Crop Breeding and Applied Biotechnology 9: 67-74, 2009
Brazilian Society of Plant Breeding. Printed in Brazil

Genetic divergence in Conilon coffee revealed by RAPD markers

Maria Amélia Gava Ferrão[1*], Aymbiré Francisco Almeida da Fonseca[2], Romário Gava Ferrão[3], Wellington Marota Barbosa[4], and Elaine Manelli Riva Souza[3]

Received 01 September 2007

Accepted 13 May 2008

ABSTRACT – *This study aimed to evaluate the genetic variability of 49* Coffea canephora *clones of the breeding program of the Capixaba Institute of Research, Technical Assistance and Rural Extension (Incaper) based on RAPD markers. Thirty-one primers were used with polymorphism patterns that generated 333 markers, of which 231 (69.4%) were polymorphic. The group of genotypes based on the UPGMA algorithm and Tocher optimization methods detected high divergence in the genotypes. It was found that the component clones of each clone variety recommended by Incaper are distributed in various genetically dissimilar groups, in spite of common phenotypic traits. The relatively wide genetic diversity observed here demonstrates the importance of hybridizations between these germplasms. The RAPD markers efficiently estimated the genetic divergence in the 49* C. canephora *genotypes studied.*

Key words: *Coffea canephora*, clonal cultivars, Espirito Santo, genetic variability, genetic improvement.

INTRODUCTION

The state of Espirito Santo is the largest producer of *Coffea canephora* in Brazil, with an average production of 7.4 million bags (Conab 2007). The species is diploid (2n = 22 chromosomes), allogamous, with gametophytic self-incompatibility (Carvalho 1952, Conagin and Mendes 1961, Berthou et al. 1983, Charrier and Berthaud 1985).

The Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper) began studying *C. canephora* in 1985, investigating different areas, including plant breeding and molecular biology. The direct results of these studies enabled the development and deployment of six cultivars with important agronomic traits for coffee cultivation in the state of Espirito Santo. Five of these clonal cultivars were formed by the grouping of 9-14 compatible clones, and one seed-propagated cultivar, designated: a) clone cultivars: Emcapa 8111-early maturing, Emcapa 8121-medium maturing and Emcapa 8131 late-maturing (Braganza et al. 1993); Emcapa 8141-Robustão Capixaba (Ferrão et al. 2000a) and; Vitória-Incaper 8142 (Fonseca et al. 2004) and b) seed-propagated cultivar: Emcaper 8151- Robusta Tropical (Ferrão et al. 2000b).

Studies on the genetic improvement of coffee, a

[1] Embrapa Café/Incaper, Rua Afonso Sarlo, 160, Bento Ferreira, 29052-010, Vitória, ES, Brazil. *E-mail: mferrao@incaper.es.gov.br
[2] Embrapa Café, Parque Estação Biológica, Ed. Sede, s/nº, Av. W3 Norte (Final), 70770-901, Brasília, DF, Brazil
[3] Incaper (Instituto Capixaba de Pesquisa Assistência Técnica e Extensão Rural), Rua Afonso Sarlo, 160, Bento Ferreira, 29052-010, Vitória, ES, Brazil
[4] Escola Agrotécnica Federal de Machado, KM 3, 37750-000, Machado, MG, Brazil

perennial crop with a long juvenile period, are often time-consuming. This calls for the implementation of techniques to facilitate and accelerate the selection and evaluation of superior genotypes (Cabral 2001). In this approach, the detection and precise quantification of genetic variation is a prerequisite for success in the conservation and exploitation of genetic plant resources and the estimation of genetic gains in shorter periods.

Molecular markers at the DNA level have been largely used in the last years as a powerful tool in genetic plant improvement and studies, since they allow mapping genes of interest with their qualitative and quantitative traits, detection and quantification of polymorphism between individuals, the construction of genetic maps, among others, which facilitate indirect selection of desirable traits in early segregating generations.

Among the different molecular markers available, the RAPD (Random Amplified Polymorphic DNA) markers are widely used due to the ease of use, fast data collection, relatively low cost compared to other molecular techniques and immediate applicability to any type of organism (Caixeta et al. 2006, Lanza et al. 2000, Cruz and Milach 1998). This marker type, developed simultaneously by Williams et al. (1990) and Welsh and McClelland (1990), is based on the PCR (Polymerase Chain Reaction) technique and uses random sequence primers, which makes basic research in different directions easier, e.g., studies into the genetic structure and diversity in genebanks (Ferreira and Grattapaglia 1998).

