado foi observado para a maioria das características, o que implica que estas características parecem ser altamente influenciadas pela variação ambiental. A repetibilidade estimada para a maioria das características foi menor, indicando a irregularidade da superioridade dos indivíduos entre as medições para esses caracteres no caso de ambos os clones com elevada irregularidade do desempenho por meio de medição, o que demonstra que a seleção do genótipo com base nessas características é uma estratégia não confiável. Em geral, para ambos os grupos, houve baixa interação com ano, como foi observado por meio da correlação entre genótipos de medição ( $r_{\text{gmed}}$ ) para a maioria dos caracteres avaliados. Esse resultado demonstrou que a seleção pode ser realizada em qualquer fase de desenvolvimento usada para a medição. Para estudo de diversidade genética foi utilizado 46074 marcadores SNP polimórficos que cobrem todo o genoma de 50 clones de *C. canephora* (24 Conilon e 26 Robusta). A estimativa de similaridade genética entre cada par de indivíduos foi calculada pelo coeficiente de Jaccard e diversidade entre clones foi obtida pela construção do dendrograma pelo método UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) usando o programa NTSYS pc2.1. Por meio do dendrograma, os clones foram divididos em seis grupos. A análise mostrou

que os genótipos de *C. canephora* foram divididos em grupos de diversidade que podem ser usados para outros programas de melhoramento genético.

## 1. GENERAL INTRODUCTION

Coffee belongs to the family, Rubiaceae and the genus *Coffea* L. which comprises over 104 species that have been identified so far. Commercial coffee production relies mainly on two species, *Coffea arabica* L. (63%) and *Coffea canephora* Pierre (36%). *Coffea arabica* is a natural allotetraploid ( $2n=4X=44$ ), and is self-fertile (Coste, 1989). Whereas, other species are diploid ( $2n=22$ ) and generally self-incompatible. The cup quality made from *C. canephora* is generally regarded as inferior to that made of *C. arabica*. However, *C. canephora* does not need to grow at high altitude, requires less care to grow because it is hardier, and it tends to be less susceptible to pests and rough handling (Coste, 1989). *C. canephora* presents a wide genetic variability, with one of the widest geographic natural distribution within the subgenus *Coffea* (Maurin et al. 2007).

Evaluation of the genetic diversity and available resources within the genus coffee is an important step in Coffee breeding (Cubry et al. 2008). Hence, genotypic parameters analysis in *Coffea canephora* has importance, especially for genetic materials from Brazil. As new coffee varieties are continuously being developed through hybridization, there is a need to determine the level and sources of genetic variation within and between new and existing coffee varieties (Gichimu and Omondi, 2010). In view of this, characterization and evaluation of its gene pool is necessary for effective crop improvement programs and for better conservation and management of genetic resources (Prakash et al., 2005). Morphological and agronomical traits as well as resistance to biotic and abiotic stresses that are known to individual accessions increase the importance of the germplasm. Efficient utilization of indigenous germplasm required knowledge of biodiversity of economic interest (Beer et al., 1993). According to De Vienne et al. (2003), morphological characters are a classical method to distinguish variation based on the observation of the external morphological differences.

To study the genetic variability, morphological and molecular techniques can be used. With the advent of SNP (Single Nucleotide Polymorphism) markers, the possibility of simultaneous analysis of a set of loci becomes more real. A SNP is created when a single nucleotide base in a DNA sequence is replaced with a different nucleotide base.

The SNP markers are based on the most fundamental alterations of the DNA molecule, mutations in the bases of unique chain of nitrogenous bases. The SNP are extremely abundant in genomes, studies show that there may be millions in an individual genome (Li et al., 2009). Thus, the availability of dense marker maps has opened new opportunities for genetic evaluation of individuals with high accuracy. As genetic markers, SNPs represent sites in the genome where DNA sequences differ by single base when two or more individuals are compared. They may be individually responsible for specific traits, or phenotypes, or may represent neutral variation that is important for evaluating diversity in the context of evolution (Li et al., 2009).

Hence, the objectives of this study were to assess the genetic parameters, genetic diversity and relationship among clones of *C. canephora* to identify genetically divergent genotypes for future coffee improvement program.

## **2. LITERATURE REVIEW**

### **2.1. Major Coffee Species and their distribution**

Coffee is an important agricultural commodity produced in more than 60 countries. Coffee, one of the most popular non-alcoholic beverages, is consumed regularly by 40% of the world population mostly in the developed world, and thus occupies a strategic position in the world socio-economy. It ranks second on international trade exchanges, representing a significant source of income to several developing countries in Africa, Asia and Latin America. Brazil, Vietnam and Colombia are responsible for about 50% of the world-coffee production, and Brazil is the main producer and exporter of coffee and constitutes its second biggest consumer market (ABIC, 2009). This fact ranks coffee amongst the most important commodities in the Brazilian trade balance.

There are around 104 different species of coffee of which only two dominate the world trade -the *Coffea arabica* and the *Coffea canephora*. The species *C. arabica* L. is endemic in Southwest Ethiopia and probably originates from a relatively recent cross between *Coffea eugenoides* and *Coffea canephora* (Lashermes et al., 1993). *Coffea arabica* do best at higher altitude where the slower growing process concentrates their

flavor. As it is susceptible to disease, frost, and drought, it requires very careful cultivation with the right climatic conditions (Lashermes et al., 1993).

The cultivation of the *C. arabica* began about five hundred years ago in Yemen and reached the southeast of Asia approximately in 1700. In the beginning of the 18<sup>th</sup> century, progenies of a single plant were taken from Indonesia to Europe and later to America (Carvalho and Fazuoli, 1945). Originating from other introductions that took place from Yemen to Brazil, seeds of two different cultivars, Typica and Bourbon, constitute the main genetic basis of all cultivated coffee arabica grown in Brazil and other countries (Krug et al., 1939; Carvalho et al., 1993). Many cultivars have been developed for *C. arabica*, but because of the narrow genetic basis of the species, phenotype differences among them are due mainly to single gene mutations. Analysis of *C. arabica* varieties in Brazil has revealed that the material employed is derived from few ancestral varieties (Typica, Bourbon and Sumatra), which themselves have undergone mutual spontaneous mutations and crossings (Mendes and Guimarães, 1998). However, Setotaw et.al., (2009) demonstrated that the genetic variability among híbrido de Timor germplasm which is potentially useful for breeding program especially because of the well-known narrow genetic basis of *C. arabica*.

*C. canephora* (from Congo, 1898) is diploid coffee species most widely cultivated around the world. It is self-incompatible and cross-pollinated and consequently displays much more variability than *C. arabica*. It is grown in West and Central Africa, throughout Southeast Asia and in Brazil, where it is also known as Conilon. The cup quality made from *C. canephora* is generally regarded as inferior to that made of *C. arabica*. However, *C. canephora* is more resistant to adverse conditions than *C. arabica*, particularly to several diseases and pests. Another diploid coffee species originating from Mozambique, *C. racemosa*, is characterized as having low caffeine content, high drought tolerance and resistance to coffee leaf-miner (*Leucoptera coffeella*) (Clarke et al., 1983), and has been used in breeding programs for introgression of important agronomic traits to *C. arabica* (Guerreiro Filho et al., 1991). *Coffea Liberica* (from Western Africa) is the third commercial species but has no great importance in coffee trade.



Globally, the *Coffea canephora* gene pool is conserved in ex-situ collection plots in several countries. In cognizant of this fact and in order to alleviate the production problems, concerted effort were undertaken to utilize its germplasm. As result several cultivars and accessions were collected and maintained at the different sites in Brazil. Efforts undertaken globally to improve coffee, though successful, have proven to be too slow and severely constrained owing to various factors. The latter includes: genetic and physiological make up (low genetic diversity and ploidy barrier in *C. arabica*, and self-incompatibility/easy cross-species fertilization in Robusta) and long generation cycle for screening/selection (Carvalho et al., 1993). The situation warrants recourse to newer, easy, practical technologies that can provide acceleration, reliability and directionality to the breeding efforts and allow characterization of cultivated/secondary gene pool for proper utilization of the available germplasm in genetic improvement programs. Hence, DNA-based markers besides facilitating the analysis of variation present in DNA itself can also be used for variety identification and accelerated breeding of improved coffee genotypes (Arens et al., 1995; Ferreira and Grattapaglia, 1998).

## **2.2. Phenotypic analysis in Coffee**

Morphological and agronomical traits as well as resistance to biotic and abiotic stresses that are known to individual accessions increase the importance of the germplasm. The economic value of a population is related to plant morphology, agronomic performance, seed quality and nutritional qualities. Efficient utilization of indigenous germplasm required knowledge of biodiversity of economic interest (Beer et al., 1993). Morphological markers are a classical method to distinguish variation based on the observation of the external morphological differences such as the size and shape of the leaf and of the plant form, the color of the shoot tip, the characteristics of the fruit, the angle of branching and the length of the internodes (De Vienne et al., 2003).

Two breeding populations have been identified with *Coffea canephora*, based on geographical and genetic differences: the Guinean group from West Africa and the Congolese group from central Africa (Leroy T., et al., 1993). Phenotypic and genotypic variation between and within groups was found to be large. Based on isozyme analysis and

phenotypic observations, two subgroups were identified within the Congolese group. Furthermore, the variation observed indicates that recurrent selection would allow progress for selection traits for both populations and intergroup hybrids (Leroy T., et al 1993).

According to the study to estimate the extent of genetic variation and association among yield and yield-related traits among Forty nine *Coffea arabica* accessions from Limu, Ethiopia (2004 to 2009), the germplasm accessions differ significantly for most of the traits (Olika Kitila, et al. 2011). Thus, relatively high phenotypic (45.11 and 30.18%) and genotypic coefficient of variation (25 and 24.90%) were observed for yield and number of secondary branches in the order of magnitude. The study confirmed the presence of trait diversity in Limu coffee accessions and this could be exploited in the genetic improvement of the crop through hybridization and selection (Olika Kitila, et al. 2011). On the other hand, minimal morphological variation within varieties was obtained which indicatives the high genetic consistency within Kenyan Arabica coffee varieties according to Gichimu, et al.( 2010).

