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# Transferability of microsatellite loci in *Coffea canephora*

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**Abstract**

Coffee is one of the most important international trade commodities. Good prospects presented by the application of SSR markers to access the diversity in *Coffea*, and the small number of SSR primers available for *C. canephora*, encouraged the realization of this study. Thus, we tested the heterologous amplification of 71 microsatellite markers, originally developed for *Coffea arabica*, in *Coffea canephora*. Heterologous amplification occurred with 38 of the 71 primers. Approximately 52.63% of the primers used were polymorphic; 35% of the primer sets were classified as non-informative, 50% as moderately informative and 15% as highly informative. The loci evaluated by SSRCa 088, M793, SSRCa 054, SSRCa 085, CM5, M25, CFGA54 were low informative; SSRCa 084, SSRCa 052 and SSRCa 040 were highly informative. The validated primer sets can be used in molecular studies for different purposes once they were efficient in the genotypes discrimination.

**Keywords:** coffee; molecular characterization; molecular markers; SSR; transferability.
**Abbreviations:** Ho_Observed average heterozygosity**;** PIC_Polymorphism Information Content; SSR_Simple Sequence Repeat; UPGMA_Un-weighted Pair Group Method with Arithmetic Mean.

## Introduction

Although SSR markers are developed for use in a single species, it is possible to extend known markers for use in related species. This is possible because the flanking regions are conserved and the number of duplications is variable (Hendre and Aggarwal 2007; Wang et al., 2009). Therefore, once an SSR marker is available in a related species, attempting to transfer known markers can be advantageous (Sudheer et al., 2011). SSR markers are highly informative due to co-dominance, multiallelism, heritability, abundance and wide coverage of the genome. Another advantage is the conservation of flanking regions across generations, which allows repeated use of the technique (Powell et al., 1996; Yamamoto et al., 2002; Buso et al., 2003). The availability of new microsatellite markers is important to effectively contribute to genetic analysis and breeding programs for coffee (Hendre et al., 2008; Dereeper et al., 2013; Gartner et al., 2013; Romero et al., 2014). However, the development of microsatellite markers involves the development of SSR-enriched libraries. This process is still time-consuming and expensive (Cubry et al., 2014). Studies concerning the transferability of microsatellite markers are important in species that have few validated primers available. Transferability can be explained due to the evolutionary proximity between taxa (Hendre and Aggarwal, 2007; Wang et al., 2009). The good results with the use of SSR markers to access the diversity in *Coffea*, and the small number of primers available for *Coffea canephora*, encouraged this study was done. There is evidence that the allotetraploid *Coffea arabica* (2n=4x=44) originated by crossing diploids *C. canephora* (2n=2x=22) and *C. eugenioides* (2n=2x=22) from a recent event, less than 500,000 ya (Lashermes et al., 1999; Cenci et al., 2012). *C. arabica* is characterized by low genetic diversity (Lashermes et al. 1996), which is attributable to its autogamy. On the other hand, *C. canephora* is a diploid species with considerable genetic variability to be studied (Charrier and Berthaud, 1985; Lashermes et al., 1999). *C. canephora* presentes varietal groups named Robusta and Conilon. Robusta group consists of more vigorous genotypes, which have larger leaves and fruits, but are sensitive to drought. In contrast, Conilon consists of genotypes with small leaves and fruits, less vigorous, and greater drought tolerance (Ferrão, 2013). *C. arabica* and *C. canephora*, (Robusta and Conilon) are mainly used in commercial production of coffee, a really important global trade commodities (Salami et al., 2014). Thus, in this study, 71 microsatellite markers originally developed for *C. arabica* were used in heterologous amplification tests for *C. canephora* .

**Table 1.** List of *Coffea canephora* polymorphic SSR primer sets, observed heterozygosity (Ho), allele frequencies and average polymorphic information content (PIC).

| Locus | Allele frequency | | | | Ho | PIC |
|---|---|---|---|---|---|---|
| | A1 | A2 | A3 | A4 | | |
| M25 | 0.1429 | 0.8571 | - | - | 0.2857 | 0.2149 |
| M32 | 0.1667 | 0.1667 | 0.6667 | - | 0.6667 | 0.4491 |
| M47 | 0.3333 | 0.6667 | - | - | 0.3333 | 0.3457 |
| M20 | 0.625 | 0.375 | - | - | 0.25 | 0.3589 |
| SSRCa 018 | 0.1429 | 0.2143 | 0.6429 | - | 0.4286 | 0.4637 |
| SSRCa 021 | 0.25 | 0.75 | - | - | 0.1667 | 0.3047 |
| SSRCa 040 | 0.1429 | 0.5714 | 0.2143 | 0.0714 | 0.5714 | 0.5528 |
| SSRCa 052 | 0.0714 | 0.2143 | 0.4286 | 0.2857 | 0.4286 | 0.6261 |
| SSRCa 054 | 0.0833 | 0.9167 | - | - | 0.1667 | 0.1411 |
| SSRCa091 | 0.25 | 0.75 | - | - | 0.5 | 0.3047 |
| SSRCa 006 | 0.4167 | 0.5833 | - | - | 0.5 | 0.368 |
| SSRCa 084 | 0.5833 | 0.25 | 0.1667 | - | 0.5 | 0.5045 |
| SSRCa 085 | 0.1667 | 0.8333 | - | - | 0.3333 | 0.2392 |
| SSRCa 087 | 0.2 | 0.8 | - | - | 0 | 0.2688 |
| SSRCa 088 | 0.9286 | 0.0714 | - | - | 0.1429 | 0.1239 |
| SSRCa 095 | 0.2143 | 0.7857 | - | - | 0.4286 | 0.28 |
| M764 | 0.1667 | 0.75 | 0.0833 | - | 0.5 | 0.3633 |
| CM5 | 0.9 | 0.1 | - | - | 0.2 | 0.1638 |
| M793 | 0.0714 | 0.9286 | - | - | 0.1429 | 0.1239 |
| CFGA54 | 0.8333 | 0.1667 | - | - | 0.3333 | 0.2392 |