In the genus *Coffea*, several studies have shown the efficiency of RAPD markers for the genotype characterization, assessment and identification and for genetic mapping (Maluf et al. 2005, Teixeira-Cabral et al. 2004, Silveira et al. 2003, Ruas et al. 2001, Fontes et al. 2000, Diniz et al. 2001, Lashermes et al. 1996, Orozco-Castillo et al. 1994). A disadvantage of this marker type is its dominance, which impairs the distinction of polymorphism of heterozygous from homozygous dominant genotypes. The presence of a certain band in the gel may be result of the amplification of the two alleles of a locus or of only one allele, respectively, in the case of diploid heterozygous and homozygous individuals. Little information can therefore be obtained per genetic locus. This feature may not be relevant in one study but represent a restriction that prevents a more detailed genetic analysis in another (Ferrão et al. 2007).

This study aims to assess the genetic divergence of 49 *Coffea canephora* genotypes of the Incaper breeding program through RAPD markers, of which 45 were components of the five clonal varieties developed by the Institute.

MATERIAL AND METHODS

Forty-five components genotypes of five clonal cultivars of conilon coffee developed and recommended by Incaper, along with four other genotypes were used here (Table 1). Leaf samples of healthy adult plants of each of these genotypes were collected on an experimental farm of Incaper in Marilândia and deep-frozen at -80 °C in a laboratory of molecular biology of Incaper, at the Centro Regional de Desenvolvimento Rural - Centre Serrano, Venda Nova do Imigrante, ES.

For DNA extraction, the protocol proposed by Doyle and Doyle (1990) was used with some modifications. Approximately two grams of leaf tissue were ground to a fine powder in a porcelain mortar containing liquid nitrogen and transferred to 2 mL tubes. Nine hundred mL extraction buffer previously heated in water bath to 65 °C was added, prepared with 5% CTAB, NaCl 5M, EDTA 0.5M, 1M Tris-HCl pH 8.0, 1% polyvinylpyrrolidone and 1% b-mercaptoethanol. The tubes containing the suspension were incubated in water bath for 40 minutes at 65 °C and turned upside down gently three times to ensure the homogenization of the solution.

After the incubation period, the samples were deproteinized twice, using chloroform: isoamyl alcohol (24:1) and centrifuged for 5 minutes at 14,000 rpm in an Eppendorf microcentrifuge, to remove fragments of leaf tissue, lipids and proteins. The supernatant was transferred to 1.5 mL tubes with 600 mL frozen isopropanol and maintained for three hours at -20 °C for precipitation of the nucleic acids. After centrifugation at 14,000 rpm for 10 minutes, the precipitated nucleic acids were recovered and washed twice with 70% ethanol and a third time with 95% ethanol. The precipitates were dried at room temperature, resuspended in TE buffer pH 8.0 (10 mM Tris-HCl + 1 mM EDTA) with RNAse A (40 mg mL^{-1}) and incubated at 37 °C for 30 minutes for RNA digestion. Thereafter DNA was precipitated with frozen absolute ethanol and centrifuged again at 14,000

rpm for separation from the aqueous phase. After another washing the DNA was finally resuspended in TE pH 8.0. The DNA was quantified by horizontal electrophoresis on 0.8% agarose gel, stained with ethidium bromide (0.5 mg mL^{-1}) for 30 minutes, destained in distilled water for 30 minutes and photographed using an Eagle Eye II (Stratagene) image system. DNA samples of good quality were diluted to10 hg mL^{-1} and stored at -20 °C.

The RAPD amplification reactions occurred in a volume of 25 mL, containing 25 hg DNA, 100 mM of each desoxinucleotide (dATP, dCTP, dGTP and dTTP); 2 mM $MgCl_2$; 10 mM Tris-HCl (pH 8.3); 50 mM KCl; 0.4 rM of a decamer primer (*USBiological – Biochemicals & Biological Reagents* and *Operon*) and one unit of Taq polymerase. The amplification reactions were performed in a thermocycler (GeneAmp PCR System 9700), according to Williams et al. (1990). The amplification consisted of a DNA denaturing step at 94 °C for 15 seconds, a linking stage of the primer to the DNA template at 35 °C for 30 seconds, and an extension step at 72 °C for one minute and a after 40 cycles a final extension step at 72 °C, for seven minutes.