### **2.3. Simultaneous Selection of characters**

To select cultivars for breeding procedures based on only one trait is nowadays not ideal, since on the one hand producers are interested in the yield capacity of the plant material, while the consumer on the other hand is concerned with the quality of the food product. Thus, selection indexes are multivariate techniques that combine information of different traits of agronomic interest with the genetic properties of a population. Using the selection indexes, numerical values are determined serving as an additional, theoretical trait, resulting from the combination of particular traits defined by the breeder, which should be maintained under simultaneous selection (Cruz and Carneiro, 2003; Bezerra Neto et al., 2006; Santos et al., 2007). Estimates of phenotypic and genotypic variances for a number of quantitative characters in arabica coffee, obtained from a variety trial in Kenya, were used to construct selection indices and indicated that the expected genetic advance in yield based on a selection index, containing the first two years yield and measurements of girth at base of stem and percentage bearing primaries and would imply

that a breeding cycle of 5 years will be sufficient for efficient selection for higher productivity in arabica coffee (Walyaro D.J. et al. 1979).

The selection indexes used to predict the gains are those of Mulamba and Mock (1978), and the classic index of Smith (1936) and Hazel (1943). The index of Mulamba and Mock (1978) ranks the genotypes, initially, for each trait, by assigning higher absolute values to those of better performance. Finally, the values assigned to each trait are added, obtaining the sum of the ranks, which indicates the classification of genotypes (Cruz and Carneiro, 2003). The index of Smith (1936) and Hazel (1943), on the other hand, is based on the solution of the matrix system:  $b = P^{-1} Ga$ , where  $b$  is the vector of the weighting coefficients of the index to be estimated;  $P^{-1}$  is the inverse of the matrix of phenotypic variances and co-variances between traits;  $G$  is the matrix of genotypic variances and co-variances between the genetic traits, and  $a$  is a vector of economic weights (Cruz and Carneiro, 2003).

#### **2.4. Application of Mixed models based on REML/BLUP in coffee**

Perennial plant species such as coffee exhibit unique biological aspects such biennial cycle; overlapping generations; expression of characters over several years and differences in earliness and productive longevity (Sera, 2001). Those characteristics lead to some consequences, such as the use of selected genetic material for several years, reduction in its useful life survival rate during the experiments, a fact that tends to generate unbalanced data for use in the estimation of genetic parameters and prediction of the breeding and genotypic values (Resende et al.; 2001). Because of these agronomic peculiarities, genetic improvement of coffee is difficult, and recommended the use of special methods to estimate genetic parameters and predict the genetic values (Oliveira et al., 2011; Petek et al., 2008).

Since the 1930s, several methodologies of genetic evaluation have been proposed, among these, the least squares for unbalanced data (Yates, 1937). The application of this method is not free of problems, since the variance of the prediction error is not minimal, the functions of the prediction are not always estimable and, depending on the degree of

data unbalancing, the values of some genotypes may be overestimated (Henderson, 1974). The mixed models equation described by Henderson (1963) introduced changes in the estimation of variance components and breeding values (Searle, 1971). The method consists basically in the predication of genetic values adjusting the data to fixed effects, to the unequal number of subclasses and to coefficients of relatedness of genotypes (Bernardo, 2002). The method proposed by Henderson (1963) therefore has flexible properties, since it can be applied to unbalanced data of different generations and to estimate the breeding values of unevaluated genotypes (Henderson 1984, Piepho et al. 2008). There is little information in the literature about the reliability of REML/BLUP (Restricted Maximum Likelihood/Best Linear Unbiased Prediction) for the prediction of genotypic values in unbalanced experiments for breeding program of annual crops (Bernardo 2002).

Currently, the standard analytical procedure recommended for studies in quantitative genetics and also to the practice on perennials is the methodology of mixed models (Henderson, 1984). This approach allows accurate prediction of genetic and unbiased even under unbalance values and also facilitates the simultaneous use of the information from the individual, family and repeated measurements over time, providing more precise estimates of the genetic variation and individual genetic values.

Another justification for using mixed models in coffee constitutes in selection of cultivar of the lines types, this being made from individual plants within segregating progenies. Thus, the prediction of additive genetic value of each individual will lead to maximizing accuracy in selecting the best among two individuals and thus to maximize the genetic gain with the selection of new strains, thus capitalizing on the properties of BLUP predictors (Resende, 2002). Ramalho et al. (2013) also emphasize the advantages of the application of BLUP in the improvement of arabica coffee. This methodology has been used by other authors in various crops such as corn, rice, sugar cane among others. However, there are few reports in the literature of the use of this methodology in the selection of individual plants of *Coffea canephora* species (Resende et al., 2001).

## **2.5. Molecular analysis of Genetic diversity in Coffee**

Efforts undertaken globally to improve coffee, though successful, have proven to be too slow and severely constrained owing to various factors. The latter includes: genetic and physiological make up (low genetic diversity and ploidy barrier in arabicas, and self-incompatibility/easy cross-species fertilization in Robusta) and long generation cycle for screening/selection (Poncet et al., 2006).

The situation warrants recourse to newer, easy, practical technologies that can provide acceleration, reliability and directionality to the breeding efforts, and allow characterization of cultivated/secondary gene pool for proper utilization of the available germplasm in genetic improvement programs (Arens et al., 1995). In this context, development of DNA marker tools and availability of markers-based molecular linkage maps becomes imperative for MAS-based could accelerate breeding of improved coffee genotypes. DNA-based markers have been used for studying genetic diversity in many plant species. This type of marker, besides facilitating the analysis of variation present in DNA itself, can also be used for variety identification. In addition, they are environmentally independent, and may be detected in any type of tissue and developmental phase of the plant (Arens et al., 1995; Ferreira and Grattapaglia, 1998).

To study the genetic variability, morphological, iso-enzyme and molecular techniques can be used. Among them, the DNA molecular technique is the best and able to detect differences at the genome level. Among the molecular technique Simple Sequence Repeats (SSR), Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) are more frequently used for genetic diversity study and proved to be efficient in establishing the core collection. Assessment of genetic variability within and among arabica coffee populations using molecular markers such as RAPD, ISSR, ALFP, and SSR has been the subject of several studies (Aga et al., 2005; Agwanda et al., 1997; Anthony et al., 2002; Chaparro et al., 2004; Lashermes et al., 1993; and Masumbuko and Bryngelsson, 2006). Nuclear DNA variation in coffee has been evaluated by using molecular markers such as RFLP (Lashermes et al., 1999), RAPD (Diniz et al., 2005; Anthony et al., 2002, Silveira et al., 2003), AFLP (Steiger et al., 2002; Anthony et al., 2002) and SSRs (Combes et al., 2000; Anthony et al., 2002; Moncada and McCouth 2004;

Maluf et al., 2005; Poncet et al., 2006; Silvestrini et al., 2007), whereby it has been shown that genetic variation in the genus *Coffea* is low, especially among cultivated *C. arabica* tetraploid varieties. Maluf et al. (2005) used RAPD, AFLP and SSR molecular markers to study the genetic diversity of *arabica* coffee and detected significant polymorphism among lines.

Maluf et al. (2005) and Silvestrini et al. (2007) confirmed the low genetic diversity in coffee, mainly in tetraploid varieties, although none were related to the varieties under study. On the other hand, Setotaw et al. (2009) investigated the existing genetic diversity among the accessions of “Híbrido de Timor” germplasm of Brazil using RAPD, AFLP and SSR molecular markers. EST-SSR molecular markers were also used to study the genetic diversity of coffee trees including accessions of “Híbrido de Timor” (Missio et al. 2009).

Teixeira-Cabral et al. (2004) used RAPD molecular markers to study the genetic diversity and characterize the coffee differentials of *Hemileia vastatrix* races. Lashermes et al. (2000), Orozco-Castillo et al. (1994) and Silvestrini et al. (2007) used AFLP, RAPD and SSR techniques to study the genetic diversity of coffee species. Similarly, Souza et al., 2013 analyzed the genetic diversity, population structure and association mapping among 130 *Coffea canephora* accessions of Brazil and detected those two major genetic groups with expressive limited variability among the groups.

Among the molecular markers currently available, the SSRs (Simple Sequence Repeats), or microsatellite, have been extensively used due to their resolution and polymorphism levels. These characteristics make these molecular markers efficient tools for the genetic mapping, linkage studies, genotype identification and conservation of germplasm, pedigree analyses, marker assisted selection, and analysis of DNA libraries for gene cloning (Rufino et al., 2005). Furthermore, they can be efficiently analyzed by rapid and simple polymerase chain reactions, besides being co-dominant, highly reproducible and multi-allelic, and capable of being automated (Ferreira and Grattapaglia, 1998).

By using markers developed for *C. arabica*, Moncada and McCouth (2004) showed the particular value of SSR markers for discriminating closely related commercial varieties of coffee. Microsatellites have also been applied in coffee to identify *C. arabica*, *C. canephora* and related species (Combes et al., 2000). They have also been used to

investigate polymorphisms among wild and cultivated *C. arabica* accessions (Anthony et al., 2002; Moncada and Couch 2004) and to analyze the introgression of DNA fragments from *C. canephora* and *C. liberica* into *C. arabica* (Lashermes et al., 2000, Lashermes, et al., 2010; and Gichuru et al., 2008).

## **2.6. Single Nucleotide Polymorphism (SNP) markers**

Within a chromosome, DNA segments can be inserted, deleted, replicated and moved to a different location in the same or in a different chromosome, or inverted in place. A SNP is created when a single nucleotide base in a DNA sequence is replaced with a different nucleotide base. SNP is the simple and common type of variant. The nucleotide base variant most common in a population is called the major allele, while the less common base is the minor allele (Meuwissen et al., 2001).

During the last few years, the dominance of medium-throughput SSRs was eventually broken by SNP markers. The introduction of new sequencing technologies has dramatically changed the landscape for detecting and monitoring genomic-wide polymorphism. Today, single nucleotide polymorphisms (SNPs) are rapidly replacing SSRs as the DNA marker of choice for applications in plant breeding and genetics because they are more abundant, stable, efficient and increasingly cost-effective (Duran et al. 2009, Edwards and Batley, 2010). Advances in genotyping technology enabling large-scale genotyping marker automation, especially for new types of molecular markers, such as SNPs (Bernardo and Yu, 2007), promise to reduce prices per data point for extensive use in various crops.