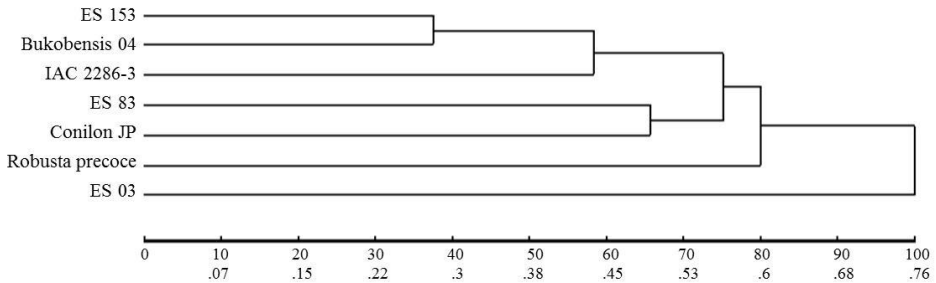


**Fig 1.** Dendrogram derived from UPGMA clustering analysis using Weighted index of SSR marker in seven genotypes of *C. canephora*.

**Results and Discussion**

Of the 71 pairs of SSR primers designed for *C. arabica*, 38 (53.52%) were successfully used for *C. canephora* and 20 primers were polymorphic. This result is of great importance for studies in *C. canephora*, since it provides 38 validated primers that can be used in genetic studies of this species (Supplementary table 1). Considering only the polymorphic primers, the average number of alleles/primer was 2.4 (2-4) in this study, which corresponds to a total of 48 alleles (Table 1). Other studies using SSR sequences in *C. canephora* have reported the number of alleles/primer as 3.78 (Hendre et al., 2008), 3.55 (Baruah et al., 2003), 1.86 (Missio et al., 2009), or 3.3 (Missio et al., 2010). This value varies as a function of both the genetic material used and the primer set. Although a sufficient amount of loci have been evaluated for the genotyping of seven genetic material of interest, these individuals do not represent the allelic pool of *C. canephora* species. Once the same loci are evaluated in more genotypes, the number of alleles detected probably will increase. Thirty-three (46.48%) evaluated primers did not amplify a fragment or did not meet a satisfactory standard for analysis of the fragments. Among the 38 primers that amplified a fragment, 18 (47.37%) showed a monomorphic pattern (Supplementary table 1). This means that the analyzed genetic material share the same alleles for these 18 loci evaluated. The inclusion of other genotypes in the analysis with these primers can allow different alleles are found in these loci. Therefore, even though the primers have not been effective at evaluating the genetic diversity of seven genotypes of this study, they can be very important for further research with other genetic materials. The mean PIC in the present study was 0.3218 (from 0.1239 to 0.6261), and the sorted primer set was moderately informative (0.5> PIC> 0.25) (Botstein et al., 1980). Individually, 35% of the primers were classified as having low informative value, 50% as moderately informative and 15% as highly informative for the set of genetic materials evaluated (Table 1). It is noteworthy that 65% of validated primers were moderately or very informative. Therefore, these become important sources to be applied in genetic studies in *C. canephora*, which still has a limited number of SSR primers developed information. The observed average heterozygosity (Ho) was 0.3439 (from 0 to 0.6667) (Table 1). According to the Ho, 19 loci of the 20

**Table 2.** List of the SSR primers that contributed the most to distinguish the seven genotypes of *C. canephora*.

| Markers | alleles present at each locus / number of genotypes in which it was found |
|---|---|
| SSRCa 018 | 33/3, 23/3, 11/1 |
| SSRCa 040 | 22/2, 23/3, 24/1, 11/1 |
| SSRCa 052 | 33/3, 24/2, 12/1, 44/1 |
| M25 | 22/5, 12/2 |
| M32 | 44/2, 34/1, 24/2, 14/2 |