The amplified products were separated by

Table 1. *Coffea canephora* genotypes used to study the genetic diversity

Genotype	Cultivar	Genotype	Cultivar
1. ES 01	Emcapa 8111, Emcapa 8141 Robustão Capixaba and Vitória Incaper 8142	26. ES 92	Emcapa 8131
2. ES 02	Emcapa 8111 and Emcapa 8141 Robustão Capixaba	27. ES 39	Emcapa 8131
3. ES 05	Emcapa 8111	28. ES 21	Emcapa 8111
4. ES 22	Emcapa 8111	29. ES 34	Emcapa 8111
5. ES 10	Emcapa 8111	30. ES 36	Emcapa 8111
6. ES 07	Emcapa 8111	31. ES 38	Emcapa 8111 and Vitória Incaper 8142
7. ES 09	Emcapa 8111	32. ES 31	Emcapa 8111
8. ES 37	Emcapa 8111	33. ES 85	
9. ES 08	Emcapa 8111	34. ES 77	
10. ES 25	Emcapa 8121	35. ES 40	Emcapa 8111
11. ES 12	Emcapa 8121	36. ES 125	Vitória Incaper 8142
12. ES 15	Emcapa 8121	37. ES 113	Vitória Incaper 8142
13. ES 14	Incaper 8121 and Emcapa 8141Robustão Capixaba	38. ES 102	Vitória Incaper 8142
14. ES 19	Emcapa 8121	39. ES 123	Emcapa 8141 Robustão Capixaba
15. ES 24	Emcapa 8121	40. ES 146	Emcapa 8141 Robustão Capixaba
16. ES 11	Emcapa 8121 and Emcapa 8141 Robustão Capixaba	41. ES 121	
17. ES 30	Emcapa 8121	42. ES 109	Vitória Incaper 8142
18. ES 23	Emcapa 8121 and Emcapa 8141 Robustão Capixaba	43. ES 212	Vitória Incaper 8142
19. ES 19	Emcapa 8121 and Emcapa 8141 Robustão Capixaba	44. ES 347	Vitória Incaper 8142
20. ES 20	Emcapa 8121	45. ES 346	Vitória Incaper 8142
21. ES 13	Emcapa 8121	46. ES 328	Emcapa 8141 Robustão Capixaba and Vitória Incaper 8142
22. ES 28	Emcapa 8121	47. ES 329	Emcapa 8141 Robustão Capixaba and Vitória Incaper 8142
23. ES 18	Emcapa 8121	48. ES 327	Vitória Incaper 8142
24. ES 27	Emcapa 8111	49. ES 309	
25. ES 26	Emcapa 8111		

horizontal electrophoresis on 1.2% agarose gel immersed in TBE buffer (90 mM Tris-borate, 1 mM EDTA, pH 8.0) at 100 volts for two hours and 45 minutes. The gel was stained with ethidium bromide (0.5 L) for 30 minutes and destained in distilled water for 30 minutes. The DNA fragments on the gel were visualized on a UV transilluminator, photgraphed and analyzed in an Eagle Eye II Image System (Stratagene).

To construct the binary matrix, 31 polymorphic primers were used with consistent bands, where 1 scored the presence and 0 the absence of a DNA band on the gel. The analyses were performed using the statistical program Genes (Cruz 2001), version 2006.4.1. The genetic dissimilarity matrix was based on the mathematical complement (Dii ') of the Jaccard Index (Sii'), and Dii '= 1 - Sii' and Sii '= a / (a + b + c), where a is the number of concordances of type 1 1; b is the number of disagreements of type 1 0; and c is the number of disagreements of type 0 1 (Cruz and Carneiro 2003). The genotypes were grouped by the Unweighted Pair-Group Method based on Arithmetic Averages (UPGMA) and the Tocher optimization method.