As genetic markers, SNPs represent sites in the genome where DNA sequences differ by single base when two or more individuals are compared. They are widely used in breeding programs for marker-assisted and genome-selection, association and QTL mapping, haplotype and pedigree analysis (Jannink et al. 2010). Using genome-wide SNP data, a breeder can examine linkage relationships among alleles along a chromosome or across the genome as whole. They may be individually responsible for specific traits, or phenotypes, or may represent neutral variation that is important for evaluating diversity in the context of evolution.

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## CHAPTER 1

### Estimation of Genetic parameters in *Coffea Canephora*

#### ABSTRACT

The objective of this work was to assess the genetic parameters in *Coffea canephora* (Robusta and Conilon groups) clones using mixed model. The experiments were carried out during four years, in complete block design, and one plant per plot, at Oratorio of Minas Gerais state, Brazil. The clones were evaluated for vigor, reaction to rust, reaction to cercospera, number of ortotropicos branches, number of plegiotropics branches, plant height, diameter of stem, fruit maturity, diameter of canopy, fruit size and production of fruits. The data were analyzed using the mixed model methodology (REML/BLUP) of Selegen software for estimation of genetic parameters in *C. canephora* breeding. The results showed a low genetic variability among the clones of Robusta and Conilon for all the evaluated traits. On the other hand, relatively high residual coefficient of variation for most of the traits was recorded implying that these traits seem to be highly influenced by the environmental variation. However, in this study the estimates of individual heritability in the broad sense ( $h^2_g$ ) was of low magnitude, but were significant for all traits except yield (sac/ha). The estimated repeatability for most of the traits was low indicating the irregularity of the superiority of the individuals among the measurements showing that genotype selection based on these traits is not reliable strategy. Generally, for both clones, there was low interaction with year, as observed by the genotypic correlation across measurement ( $r_{gmed}$ ) for most of the characters evaluated demonstrating that selection can be performed at any of the development stages used for measurement.

Keywords: heritability, mixed model, repeatability

## Estimativas de parâmetros genéticos em *Coffea canephora*

### RESUMO

O objetivo deste trabalho foi avaliar os parâmetros genéticos em *Coffea canephora* (Robusta e Conilon) clones usando modelo misto. Os experimentos foram realizados durante quatro anos, em delineamento de blocos, e uma planta por parcela, em Oratórios, estado de Minas Gerais, Brasil. Os clones foram avaliados quanto em vigor vegetativo e reação à ferrugem, reação a cercospora, número de ramos ortotropicos, número de ramos plegiotropicos, altura da planta, diâmetro do caule, época de maturação de frutos, diâmetro da copa, tamanho dos frutos e produção de frutos. Os dados foram analisados utilizando a metodologia de modelos mistos (REML/BLUP) por meio do Selegen software para a estimativa de parâmetros genéticos no melhoramento de *C. canephora*. Os resultados mostraram que baixa variabilidade genética entre os clones de Robusta e Conilon para todas as características avaliadas. Por outro lado, relativamente elevado coeficiente de variação para a maioria das características foi observado, o que implica que estas características parecem ser altamente influenciadas pela variação ambiental. No entanto, neste estudo, as estimativas de herdabilidade individual no sentido amplo ( $h^2_g$ ) foi de baixa magnitude, mas foram significativas para todos os caracteres, exceto para a produção de frutos (sac/ha). A repetibilidade estimado para maioria das características foi baixa, indicando a irregularidade da superioridade dos indivíduos entre as medições que mostra que a seleção do genótipo com base nestas características não é confiável estratégia. Geralmente, para ambos os clones, houve baixa interação com anos, como foi observado por meio da correlação entre genótipos de medição ( $r_{gmed}$ ) para a maioria dos caracteres avaliados demonstrando que a seleção pode ser realizada em qualquer das fases de desenvolvimento utilizadas para a medição.

Palavras-chave: herdabilidade, modelo misto, repetibilidade

## 1. INTRODUCTION

Coffee belongs to the family, Rubiaceae and the genus *Coffea* L. which comprises over 104 species that have been identified so far. Commercial coffee production relies mainly on two species, *Coffea arabica* L. (63%) and *Coffea canephora* Pierre (36%). *C. arabica* is a natural allotetraploid ( $2n=4X=44$ ), and is self-fertile (Coste, 1989). Whereas, other species are diploid ( $2n = 22$ ) and generally self-incompatible. The cup quality made from *C. canephora* is generally regarded as inferior to that made of *C. arabica*. However, *C. canephora* does not need to grow at high altitude, requires less care to grow because it is hardier, and it tends to be less susceptible to pests and rough handling (Coste, 1989). *C. canephora* presents a wide genetic variability, with one of the widest geographic natural distribution within the subgenus *Coffea* (Maurin et al., 2007). Hence, genotypic parameters analysis in *Coffea canephora* has importance, especially for genetic materials from Brazil.

As new coffee varieties are continuously being developed, there is a need to determine the level and sources of genetic variation within and between new and existing coffee varieties (Gichimu and Omondi, 2010). Like it is for many crops, evaluation of the genetic diversity and available resources within the genus coffee is an important step in Coffee breeding (Cubry et al., 2008). In view of this, characterization and evaluation of its gene pool is necessary for effective crop improvement programs and for better conservation and management of genetic resources (Prakash et. al, 2005). According to De Vienne et al. (2003), morphological characters are a classical method to distinguish variation based on the observation of the external morphological differences. Morphological and agronomical traits as well as resistance to biotic and abiotic stresses that are known to individual accessions increase the importance of the germplasm. Efficient utilization of indigenous germplasm required knowledge of biodiversity of economic interest (Beer et al., 1993).

Perennial plant species such as coffee exhibit unique biological aspects such a biennial cycle; overlapping generations; expression of characters over several years and differences in earliness and productive longevity (Sera, 2001). Those characteristics lead to some consequences, such as the use of selected genetic material for several years, reduction in its useful life survival rate during the experiments, a fact that tends to generate

unbalanced data for use in the estimation of genetic parameters and prediction of the breeding and genotypic values (Resende, 2001). Because of these agronomic peculiarities, genetic improvement of coffee is difficult, and recommended the use of special methods to estimate genetic parameters and predict the genetic values (Oliveira et al., 2011; Petek et al., 2008).

Estimations of genetic parameters permit the understanding of the nature of the gene action involved in trait inheritance and lead themselves to assessment of expected progress with selection, besides defining the best selection method to be adopted (Sampaio et al., 2002, Oliveira et al., 2008). Hence, in the *Coffea canephora* improvement program, the best clones can be selected considering good performance for a number of breeding target traits, so the use of a selection index is a promising method for simultaneous selection. The use of additive selection indices allows a clear visualization of the performance of the progenies combining most of the agronomic allowing for a better selection of the most promising progenies. The reason is that the correlation may be caused by the action of pleiotropic and/or closely linked genes that affect the traits under study (Falconer and Mackay 1996).

Since the 1930s, several methodologies of genetic evaluation have been proposed, one of them is the least squares for unbalanced data (Yates, 1934). The application of this method is not free of problems, since the variance of the prediction error is minimal, the functions of the prediction are not always estimable and, depending on the degree of data unbalancing, the values of some genotypes may be used overestimated (Henderson, 1974). Whereas, the mixed models equation described by Henderson (1963) introduced changes in the estimation of variance components and breeding values (Searl, 1971). This method consists basically the predication of genetic values considered random to the unequal number of subclasses and to coefficients of relatedness of genotypes (Bernardo, 2002).

Since the prediction of genetic values of superior materials is one of the main problems in the breeding of any species, once it requires the true values of variance components, the use of more sophisticated methods, such as BLUP, allows obtaining better estimates for these parameters (Resende, 2002). This approach takes into account the treatment effects as random, which enables to carry out the genotypic selection instead of the phenotypic one (Resende and Duarte 2007) and their implications in plant

selection is presented by several authors in the literature (Duarte and Vencovsky 2001, Resende and Duarte 2007). Ramalho et al. (2013) also emphasize the advantages of the application of BLUP in the improvement of Arabica coffee. This methodology has been used by other authors in various crops such as corn, rice, sugar cane among others. However, there are few reports in the literature of the use of this methodology in the selection of individual plants of *Coffea canephora* species. Hence, the objective of this study was to estimate genetic parameters and identify the existing variability among *C. canephora* specie using the mixed model methodology.

## **2. MATERIALS AND METHODS**

### **2.1. Plant materials**

A total of 121 *Coffea canephora* clones, of which 69 Conilon variety and 52 Robusta variety, were used for this study which are maintained at the Coffee Germplasm Collection of EPAMIG(Empresa de Pesquisa Agropecuária de Minas Gerais)/UFV(Universidade Federal de Vicosa) at Oratorios, Minas Gerais, Brazil. The trail was established as a randomized complete block design with 5 replications and one plant per plot was used.

### **2.2. Data collection**

Data on 11 quantitative traits recorded on tree basis include vegetative vigor, reaction to rust, reaction to cercospora, number of ortotropics branches, number of plagiotropics branches, plant height, canopy diameter, stalk diameter, fruit maturity, fruit size and production of fruits.

Vegetative vigor average plant scored by the general appearance of the plant, observing the leafiness, the number of orthotropic and reproductive branches, nutritional status and health of coffee, adopting scores from 1 (completely depleted plant) to 10 (highly vigorous plant); reaction to the coffee rust - measured in the peak months of the disease in the field (between March and July), considering grades 1-5, where 1- immune plants without any signs of infection; 2-plants hypersensitivity reaction visible macroscopically, chlorotic lesions, small swellings, without occurrence of sporulation; 3-plants hypersensitivity reaction visible macroscopically, chlorotic lesions usually

sporulation the edge and small swellings; 4-plants hypersensitivity reaction visible macroscopically, chlorotic lesions, swellings, occurring average sporulation; and 5 - plants with lesions with intense sporulation and the presence of many large pustules; reaction with *Cercospora* (RC) - evaluates the scale from 1 to 3, wherein the note 1 refers to plants that showed no disease incidence and grade 3 for plants with high rates of disease; average number of orthotropic branches per plant (NR<sub>Ort</sub>); average number of reproductive branches per plant (NR<sub>Pla</sub>); and average diameter of the tree canopy (DCO)- given in centimeters (cm).