**Table 3.** List of *Coffea canephora* genetic material used for cross-amplification tests.

| Genetic Material | Short Description |
|---|---|
| ES 03 | Characteristic features of Conilon, early maturation |
| ES 83 | Intermediate characteristic features of Conilon and Robusta, medium to late maturation |
| ES 153 | Characteristic features of Conilon, late maturation |
| Bukobensis 04 | Characteristic features of Conilon, early maturation |
| Robusta IAC 2286-3 | Characteristic features of Robusta, late maturation |
| Robusta Precoce | Intermediate characteristic features of Conilon and Robusta, very early maturation |
| Conilon JP | Characteristic features of Conilon, early maturation |

evaluated were considered polymorphic (Ho ≥ 0.1) (Susol et al., 2000; Cruz, 2011). The primers used were able to distinguish the genetic material of *C. canephora* (Fig 1). A high cophenetic correlation (r = 0.89) were found, indicating the existence of a great fit between the similarity matrix and the dendrogram. In the Genes software analysis, we found that among the 20 polymorphic primers, SSRCa 018, SSRCa 040, SSRCa 052, M25 and M32 were more informatives (Table 2). Those four loci contributed the most to distinguish the seven genotypes of *C. canephora*. The possibility of cross-amplification in the genus *Coffea* have already been demonstrated. Primers showed efficient cross-amplification in many *Coffea* and *Psilanthus* species (Combes et al., 2000, Baruah et al., 2003, Poncet et al., 2007). Therefore, we strongly recommend the use of these microsatellite sequences in studies who wish to access genetic diversity in Robusta plants.

## Material and Methods

### *Plant materials*

We used 7 genotypes *C. canephora* from the coffee breeding program of the Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper) (Table 3). The leaves of 3 plants/genotype used for DNA extraction were collected at the Experimental Farm of Bananal do Norte (FEBN), Cahoeiro do Itapemirim and at the Experimental Farm of Marilândia (FEM), Espírito Santo, Brazil.

### *Molecular procedures*

DNA extraction was performed according to the Doyle and Doyle (1990) protocol as modified by Abdelnoor et al., (1995). The quality and quantity of extracted DNA were tested on an 0.8% agarose gel. The cross-amplification test consisted of amplification using 71 microsatellite primers (Bioneer) originally developed for *C. arabica* species (Rovelli et al., 2000; Combes et al., 2000; Poncet et al., 2004; Coulibaly et al., 2003; Baruah et al., 2003; Moncada and McCouch 2004; Missio et al., 2009; Vieira et al., 2010). PCR reactions were performed in 20 µL, containing 2 µL 10x buffer, 150 µmol/L each dNTP, 0.1 µmol/L each primer, 50 ng of DNA, 1 mol/L of $MgCl_2$, and 0.6 U of Taq DNA polymerase (Fermentas), and the remaining volume was water (Missio et al., 2009). The amplification reactions with the SSR primer sets were performed on a Veriti 96 Applied Biosystems thermocycler using the touchdown PCR procedure proposed by Missio et al., (2009). The samples were run on a native polyacrylamide gel (6%) in the presence of 1X TBE buffer for 2 h 30 min at 100 volts. At the end of the run, the gels were stained in ethidium bromide solution (0.25 mg/mL) and photographed under ultraviolet light. Electrophoretic pattern analysis was used to assess the number and size of fragments that showed satisfactory amplification patterns.

### *Statistical analyses*

The results of the genotyping of microsatellite markers for *C. canephora* were recorded from coding the detected polymorphism of the loci in each genotype. The homozygotes were coded as 11, 22, 33 ... nn, according to allele showed, and for heterozygotes 12, 13, 23, ... nn (Cruz, 2008). The average number of alleles/primer, PIC, heterozygosity, and the loci that contributed most to the discrimination of genotypes were analyzed. The estimate of genetic dissimilarity was also made, according to the arithmetic complement of Weighted Index. The dissimilarity values obtained were arranged in matrices to be used in the cluster analysis by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The cophenetic correlation was calculated for information about the adjusting of the the original dissimilarity matrix with the dendrogram. Statistical analyses were performed using Genes software (Cruz, 2013).

## Conclusions

It was found that 38 SSR primers were capable of cross amplification in *C. canephora*. Among the primer sets, 20 loci used in the cross-amplification analysis were polymorphic, and the average heterozygosity (Ho) was 0.3439 (0 to 0.6667). The average PIC of the tested primers was 0.3218 (from 0.1239 to 0.6261). CFGA54, M25, SSRCa 085, SSRCa 088, M793, SSRCa 054, CM5 were classified as being of low informative value; SSRCa 021, SSRCa 087, M47, SSRCa 095, SSRCa 018, SSRCa 091, M764, SSRCa 006, M32 and M20 were moderately informative, SSRCa 084, SSRCa 040 and SSRCa 052 were highly informative. The findings of this study provided available and validated

primers for Robusta coffee. These primers will facilitate further studies on the species diversity, genetic mapping, identification and protection of varieties or clones, marker-assisted selection and establishing germplasm conservation strategies.

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