RESULTS AND DISCUSSION

The 31 polymorphic primers used (Table 02) amplified 333 bands, with an average of 10.7 bands / primer, and 102 (30.7%) monomorphic (3.3 bands / primer) and 231 (69.3%) polymorphic (7.4 bands / primer). The primers 23, 15 and 16 were the most polymorphic. This result shows the existence of genetic variability among genotypes and efficiency of RAPD markers in the sampled population under study. According to Duarte et al. (1999), the intraspecific polymorphism is dependent on the level of divergence in the genotypes studied. The high polymorphism found is most likely related to the reproductive system of the species (allogamous and self-incompatible), which generally generates a rather heterozygous population.

In the analysis of the genetic dissimilarity matrix obtained from the arithmetic complement of the Jaccard index, a mean genetic distance of 0.275 (± 0.001) was found for the different genetic combinations among the 49 *C. canephora* genotypes. The greatest distance was observed between genotypes 7 and 38 (0.398), so this is the most divergent pair. Considering the genetically

Table 2. Sequence of nucleotides of 31 primers that generated polymorphism patterns in the *C. canephora* clones analyzed

Primer code[1]	5' → 3' nucleotide sequence	Primer code[1]	5' → 3' nucleotide sequence
1. OPAF-03	GAAGGAGGCA	17. KIT 2-03	CATCCCCCTG
2. OPAF-07	GGAAAGCGTC	18. KIT 2-05	TGCGCCCTTC
3. OPAF-11	ACTGGGCCTC	19. KIT 2-07	GGTGACGCAG
4. OPAF-13	CCGAGGTGAC	20. KIT 2-11	GTAGACCCGT
5. OPAF-15	CACGAACCTC	21. KIT 2-17	AGGGAACGAG
6. OPAF-17	TGAACCGAGG	22. KIT 3-01	TTCGAGCCAG
7. OPAF-19	GGACAAGCAG	23. KIT 3-05	GATGACCGCC
8. KIT 1-01	CAGGCCCTTC	24. KIT 3-07	GTCCCGACGA
9. KIT 1-07	GAAACGGGTG	25. K IT 3-09	CTCACCGTCC
10. KIT 1-09	GGGTAACGCC	26. KIT 3-11	AAAGCTGCGG
11. KIT 1-11	CAATCGCCGT	27. KIT 3-15	GACGGATCAG
12. KIT 1-13	CAGCACCCAC	28. KIT 3-19	GTTGCCAGCC
13. KIT 1-15	TTCCGAACCC	29. KIT 4-03	GTCGCCCGTCA
14. KIT 1-17	GACCGCTTGT	30. KIT 4-05	TGAGCGGACA
15. KIT 1-19	CAAACGTCGG	31. KIT 4-07	TTGGCACGGG
16. KIT 2-01	GTTTCGCTCC		

[1]OP= Operon Technologies, Alameda, CA, USA; KIT 1 a 4 = USBiological, USA

most distant and most similar genotype pairs (Table 03), it was observed that genotype 38 was present in 7 of 10 pairs, indicating greater divergence than the others. The five most similar genotype pairs were 28 and 29 (90.9% similarity), 41 and 42 (90.7% similarity), 6 and 9 (88.8% similarity), 1 and 3 (86. 2% similarity), and 4 and 15 (83.1% similarity).

The dissimilarity measures used to group the 49 genotypes ranged from 0.0913 to 0.3445. The UPGMA-based genotype ranking is shown in the dendrogram (Figure 1). It was found that the component clones of each recommended variety were dispersed in the dendrogram, which shows the existence of genetic dissimilarity in the clones and the varieties concerned. The genotypes 38 (ES 102) and 21 (ES 13) emerged as the most dissimilar, followed by genotypes 7 (ES 09) and 18 (ES 23) and 49 (ES 309). These genotypes are part of five different recommended varieties: 38 (ES 102) is in the variety Vitoria - Incaper 8142, 21 (ES 13)in the variety Emcapa 8121, 7 (ES 09) in variety Emcapa 8111and 18 (ES 23) in the varieties Emcapa 8121 and Emcapa 8141.