Traits and their years of evaluation are: Vegetative vigor (VIG), Reaction to rust (Ferr) and plant height in cm (APL), canopy diameter in cm(DCO) - 2011; 2012; 2013; 2014; reaction to *Cercospora* (CER) -2011; 2012; 2013: number of orthotropic branches (NR<sub>Ort</sub>) - 2011; 2012. number of Plagiotropicos branches (NR<sub>Pla</sub>) -2011, average stem diameter in mm (DCAU) –2013, fruit maturity (MAT), fruit size (TFR) and yield per hectare (PROD) - 2012; 2013; 2014.

### **2.3. Statistical analysis**

#### **2.3.1. Estimation of genetic parameters:**

In order to estimate genetic parameters among *Coffea canephora* clones, all the quantitative characters considered in the study were statistically analyzed using REML/BLUP by using Selegen software (Resende, 2008).

Considering the environmental of measurements (m ) as fixed, they can be adjusted together the overall average in a single vector of fixed effects. The following model was adjusted.

$$y = X_m + Z_g + W_b + S_p + T_{gm} + e$$

Where:

y, m, g, b, p, gm, e: vectors of data, of measurements (fixed), of genotypic effects (random), of block (random), of permanent environment (random), of genotypic x measurement interaction (random), and random errors, respectively.

X, Z, W, S, T: Incidences matrices for the effects in the model.

The following mixed model equations were used:

$$\begin{bmatrix} X'X & X'Z & X'W & X'T & X'S \\ Z'X & Z'Z + I\lambda_1 & Z'W & Z'T & Z'S \\ W'X & W'Z & W'W + I\lambda_2 & W'T & W'S \\ T'X & T'Z & T'H & T'T + I\lambda_3 & T'S \\ S'X & S'Z & S'W & S'T & S'S + I\lambda_4 \end{bmatrix} \begin{bmatrix} \hat{r} \\ \hat{g} \\ \hat{b} \\ \hat{p} \\ \hat{gm} \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \\ W'y \\ T'y \\ S'y \end{bmatrix},$$

where:

$$\lambda_1 = \frac{1 - h_g^2 - c_b^2 - c_p^2 - c_{gm}^2}{h_g^2}; \lambda_2 = \frac{1 - h_g^2 - c_b^2 - c_p^2 - c_{gm}^2}{c_b^2}; \lambda_3 = \frac{1 - h_g^2 - c_b^2 - c_p^2 - c_{gm}^2}{c_p^2}; \lambda_4 = \frac{1 - h_g^2 - c_b^2 - c_p^2 - c_{gm}^2}{c_{gm}^2}$$

$$h_g^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_b^2 + \sigma_p^2 + \sigma_{gm}^2 + \sigma_e^2} = \text{broad sense heritability};$$

$$c_b^2 = \frac{\sigma_b^2}{\sigma_g^2 + \sigma_b^2 + \sigma_p^2 + \sigma_{gm}^2 + \sigma_e^2} : \text{Coefficient of determination of block effects};$$

$$c_p^2 = \frac{\sigma_p^2}{\sigma_g^2 + \sigma_b^2 + \sigma_p^2 + \sigma_{gm}^2 + \sigma_e^2} : \text{Coefficient of determination of permanent effects}$$

$$c_{gm}^2 = \frac{\sigma_{gm}^2}{\sigma_g^2 + \sigma_b^2 + \sigma_p^2 + \sigma_{gm}^2 + \sigma_e^2} : \text{Coefficient of determination of interaction effects}$$

$$\sigma_g^2 = \text{genotypic variance}$$

$$\sigma_b^2 = \text{block variance}$$

$$\sigma_p^2 = \text{Permanente variance}$$

$$\sigma_{gm}^2 = \text{genotype x measurement variance}$$

$$\sigma_e^2 = \text{residual variance}$$

Accuracy was calculated using the following equation:

$$r = \sqrt{h_m^2}$$

where  $h_m^2$  = heritability at the average level of genotypes

$$h_m^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_b^2 + \sigma_{gm}^2 + \sigma_e^2}$$

Selection Index was calculated using the following equation ( Smith (1963) and Hazel (1943) ).

$$I = b_1x_1 + b_2x_2 \dots b_m x m = b'x \quad (6.2)$$

Where;

$X_i$  = an observation on the  $i^{\text{th}}$  trait and  $b_i$  is the selection index coefficient (or weight) for that trait. In vector notation:  $b' = [ b_1, b_2, \dots, b_m]$  and  $x' = [ x_1, x_2, \dots, x_m]$ .

Economic Weight: estimated from Statistics of the experimental data and the genetic variation coefficient (CVg%) as a reference, which directly proportional to the available genetic variance, which express the proportionality between the characters and it is dimensionless (Cruz, 1993).

### **3. RESULTS**

#### **3.1. Estimation of genetic and phenotypic parameters**

In general, low genetic coefficient of variation CVg(%) was observed among the clones of Robusta and Conilon varieties for all the traits evaluated (Table 1 and 2). For Robusta, the highest coefficients of genetic variation obtained were 14.27%, 17.39%, and 19.49% for fruit size (TFr), number of plagiotropics branches (NRPla) and number of Ortotropicos branches (NRort), respectively whereas, for Conilon variety, 13.58%, 17.17% and 19.522% for number of Ortotropicos branches (NRort), Rust and fruit size (TFr), respectively, indicating the relative importance of these traits for the improvement of these varieties.

On the other hand, relatively highest residual coefficient of variation (CV%) of 125.32%, 45.688%, 36.578%, 23.50% and 26.269% were observed for yield, number of plagiotropicos branches (NRPla), number of Ortotropicos branches(NRort), Cercospor and Vigor, respectively for Robusta. While, for Conilon variety, the highest CV% of 72.75%, 45.53%, 33.27% and 30.25% were recorded for yield, number of plagiotropicos branches (NRPla), rust and number of Ortotropicos branches(NRort), respectively. For Robusta variety, the highest phenotypic variance of 1018.85, 674.9, 623.2146, and 222.7696 were obtained for canopy diameter(Dco), plant height, yield (Sac/ha) and plagiotropicos branches (NRPla), respectively. While for conilon variety,



plagiotropicos branches (NRPLa), canopy diameter (Dco), plant height (API) and yield (Sac/ha), showed 839.93, 723.21, 624.016 and 487.39 phenotypic variance, respectively. The genetic correlation across measurements (accuracy) over years ( $r_{\text{med}}$ ) ranged from 0.1938 for yield (Sac/ha) to 0.8682 for Cercospora (Table 2.) for Robusta clones. While, for conilon the accuracy ranged 0.0441 for yield (sac/ha) to 0.9553 for Cercospora.

The most important function of heritability in the genetic studies on the metric characters, according Falconer (1960), is its predictive capacity and the expression of the confidence of the phenotypic values as a guide for the genetic value. However, in this study the estimates of individual heritability in the broad sense ( $h^2_g$ ) were of low magnitude, but were significant for all traits except yield (Sac/ha). Rodrigues W.P. et al (2013) also found similar results for *Coffea arabica*. The estimated repeatability for most of the traits was lowest indicating the irregularity of the superiority of the individuals among the measurements for these characters in the case of both groups of clones showing high irregularity of the performance across measurement, which demonstrate that genotype selection based on those traits is not reliable strategy. The progress expected with the selection depends on the heritability of the character, intensity of selection and phenotypic standard deviation of the character (Cruz and Carneiro, 2004). Thus, the values of heritability and repeatability achieved in this study allow the prediction of better possibilities of genetic gain (Table 1). This also implies that the selection process also provide satisfactory results for all traits (except yield and cercospora) which are economically important characters for *Coffea canephora* species.

### **3.2. Correlation among traits**

The study of correlation provide the information that how strong traits are genetically associated with one other. Thus through the estimate of genotypic and phenotypic correlation among yield components, it paved the basis for selection of superior genotypes from the diverse breeding populations. Therefore, the present study was undertaken to find association of different characters of *Coffea canephora*, Robusta

and Conilon varieties. Correlation among 11 traits was studied using 69 genotypes of Conilon and 52 Robusta varieties (Table 3).

Correlation between yield and other plant traits were computed. The result showed that positive and significant genotypic correlation were observed for vigor, diameter of stem (DCO), diameter of callus (DCA), fruits size (TFr), number of plagiotropic branches (NRPLa) with yield for Conilon variety suggesting that selection for these traits can result in more productive plants. The strong association of stem diameter with yield may be related with a greater capacity of the plants to take up water and nutrients from the soil, resulting in greater vigor and favorable performance of the yield component. Out of the 11 characters yield and vigor showed significant correlation with majority of the traits indicating that these characters are interrelated and they can be jointly considered for selection program in Conilon variety. Anim-Kwapong Esther et al. (2010) also found the interrelation of yield with most of the vegetative and reproductive plant parts in *Coffea canephora*. Most of the traits also showed negative correlation with rust and cercospora indicating that better resistance of this variety (Table 3).

Positive correlations were observed between plant height and stem diameter, plant height and number of plagiotropic branches and stem diameter and number of plagiotropic branches. Freitas (2004) reports correlations between stem diameter and plant height, number of branches, length of primary branches and number of internodes for arabica coffee. Similarly, Miranda et al. (2005) found correlations between yield and vegetative traits for crosses between Yellow Catuaí and Timor Hybrid and concluded that the vegetative attributes that contributed most to increased productivity were the length of plagiotropic branches, plant height and stem diameter.

For Robusta variety, yield was positively and significantly correlated with vigor, NRPLa, APL, DCO and DCA, which suggests that yield per plant would increase with these characters. Sureshkumar et al. (2013) also reported similar results in *Coffea canephora*. It is also observed that correlation between NRort and DCA was significant but negative suggesting that there is a negative genetic influence involved in the relationships of these variables. Thus, selection for higher NRort will directly select against DCA. On the other hand, Plant vigor was positively and significantly

associated with NRPla, APL, DCO and yield indicating that vigorous of plants expressed in these traits. Montagnon et al. (2001) noted that for young tree Robusta coffee, vigor was best correlated to competition effects, ie, vigorous clones were more aggressive than others. Aggressiveness of clones is reflected either in completion for their neighbor and or stimulating or promoting yield (Montagnon et al; 2001). Similarly, Leroy et al. (1997) reported that more vigorous young plant reflected high yields of Robusta coffee. Generally, fruit maturity showed negative association with most of the traits for both varieties indicating earliness of the varieties.