By the Tocher optimization method, the genotypes were classified into four groups, and 46 genotypes (93.9%) allocated in Group I, which was divided into 17 subgroups (Table 4). Group II contained genotype 7, group III genotype 49 and group IV genotype 38. It was found that there was agreement between the UPGMA and the Tocher method in the formation of groups with a single unit, containing the genotypes 7 and 38. Genotype 38 was represented in the most divergent pairs, which showed once more the greater genetic distance of this from the other genotypes. These results corroborate the agronomic field characterization, where the plant architecture, leaf size and shape and disease response of genotype 38, considered here as the most dissimilar, was also more divergent compared to the other genotypes; genotype 21 is characterized as taller plant height, large grains, different leaf shape and responsive to irrigation and fertilization; genotype 18 stands out as drought-tolerant and 49 with large grain. On the other hand, the morpho-agronomicas traits of the less divergent genotype pairs selected at the same time and place were less distinct.

It is noteworthy that in the composition of clonal cultivars of Incaper compatible genotypes were selected that join agronomic traits of interest, good adaptation and yield stability.

Within this context, knowledge on the genetic divergence between the genotypes selected is essential to enable the maintenance of a broad genetic base in stands to avoid the harmful process of genetic erosion in the future. Therefore, the constitution of varieties should always be examined from this viewpoint, with a view to additional information for breeding programs aiming at hybrid combinations of greater heterotic effect (Fonseca 1999). Thus, aside from a number of common agronomic traits, clones of a same cultivar should have a dissimilar genetic constitution, to ensure greater safety and yield stability of the coffee used for planting (Ferrão et al. 2004).

In *C. arabica*, Maluf et al. (2005) found that the genetic variability detected by RAPD markers is very similar to that detected using the markers and RFLP and SSR, and that RAPD and SSR are the most efficient in parentage analyses. The lines were grouped according to their genealogical origin. Orozco-Castillo et al. (1994) using RAPD markers, observed a clear separation of the diploid species *C. canephora* and *C. liberica* from the different *C. arabica* groups (germplasm of Ethiopia, Bourbon and Typica).

Table 3. Values of the greatest and smallest genetic distances observed in 49 *Coffea canephora* genotypes

Description	Genotypes	Distances	Genotypes	Distances
Greatest distances	7 and 38	0.39382	38 and 45	0.37500
	10 and 38	0.38596	5 and 49	0.37226
	8 and 38	0.38078	20 and 38	0.36996
	30 and 38	0.37828	2 and 38	0.36782
	9 and 49	0.37545	27 and 49	0.36667
Shortest Distances	28 and 29	0.09132	16 and 23	0.17209
	41 and 42	0.09322	32 and 33	0.17241
	6 and 9	0.11159	36 and 39	0.18045
	1 and 3	0.13853	5 and 6	0.18333
	4 and 15	0.16863	3 and 28	0.18828

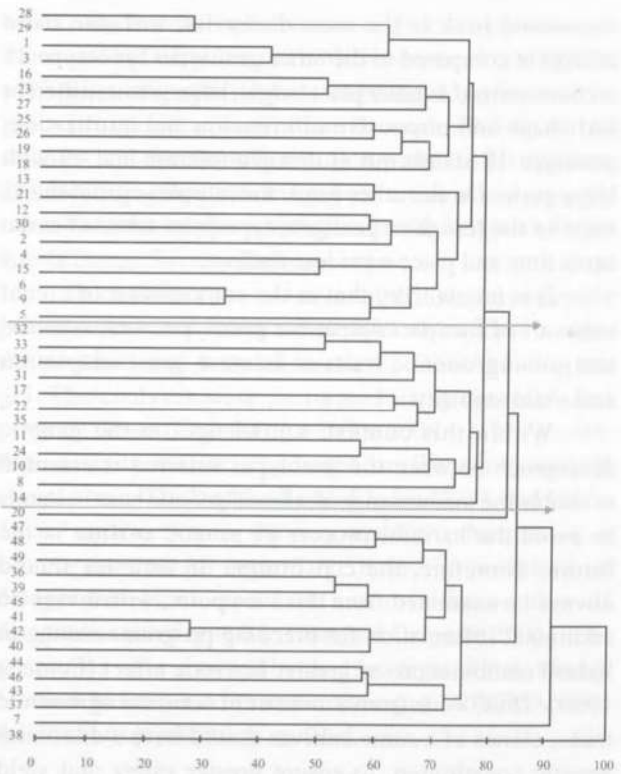

Figure 1. Dendrogram of genetic dissimilarity in 49 *Coffea canephora* accessions established by the Unweighted Pair-Group Method based on Arithmetic Averages (UPGMA), using the genetic dissimilarity matrix obtained by the arithmetic complement of the Jaccard index for binary data. Relative genetic distances on the X axis and number of genotypes on the Y axis