### **3.3. Simultaneous selection of characters and mean performances**

Using REML/BLUP, the selection gains (SG) can be obtained directly from the BLUP predictions of the progenies, since these reflect the estimated genotypic values already adjusted to the fixed environmental effects. In percentages, the SG ranged from 3.71% to 2.56% (Conilon variety) whereas, the variation was 15.39% to 6.60% (for Robusta variety) among agronomic traits based on the selection intensity of the best 20% (Table 4 and 5). For Conilon variety, response due to index selection was largest for 207 and 24 and lowest for 304 and 306. While, for Robusta variety, response due to index selection was largest for 1018 and 4021 and lowest for 401 and 4022 genotypes. Moreover, 50% of genotypes accounted for more than 40% of total advance.

When expected gains were computed as percentage of the means, genotypes responded less to selection using index. It was observed that the SGs for Conilon variety were lower than for the Robusta, but the gain was positive for both. High gains from selection for Robusta indicated the great potential of improvement expected for this variety. The lower gains in the desired direction for clones 306 and 4022 was explained by the negative genetic association between the agronomic traits. However, the negative genetic correlation does not necessarily imply that progenies combining good agronomic performances cannot be obtained. Since this correlation is determined by the action of linked genes, success in simultaneous selection could be achieved by evaluating a larger number of traits to raise the chances of finding promising recombinant genotypes within the population (Falconer and Mackay, 1996).

#### 4. DISCUSSION

Generally, the low the genetic coefficient of variation, characterizes the existence of low genetic variability between genotypes for both varieties, showing that the greater part of the total variation is due to non-genetic parameters. Resende et al. (2001) reported similar results for *Coffea arabica*. The high and very high values of CV% observed here are partly due to the ample variation for these traits in the treatments, above all these traits also seems to be very influenced by the environment which may have contributed to high CV%. According to these results, generally for both varieties, there was low interaction with year, as observed by the genotypic correlation across measurement ( $r_{gmed}$ ) for most of the characters evaluated, demonstrated that selection can be performed at any of the development stages used for measurement.

Since the environmental variance obtained in this study was low in relation to phenotypic variance, it can be concluded that genotypes with better yield will have the same response at each season while maintaining predictability in the face of environmental variations. However, the genotypic correlation across the measurement was low for both varieties for yield (sac/ha). As the environment has strong influence on productivity, the highest coefficient of variation was obtained for both varieties implying that experimental precision in different harvests was very poor. Similarly, low selective accuracy ( $r = 0.1130$  for Robusta and  $r = 0.1301$  for Conilon) showed that there was weak relationship between predicted and actual values. In contrary to this, Resende and Duarte (2007) obtained high selective accuracy for *Coffea arabica* which results in surety in the selection of agronomically superior genotypes. This statistic was preferred to the experimental variation coefficient, because it does not only relies on the magnitude of the residual variance and number of replications, but also on the proportion between the genetic variations and residual nature associated with the character under question (Resende, 2002).

The repeatability represents the maximum value that heritability may achieve in the broad sense, since the genotypic variance used to estimate the repeatability is not only of genetic origin, but still masked by the variance components of the permanent environment and among individuals (Cruz et al, 2004). The study of correlation among

yield and yield contributing traits suggests that Vigor, DCO, DCA and TFR were the most important characters which possessed positive association with yield. Therefore, these characters could be utilized in breeding program to improve varieties for higher yield. Positive correlations were also observed between most vegetative characteristics and productivity. This shows that plants that have good initial development can provide good yields. Silvarolla et al. (1997) found a correlation between productivity and vegetative characters for Timor Hybrid. On the average of four harvests, the authors obtained a high phenotypic correlation of productivity gained by plant height and canopy diameter. Carvalho et al. (2010) reported that yield showed higher correlation with the number of plagiotropic branches, plant height and length of plagiotropic branches for *Coffea arabica* Timor Hibrid.

**Table 1. Estimates of Genetic Parameters for 11 traits of Coffea canephora var. Conilon evaluated at Oratorios, Minas Gerias.**

Parameters	VIG	FER	CER	NROrt	NRPla	APL	DCO	DCA	MAT	TFR	PROD
$V_g$	0.1741**	0.2058**	0.0101**	0.7358**	26.049**	73.994**	39.376**	2.0210**	0.1253**	0.1090**	4.3362
$V_{bloc}$	0.2550	0.0101	0.0023	0.1022	186.5053	116.9057	103.7963	3.4383	0.0092	0.0053	17.6138
$V_{gm}$	0.0195	0.0771	0.0005	0.0879	26.0499	10.7779	14.5471	2.0210	0.1172	0.0560	94.0555
$V_{perm}$	0.5152	0.0661	0.0043	1.8034	91.8197	300.5269	268.7035	2.0578	0.0117	0.0104	41.4706
$V_e$	1.3929	0.4890	0.0918	1.7929	509.5095	121.8568	296.7895	12.3663	0.2159	0.1825	329.9128
$V_f$	2.3566	0.8482	0.1089	4.5221	839.9342	624.0167	723.2139	21.9045	0.4792	0.3632	487.3890
$h^2_g$	0.0739 ± 0.0232	0.2426 ± 0.0421	0.0926 ± 0.0296	0.1627 ± 0.0476	0.0310 ± 0.0295	0.1185 ± 0.0294	0.0544 ± 0.0199	0.0923 ± 0.0522	0.2615 ± 0.0509	0.3002 ± 0.0545	0.0089 ± 0.0081
R	0.4007	0.3325	0.1529	0.5841	0.3624	0.7875	0.5695	0.3432	0.3051	0.3433	0.1301
$C^2_{bloc}$	0.1082	0.0120	0.0208	0.0226	0.2220	0.1873	0.1435	0.1570	0.0192	0.0145	0.0361
$C^2_{gm}$	0.0083	0.0909	0.0043	0.0194	0.0310	0.0173	0.0201	0.0923	0.2445	0.1543	0.1930
$C^2_{perm}$	0.2186	0.0779	0.0394	0.3988	0.1093	0.4816	0.3715	0.0939	0.0244	0.0287	0.0851
CVg(%)	9.659	17.174	5.86439	13.5831	6.64836	6.67	5.73	5.1693	0.4130	19.5226	10.169
CVe (%)	21.03048	33.267306	16.61545	30.25788	45.53157	11.5776	15.38247	16.57433	0.3460	23.42	72.75
$r_{gmed}$	0.8993	0.7274	0.9553	0.8933	0.5000	0.8728	0.7302	0.5000	0.5168	0.6605	0.0441
Accuracy	0.27	0.52	0.30	0.160	0.176	0.344	0.233	0.304	0.511	0.547	0.094
Mean	5.7264	2.2773	2.0513	5.6357	49.1530	133.7517	123.4819	22.8655	2.0234	1.9249	28.5825

\*\* = Significant at 1% and 5% respectively (t test),  $V_g$  = genotypic variance,  $V_{gm}$  = Variance of progenies X measurement interaction,  $V_{perm}$  = variance of permanents effects,  $V_e$  = Residual variance,  $V_f$  = phenotypic variance,  $h^2_g$  = broad sense heritability,  $r$  = coefficient of individual repeatability,  $C^2_{gm}$  = coefficient of determination of general combining abilities in pop,  $C^2_{perm}$  = Coefficient of determination of permanents effects,  $r_{gmed}$  = genotypic correlation across the measurements, Vig = Vigor of the plant, Fer = reaction to rust, Cer = reaction to cercospera, NROrt = number of Ortotropics branches, DCA= diameter of stem, MAT= fruit maturity, NRPla= number of plagiotropics branches, Apl = plant height, Dco= Canopy diameter, TFr = fruit size, Prod. = production of fruits.

Table 2. Estimates of Genetic Parameters for 11 traits of *Coffea canephora* var. Robusta evaluated at Oratorios, Minas Gerias.

Parameter	VIG	FER	CER	NROrt	NRPla	APL	DCO	DCA	MAT	TFR	PROD
V <sub>g</sub>	0.1595*	0.0120	0.0388	0.6316	14.903*	164.056**	143.5347*	3.9513	0.1587	0.1059	28.7462
V <sub>bloc</sub>	0.1462	0.0001	0.0000	0.0162	3.8502	34.4099	85.7669	1.0420	0.0022	0.0013	18.6498
V <sub>gm</sub>	0.1346	0.0231	0.0059	0.1077	14.9043	33.4657	83.9416	3.9513	0.0943	0.0489	119.5791
V <sub>perm</sub>	0.8612	0.0006	0.0269	0.8213	28.7705	479.9266	445.1732	3.8842	0.0022	0.0031	23.0217
V <sub>e</sub>	1.2054	0.1383	0.1328	1.0893	160.3403	111.8152	260.4378	23.1055	0.1584	0.0919	433.2178
V <sub>f</sub>	2.5068	0.1742	0.2044	2.6660	222.7696	823.6749	1018.854	35.9344	0.4158	0.2510	623.2146
h <sup>2</sup> <sub>g</sub>	0.0636 ±	0.0690 ±	0.1897 ±	0.2369 ±	0.0669 ±	0.1992 ±	0.1409 ±	0.1100 ±	0.3817 ±	0.4217 ±	0.0461 ±
R	0.0291	0.0303	0.0566	0.0774	0.0584	0.0514	0.0432	0.0746	0.0828	0.0871	0.0247
C <sup>2</sup> <sub>bloc</sub>	0.4655	0.0734	0.3216	0.5510	0.2133	0.8236	0.6620	0.2471	0.3922	0.4392	0.1130
C <sup>2</sup> <sub>gm</sub>	0.0583	0.0008	0.0002	0.0061	0.0173	0.0418	0.0842	0.0290	0.0052	0.0052	0.0299
C <sup>2</sup> <sub>perm</sub>	0.0537	0.1327	0.0288	0.0404	0.0669	0.0406	0.0824	0.1100	0.2268	0.1948	0.1919
CV <sub>g(%)</sub>	0.3436	0.0036	0.1317	0.3081	0.1291	0.5827	0.4369	0.1081	0.0053	0.0122	0.0369
CV <sub>e(%)</sub>	11.40	3.6686	9.9271	19.498	17.395	10.737	10.006	5.714	14.1652	14.2763	11.76
r <sub>gmed</sub>	23.50	26.26	19.155	36.58	45.69	12.83	16.842	19.46	11.122	9.56	125.32
Accuracy	0.5423	0.3420	0.8682	0.8544	0.5000	0.8306	0.6310	0.5000	0.6272	0.6841	0.1938
Mean	0.252	0.417	0.4355	0.487	0.559	0.446	0.3753	0.332	0.6178	0.649	0.215
Mean	5.7305	1.3361	2.0309	3.635	24.734	140.4677	128.2993	24.9249	2.5459	2.5064	23.6730

\*, \*\* = Significant at 5% , 1%, respectively (t-test), V<sub>g</sub> = genotypic variance, V<sub>gm</sub> = Variance of progenies X measurement interaction, V<sub>perm</sub> = variance of permanents effects, V<sub>e</sub> = Residual variance, V<sub>f</sub> = phenotypic variance, h<sup>2</sup><sub>g</sub> = broad sense heritability, r = coefficient of individual repeatability, C<sup>2</sup><sub>gm</sub> = coefficient of determination of general combining abilities in pop, C<sup>2</sup><sub>perm</sub> = Coefficient of determination of permanents effects, r<sub>gmed</sub> = genotypic correlation across the measurements, Vig = Vigor of the plant, Fer = reaction to rust, Cer = reaction to cercospera, NROrt = number of Ortotropics branches, DCA= diameter of stem, MAT= fruit maturity, NRPla= number of plagiotropics branches, Apl = plant height, Dco= canopy diameter, TFr = fruit size, Prod. = production of fruits.

**Table 3. Genotypic correlations for *Coffea canephora* var. Conilon (above diagonal) and var. Robusta (below the diagonal), economic weight of the linear selection index and direction of selection for the variables.**

Traits	VIG	FER	CER	NROrt	NRPla	APL	DCO	DCA	MAT	TFR	PROD	Eco.Wt Conilon	Sense of seletion
VIG	1.0000	-0.3034**	-0.5946**	0.1428	0.7181*	0.669**	0.689*	0.668*	0.0485	0.1223	0.5845*	0.1929	high
FER	-0.0324	1.0000	0.5145*	0.1903	0.0326	-0.0964	-0.1350	-0.2264	-0.343*	0.1561	-0.2470	-0.0815	low
CER	-0.2632	0.0156	1.0000	0.0308	-0.3283*	-0.357*	-0.3399*	-0.362*	-0.0539	-0.0242	-0.4920*	-0.1623	low
NROrt	0.1636	0.1984	0.0248	1.0000	0.3324*	0.4953*	0.2733	0.0060	-0.2349	0.2113	-0.0976	-0.0322	low
NRPla	0.6620*	-0.0680	-0.1607	0.1936	1.0000	0.6447*	0.6309*	0.6096*	-0.1804	0.2537	0.3990*	0.1316	high
APL	0.7329*	-0.1140	-0.1976	0.2413	0.6728*	1.0000	0.8336*	0.5891*	-0.1903	0.3931*	0.4948*	0.1633	low
DCO	0.8438*	-0.0718	-0.2378	0.1812	0.6404*	0.8714*	1.0000	0.6936*	-0.1861	0.2504	0.5352*	0.1766	high
DCA	0.6485*	-0.2095	-0.2721	-0.367*	0.4879*	0.6499*	0.6836*	1.0000	-0.0889	0.0322	0.5325*	0.1757	high
MAT	-0.0541	-0.1929	-0.1650	-0.0828	-0.1928	-0.1178	-0.1816	-0.1499	1.0000	-0.325*	-0.0661	-0.0218	Low
TFR	-0.1047	-0.2196	0.1522	-0.0887	-0.1580	-0.0108	-0.0539	-0.0678	0.3067*	1.0000	0.3875*	0.1279	High
PROD	0.5075*	0.1160	-0.0862	-0.0342	0.4717*	0.4222*	0.5231*	0.5553*	-0.1329	0.0548	1.0000	0.3299	High
Eco. Wt Robusta	0.1494	0.0341	-0.0254	-0.0101	0.1388	0.1243	0.1540	0.1635	-0.0391	0.0161	0.2944		

\*\* = significance at 1% and \* = significance at 5% (t -test), Vig = vigor of the plant, NProd = Record of Production of fruits, Fer = reaction to rust, Cer = reaction to cercospera, NROrt = number of Ortotropicos branches, NRPla = number of plagiotropicos branches, Apl = plant height, DCO = Canopy diameter, TFR = fruit size, Prod. = production of fruits, DCA= diameter of stem, MAT = fruit maturity. Eco Wt = Economic weight.



**Table 4. Genotypic values and linear selection index for clones of *Coffea canephora*, Var. Conilon**

Genotype	Genetic values											Index	Gain	Gain%
	VIG	FER	CER	NROrt	NRPla	APL	DCO	DCA	MAT	TFR	PROD			
207	6.07	2.09	1.99	5.04	50.14	135.19	126.68	24.16	2.18	2.02	29.99	40.55	40.55	3.71
24	6.23	1.69	1.99	5.68	50.93	145.55	133.22	24.91	1.69	1.90	29.57	40.54	40.54	3.70
305	6.24	1.93	2.01	5.73	54.14	140.03	128.37	23.83	2.26	1.97	29.38	40.48	40.52	3.65
3034	5.98	2.69	2.02	5.22	50.57	139.09	123.53	22.82	1.79	2.61	29.82	40.25	40.46	3.48
3036	6.00	2.41	2.06	5.87	51.35	139.90	128.40	22.74	1.78	2.32	29.34	40.16	40.40	3.33
2021	5.94	3.25	2.04	7.63	55.39	143.14	127.18	23.69	1.70	2.21	28.49	40.15	40.36	3.22
2032	6.08	2.62	1.98	5.46	50.62	144.04	129.16	24.18	1.93	1.96	29.31	40.13	40.32	3.14
203	6.05	2.29	1.99	5.54	51.20	133.58	129.33	23.29	2.28	1.90	29.00	39.99	40.28	3.03
2027	5.80	2.52	2.19	6.04	51.59	139.30	128.74	23.35	1.78	2.13	28.45	39.93	40.24	2.93
3026	5.97	1.92	2.01	5.35	50.37	133.94	123.64	25.16	1.81	1.48	29.22	39.91	40.21	2.85
301	5.88	2.20	2.02	4.98	49.32	134.03	124.49	22.91	2.09	2.14	29.55	39.83	40.17	2.76
2019	6.00	2.54	2.04	6.15	52.91	139.43	125.34	23.42	1.91	2.43	28.45	39.82	40.15	2.68
304	6.05	1.60	1.96	5.28	51.80	144.47	131.99	23.97	1.59	1.75	29.17	39.81	40.12	2.62
306	5.83	1.95	1.97	4.58	50.21	134.00	125.01	23.89	2.19	2.12	29.32	39.81	40.10	2.56

Vig = vigor of the plant, Ferr = reaction to rust, Cer = reaction to cercospera, NROrt = number of Ortotropicos branches, NRPla = number of plagiotropicos branches, Apl = plant height, DCO = Canopy diameter, TFR = fruit size, Prod. = production of fruits, DCA= diameter of stem, MAT = fruit maturity

**Table 5. Genotypic values and linear selection index for clones of *Coffea canephora*, Var. Robusta.**

Genotype	Genetic value											Index	Gain	Gain%
	VIG	FER	CER	NROrt	NRPla	APL	DCO	DCA	MAT	TFR	PROD			
1018	6.09	1.27	1.65	2.62	27.49	159.20	148.39	27.74	2.89	2.42	33.10	16.05	16.05	15.39
4021	5.94	1.37	2.47	3.83	28.86	144.29	133.46	25.75	1.92	2.72	33.60	15.71	15.88	14.15
1027	5.99	1.27	1.95	3.09	27.88	161.60	139.91	28.03	2.08	2.19	26.93	15.16	15.64	12.43
408	6.19	1.24	1.84	3.33	27.68	157.63	146.57	26.54	2.87	2.88	23.88	15.01	15.48	11.30
4027	5.99	1.33	2.01	3.94	25.19	145.83	136.34	26.18	2.06	2.78	26.03	14.68	15.32	10.14
4019	6.10	1.34	1.96	4.15	27.47	153.03	141.60	25.79	1.67	1.96	24.15	14.61	15.20	9.29
4031	5.88	1.31	2.02	3.12	25.67	140.98	127.54	25.79	2.88	2.51	25.84	14.50	15.10	8.57
106	5.84	1.66	1.90	4.63	25.43	146.90	135.57	24.82	1.93	2.16	28.82	14.41	15.02	7.94
4024	5.99	1.42	2.29	3.22	24.61	154.59	142.28	25.72	2.51	2.29	24.83	14.40	14.95	7.46
401	5.81	1.31	2.02	3.56	25.14	140.64	130.06	25.31	2.29	2.50	25.67	14.31	14.88	6.99
4022	5.92	1.30	1.88	3.48	25.74	143.24	133.46	25.61	2.37	2.48	23.50	14.29	14.83	6.60

Vig = vigor of the plant, Ferr = reaction to rust, Cer = reaction to cercospera, NROrt = number of Ortotropicos branches, NRPla = number of plagiotropicos branches, APL = plant height, DCO = Canopy diameter, DCA= diameter of stem, MAT = fruit maturity, TFR = fruit size, Prod. = production of fruits.

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## CHAPTER 2

### SNPs Based Molecular Diversity of *Coffea canephora*

#### ABSTRACT

Coffee offers one of the most widely drunk beverage in the world, and is a very important source of foreign currency income for many countries. *Coffea canephora* Pierre presents a great genetic variability, with one of the widest natural geographical distribution within the subgenus. As a result, the assessment of genetic diversity and genetic structure in *C. canephora* has important implications for breeding programs and conservation of its genetic resources. Newly developed single nucleotide Polymorphism (SNP) markers are effective in genetic diversity detection. The objective of this study was to analyze the genetic diversity and group *C. canephora* accessions, which were genotyped with SNPs molecular markers. In the present study, *C. canephora* germplasm consisting of 50 clones (24 Conilon and 26 Robusta) were used. Genetic diversity was investigated using 46074 polymorphic SNP markers covering the entire genome of *C. canephora*. The estimation of genetic similarity between each pair of individuals was calculated by the Jaccard coefficient using the program NTSYS pc2.1. A simplified representation of the similarity was obtained by constructing the dendrogram using UPGMA method (Unweighted Pair-Group Method using Arithmetic Mean). The optimal number of groups in the dendrogram was determined by the relative size of distances in the dendrogram. Thus, the first group was composed of clones 1, 5, 24, 6, 9, 14, 23, 22, 10, 17, 19, 18, 20, 2, 13, 11, 15, 7, 4, 3 and 21; the second group 8, the third group 12, the fourth 16, the fifth group 26, 31, 33, 34, 37, 38, 28, 45, 46, 41 25, 29, 36, 43, 48, 49, 47, 50, 35, 39, 44, 40, 32, 30, and the sixth composed by 27 and 42. Generally, the analysis showed that the *C. canephora* clones were clearly divided into diversity groups that can be used for further breeding programs.

Key words: genetic diversity, genetic similarity, molecular markers

## **Análise da diversidade molecular de *Coffea canephora* baseado em SNP**

### **RESUMO**

O café consiste em uma das bebidas mais consumida no mundo, e é uma fonte muito importante de renda para muitos países. *Coffea canephora* Pierre apresenta uma grande variabilidade genética, com uma das mais amplas distribuições natural geográfica dentro do subgênero. Em vista disso, a avaliação da diversidade genética e estrutura genética em *C. canephora* têm implicações importantes para programas de melhoramento genético e de conservação dos recursos genéticos. Marcadores de polimorfismo de nucleotídeo único recém-desenvolvido (SNP) são eficazes na detecção de diversidade genética. Assim, o objetivo deste estudo foi a análise da diversidade genética e agrupamento dos acessos de *C. canephora*, que foram genotipados com marcadores moleculares SNPs. No presente estudo, foram utilizados um germoplasma *C. canephora* consistindo de 50 clones (24 pertencentes a variedades Conilon e 26 pertencentes a Robusta). A diversidade genética foi investigada utilizando 4607 marcadores SNP polimórficos que cobrem todo o genoma do *C. canephora*. A estimativa da similaridade genética entre cada par de indivíduos foi calculada usando o coeficiente de Jaccard utilizando o programa NTSYS pc2.1. Uma representação simplificada similaridade foi obtida construindo dendrogramas pelo método UPGMA (Uweighted pair-Group Method with Arithmetic Mean) usando o programa NTSYS pc2.1. O número ideal de grupos foi determinado por um procedimento baseado no tamanho relativo de distância no dendrograma. Assim, o primeiro grupo foi composto pelos clones 1, 5, 24, 6, 9, 14, 23, 22, 10, 17, 19, 18, 20, 2, 13, 11, 15, 7, 4, 3 e 21; o segundo grupo pelo clone 8; o terceiro grupo pelo 12, a quarto pelo 16; o quinto grupo pelos 26, 31, 33, 34, 37, 38, 28, 45, 46, 41, 25, 29, 36, 43, 48, 49, 47, 50, 35, 39, 44, 40, 32 e 30; e o sexto pelos 27 e 42. Análise mostrou que os genótipos de *C. canephora* foram divididos em grupo de diversidade que pode ser utilizado em programas de melhoramento genético.

Palavras-chave: diversidade genética, marcadores moleculares, similaridade genética



## 1. INTRODUCTION

Coffee provides one of the most widely drunk beverages in the world, and is a very important source of foreign exchange income for many countries, and ranks second on international trade exchanges, representing a significant source of income to several developing countries in Africa, Asia and Latin America.

Coffee belongs to the Family, Rubiaceae. The genus *Coffea* L. comprises 104 taxa that have been identified so far. Commercial coffee production relies mainly on two species, *Coffea arabica* L. (63%) and *Coffea canephora* Pierre (36%). *C. arabica* is a natural allotetraploid ( $2n = 4X = 44$ ), and is self-fertile (Coste, 1989). Other species are diploids ( $2n = 2x = 22$ ) and generally self-incompatible. Unlike *C. arabica*, plants of *C. canephora* does not need to grow at high altitude, requires less care to grow because it is hardier, and it tends to be less susceptible to pests and rough handling (Coste, 1989). Its area of distribution is variable and corresponds to hot and humid climatic regions.

Globally, the *C. canephora* gene pool is conserved in ex-situ collection plots in several countries. In cognizant of this fact and in order to alleviate the production problems, concerted effort were undertaken to utilize coffee canephora germplasm, as result several cultivars and accessions were collected and maintained at the different centers in Brazil. The main coffee germplasm collections are placed in governmental institutions, in São Paulo, at Instituto Agronômico de Campinas (IAC); in Espírito Santo, at Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper); in Minas Gerais, at the Universidade Federal de Viçosa (UFV), in a partnership with Empresa de Pesquisa Agropecuária de Minas Gerais (Epamig); and in Rondônia, at Embrapa-Rondônia. Furthermore, Espírito Santo and Rondônia response for 75% of *C. canephora* produced in Brazil. Embrapa-Rondônia has the particularity of containing a significant number of accessions resulting from exchanges with the institutions mentioned before (i.e.: IAC, UFV and Incaper), besides an expressive sample of local genotypes (Souza and Santos 2009). Consequently, its variability is representative of the germplasm commercially grown and conserved in Brazil.

*C. canephora* Pierre presents a wide genetic variability, with one of the widest geographic natural distribution within the subgenus *Coffea* (Maurin et al. 2007). In view of this, characterization and evaluation of its gene pool is necessary for effective crop improvement programmes and for better conservation and management of genetic resources (Prakash et al., 2005). Assessment of genetic variability within and among arabica coffee populations using molecular markers such as RAPD, ISSR, ALFP, and SSR has been the subject of several studies (Aga et al., 2005; Agwanda et al., 1997; Anthony et al., 2002; Chaparro et al., 2004 and Lashermes et al., 1993). Similar analysis in *C. canephora* has paramount importance, especially for genetic materials from Brazil.

With the advent of SNP (Single Nucleotide Polymorphism) markers, the possibility of simultaneous analysis of a set of loci becomes more real. A SNP is created when a single nucleotide base in a DNA sequence is replaced with a different nucleotide base. The SNP markers are based on the most fundamental alterations of the DNA molecule, mutations in the bases of unique chain of nitrogenous bases (Adenine, Cytosine, Guanine and Thymine). The SNP are extremely abundant in genomes, studies show that there may be millions in an individual genome (Li et al., 2009).

The genetic variation of a quantitative trait is controlled by segregation of multiple genes described by the infinitesimal model of Fisher (1918), which assumes that the number of loci is infinitely large and each with small effect. The genetic variances of individual loci are so small that they cannot be investigated individually and necessary to analysis sets of these loci. Thus, the availability of dense marker maps has opened new opportunities for genetic evaluation of individuals with high accuracy. Hence, this study was designed to estimate the level of genetic diversity and relationship among and within the clones of *C. canephora* conserved at research station of UFV using SNPs markers which can capture the whole genetic variability and useful for future coffee improvement program.

## 2. MATERIALS AND METHODS

### 2.1. Plant materials

A total of 50 *C. canephora* genotypes including two varietal groups, 26 Robusta and 24 Conilon, maintained at the Coffee Germplasm Collection of UFV, Minas Gerais, Brazil, were used for this study (Table 6).

**Table 6. List of *Coffea canephora* clones used for genetic diversity study**

No.	Conilon (UFV code)	No.	Robusta (UFV code)
1	UFV513	25	UFV 3365-144
2	UFV 3627-31	26	UFV 3366-139
3	UFV 3628-2	27	UFV3373-36
4	UFV 3629-11	28	UFV3374-28
5	UFV 3629-25	29	UFV514
6	UFV3627-20	30	UFV3356-71
7	UFV3627-24	31	UFV3357-93
8	UFV 3627-27	32	UFV3358-88
9	UFV 3627-29	33	UFV3360-169
10	UFV 3627-30	34	UFV3361-148
11	UFV 3628-1	35	UFV3363-118
12	UFV 3628-3	36	UFV3366-134
13	UFV 3628-5	37	UFV3367-101
14	UFV 3628-16	38	UFV3368-58
15	UFV 3628-29	39	UFV3370-47
16	UFV 3628-37	40	UFV3371-19
17	UFV 3628-45	41	UFV3373-43
18	UFV 3629-4	42	UFV3374-29
19	UFV 3629-7	43	UFV3375-65
20	UFV 3629-10	44	UFV3376-8
21	UFV 3629-17	45	UFV3630-2
22	UFV 3629-27	46	UFV3631-1
23	UFV 3629-29	47	UFV 3631-6
24	UFV 3629-30	48	UFV3356-74
		49	UFV3631-9
		50	UFV3631-13

## 2.2. Genotyping with SNP markers

For DNA extraction, young and completely extended leaves were collected from each accession, frozen at -80 °C, lyophilized, ground to make a fine powder and kept at -20 °C until used. Genomic DNA was extracted using the method described by Diniz et al (2005). SNPs were genotyped in Florida, USA using the RAPiD-Seq technology platform.

## 2.3. Statistical Analysis

The genetic similarity ( $S_{g_{ij}}$ ), between pairs of *C. canephora* individuals was calculated using the Jaccard coefficient. The similarities were calculated employing 4607 SNPs markers, using the following expression:

$$S_{g_{ij}} = \frac{a}{a + b + c}$$

where:

a = Number of cases in which the presence of the band occurs on both subjects simultaneously;

b = Number of cases in which the presence of the band occurs only in individual i;

c = Number of cases in which there is only the presence of the band in the individual j;

A simplified representation of the similarities was obtained by constructing dendrogram by UPGMA method (Unweighted Pair-Group Method with Arithmetic mean) (Sneath and Sokal, 1973). The analysis of similarity and clustering were performed with software using the Software NTSYS pc2.1 program (Numerical taxonomy and multivariate analysis system in PC) (Rohlf, 2000). By the method of Mojena (1977), the dendrogram was cut at the point of  $\theta = 0.44$  (general criteria:  $\theta - k = \alpha \pm 1.25\sigma - \alpha$ ). Where,  $\theta$  = correction for trend lag in stages,  $\alpha$  = value of criterion in stages (unbiased standard deviation) and  $K$  = mean of  $\alpha$  distribution.

### 3. RESULTS

#### 3.1. Genetic diversity among the genotypes

Measurements of genetic diversity in crops have important implications for plant breeding programs and the conservation of genetic resources. In the present study, genetic variation among *C. canephora* clones was analyzed.

The relationships among *C. canephora* accessions were evaluated with UPGMA clustering technique (Figure 1). The greatest similarity was between 34 and 31 (98%). The cophenetic correlation coefficient was  $r = 0.98$ , indicating a good fit. By the method of Mojena (1977) it is observed that it is possible to cut in the dendrogram at the point of  $\theta = 0.44$  (general criteria:  $\theta - k = \alpha \pm 1.25\sigma - \alpha$ ), indicating that the ideal number of groups should be equal to six. Hence, the dendrogram divided the genotypes into six groups, with group I composed of twenty one clones; group II composed by only one ; and group III composed only one, the fourth is composed by one clone; the fifth consists of by twenty four; and the sixth by two clones (Table 7). Based on the similarity matrix and dendrogram, we can infer that there is great genetic diversity among clones of *C. canephora* and that these genetic materials are promising for use in breeding programs. However, not all clones from the same varietal group were clustered in just one group.

According to the result, genotypes which showed the most similarity pair were 34 with 31 and 33, 31 with 33, 49 with 48, 46 with 45 (Table 8). Moreover, the phenotypic data from field evaluation showed that low variability among clones. On other hand, SNPs markers were able to classify the two varieties clearly implying that Robusta and Conilon are divergent heterotic groups with complementary characteristics. Thus, this marker revealed a high degree of polymorphism, which allowed a satisfactory understanding of genetic diversity among the *C. canephora* accessions. Moreover, they allowed the proper identification of the main current phenotypic varietal groups and showed to be able to resolve doubts about the accession classification. This ability represents a great advantage, because the high intra-specific variability and the environmental effects produce a great

amount of types, which can hinder the identification of Robusta and Conilon based only on the phenotypic evaluation.

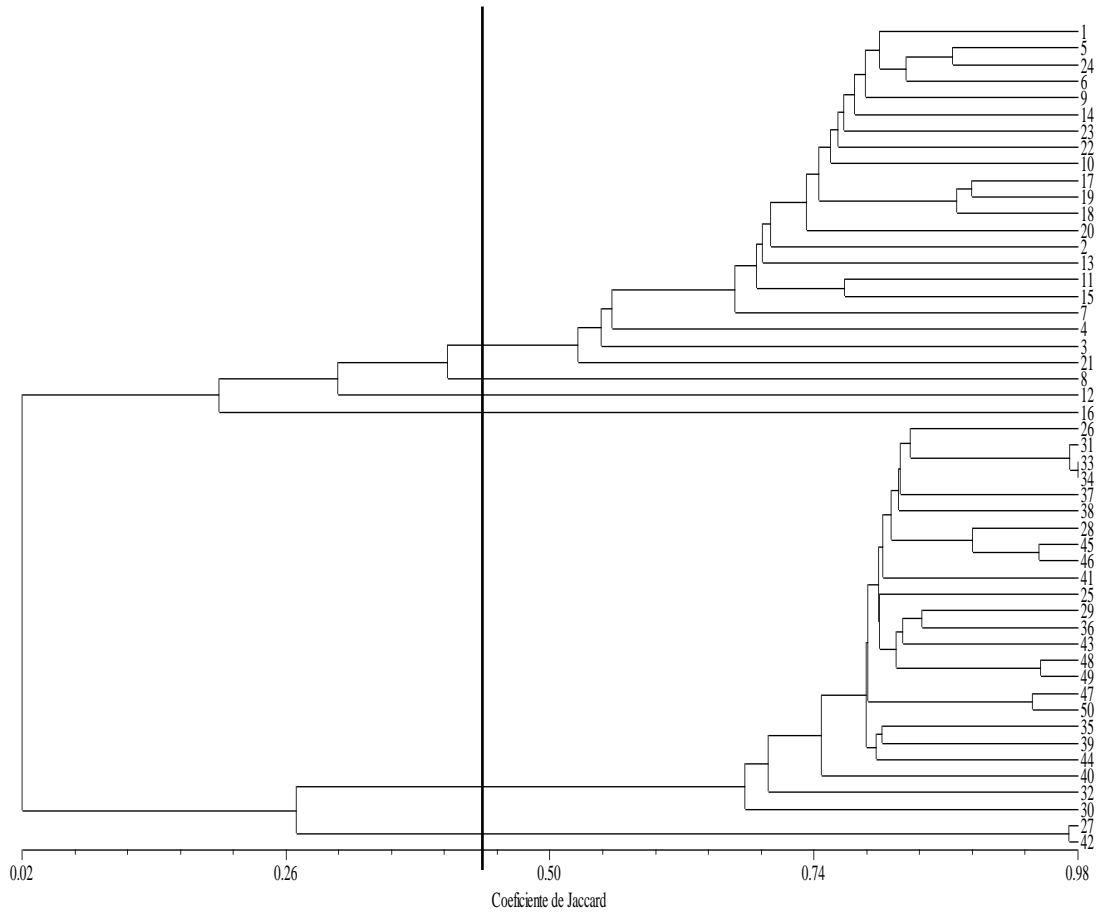


Figure 1. Dendrogram of genetic similarity among 50 cultivars *C. canephora*, obtained from SNPs markers, using the UPGMA method.

**Table 7. Grouping Coffea canephora clones using Unweighted Pair-Group Arithmetic Mean Method (UPGMA). 1 to 24 = Conilon and 25 to 50 = Robusta**

Groups	Genotypes
1	1, 5, 24, 6, 9, 14, 23, 22, 10, 17, 19, 18, 20, 2, 13, 11, 15, 7, 4, 3 and 21
2	8
3	12
4	16
5	26, 31, 33, 34, 37, 38, 28, 45, 46, 41, 25, 29, 36, 43, 48, 49, 47, 50, 35, 39, 44, 40, 32 and 30
6	27 and 42

**Table 8. Genetic similarity among 13 clones of *C. canephora* based on SNPs molecular markers calculated using the Jaccard coefficient**

<b>Clones</b>	<b>Clones</b>	<b>Jaccard Similarity coeficiente</b>
34	31	0.9889
34	33	0.9868
31	33	0.9854
49	48	0.9651
46	45	0.9618
46	28	0.9167
24	5	0.9157
19	17	0.9135
29	28	0.9125
18	17	0.9107
18	19	0.89917
36	29	0.8816
30	26	0.8813



#### 4. DISCUSSIONS

The present study adds a SNP markers system to the marker repertoire of *C. canephora*, which is cost-effective and highly flexible. It clearly revealed that there are dissimilarities between of Conilon and Robusta. Within Robusta variety, clones presented a more similarity, that is, almost grouped in group 5. However, Prakash et al. (2005) reported a high amount of diversity in a sample of Indian Robusta coffee. However, the Conilon showed more divergence. Thus, increasing the use of Conilon accession in breeding program would have a lot of benefits. Moreover, the exploitation of heterosis resulting from crosses of the two groups can be very advantageous. This strategy has been used successfully in some breeding programs around the world (Bouharmont et al. 1986, Leroy et al.,1997).

Furthermore, Conilon and Robusta have complementary characteristics. Robusta plants present high resistance to rust and nematodes, and give good beverage. Whereas, Conilon plants are tolerant to drought and they are easier to cultivate due to smaller size. So, these populations compose a perfect combination to use in a reciprocal recurrent selection program, as it has been already performed in Ivory Coast, since 1984(Leroy et al. 1993, 1994 and 1997). Although higher genetic gains could be obtained using Guinean versus Congolese strategy, considerably progress has been also achieved between Congolese subgroups (Leroy et al. 1993).

Even though, clones used in this study represent a minimal part of the natural variability of *C. canephora* germplasm, SNPs markers were able to classify the two clones clearly implying that Robusta and Conilon are divergent groups with complementary characteristics.

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## GENERAL CONCLUSION

A low genetic variability among the clones of Robusta and Conilon for all the evaluated traits but high residual coefficient of variation for most of the traits was recorded implying that these traits seem to be highly influenced by the environmental variation. But given the number of replications, high selective accuracy was achieved which allowed gains in several important traits. The strong association of stem diameter with production may be related with a greater capacity of the plants to take up water and nutrients from the soil, resulting in greater vigor and favorable performance of the yield component.

It was observed that the selection gains for Conilon variety were lower than for the Robusta, but the gain was positive for both. High gains from selection for Robusta indicated the great potential of improvement expected for this variety.

SNPs markers were able to classify clearly the two varieties into different groups implying that Robusta and Conilon are divergent heterotic groups with complementary characteristics. Moreover, they allowed the proper identification of the main current phenotypic varietal groups which can be used for coffee improvement programs. It could be recommended that using SNPs marker Brazilian coffee breeding programs, especially UFV and EPAMIG can capitalize the genetic diversity among *C. canephora* and utilize for further breeding program.

## APPENDIX

### Appendix 1. Origin of clones of Robusta variety

No.	Robusta (UFV code)	Origin of the clones
25	UFV 3365-144	T3581(2-2)
26	UFV 3366-139	T3751(1-1)
27	UFV3373-36	T3753(1-3)
28	UFV3374-28	T3754(1-1)
29	UFV514	-
30	UFV3356-71	T3564(1-1)
31	UFV3357-93	T3564(1-2)
32	UFV3358-88	T3564(1-3)
33	UFV3360-169	T3580(1-2)
34	UFV3361-148	T3580(1-3)
35	UFV3363-118	T3581(1-3)
36	UFV3366-134	T3751(1-1)
37	UFV3367-101	T3751(1-2)
38	UFV3368-58	T3751(1-3)
39	UFV3370-47	T3752(1-1))
40	UFV3371-19	T3752(1-3)
41	UFV3373-43	T3753(1-3)
42	UFV3374-29	T3754(1-1)
43	UFV3375-65	T3754(2-1)
44	UFV3376-8	T3755(1-1)
45	UFV3630-2	C. canephora IAC 1652-15
46	UFV3631-1	C. canephora IAC 2258. Apoatao
47	UFV 3631-6	C. canephora IAC 2258. Apoatao
48	UFV3356-74	T3564 (1-1)
49	UFV3631-9	C. canephora IAC 2258. Apoatao
50	UFV3631-13	C. canephora IAC 2258. Apoatao