Table 4. Groups established by the Tocher optimization method, based on the genetic dissimilarity matrix obtained by the arithmetic complement of the Jaccard index for binary data, in 49 *Coffea canephora* accessions

Groups	Subgroups	Genotypes
I	I	28, 29, 25, 3 and 1
	II	41, 42, 40, 45, 47, 46, 39 and 36
	III	6, 9, 5, 23, 4, 15, 13, 12 and 30
	IV	32, 33, 34, 24 and 11
	V	43 and 44
	VI	17, 22, 26 and 35
	VII	19, 27 and 18
	VIII	2
	IX	21
	X	16
	XI	20
	XII	14
	XIII	8
	XIV	37
	XV	31
	XVI	48
	XVII	10
II		7
III		49
IV		38

Our results indicate that RAPD markers are promising to detect genetic variability in the group of genotypes analyzed and generate data for a direct application in improvement programs, such as the selection of the most divergent genotypes for cross scheduling. This knowledge on the distribution of genetic variability within and among the clonal *Coffea canephora* varieties is essential to determine strategies for germplasm improvement and conservation for Incaper. Simultaneously, 326 other genotypes of the Active Germplasm Bank of Incaper are being analyzed, based on morpho-agronomic and molecular tools.

ACKNOWLEDGEMENTS

The authors thank the Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café - CBP&D Café (Brazilian Consortium of Coffee Research and Development) for financial support and scholarships.

Divergência genética em café Conilon usando marcadores RAPD

RESUMO – *Marcadores moleculares têm sido utilizados para auxiliar pesquisas com recursos genéticos. Este estudo teve como objetivo avaliar a variabilidade genética, por meio de marcadores do tipo RAPD, de 49 clones de* Coffea canephora *do programa de melhoramento do Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper). Foram utilizados 31 primers com padrões de polimorfismo que geraram 333 marcadores, dos quais 231 (69,4%) apresentaram-se polimórficos. O agrupamento dos genótipos com base no algoritmo UPGMA e no método de otimização de Tocher mostrou*

elevada divergência entre os genótipos. Verificou-se que os clones componentes de cada variedade clonal recomendada pelo Incaper encontram-se distribuídos em vários grupos geneticamente dissimilar, apesar de possuírem características fenotípicas em comum. A diversidade genética relativamente ampla observada neste estudo demonstra a importância da realização de hibridações entre estes germoplasmas. Os marcadores RAPD foram eficientes para caracterizar a divergência genética entre os 49 genótipos de C. canephora *estudados.*

Palavras-chave: *Coffea canephora*, cultivares clonais, Espírito Santo, variabilidade genética, melhoramento genético.

REFERENCES

Berthou F, Mathieu C and Vedel F (1983) Chloroplast and mitochondrial DNA variation as indicator of phylogenetic relationships in the genus *Coffea* L. **Theoretical and Applied Genetics 65**: 77-84.

Bragança SM, Carvalho CHS, Fonseca AFA da and Ferrão RG (1993) Emcapa 8111, **Emcapa 8121, Emcapa 8131: primeiras variedades clonais de café conilon lançadas para o Estado do Espírito Santo**. Vitória, ES: EMCAPA. (EMCAPA, Comunicado Técnico, 68).

Cabral TAT (2001) **Padrões moleculares, diversidade genética e mapa parcial de ligação do cafeeiro.** Tese de Doutorado, Universidade Federal de Viçosa, Viçosa, 110p.

Caixeta ET, Oliveira ACB, Brito GG and Sakiyama NS (2006) Tipos de marcadores moleculares. In: Borém A, Caixeta ET (eds.). **Marcadores moleculares**. Editora UFV, Viçosa, p. 10-78.

Carvalho A (1952) Taxonomia do gênero *Coffea arabica* L. **Bragantia 12:** 171-178.

Charrier A and Berthaud J (1985) Botanical classification of coffee. In: Clifford MN and Wilson KC (eds.). **Coffee: botany, biochemistry and production of beans and beverage.** Croom Held Publ, Beckenham, UK, p.13-47.

CONAB – COMPANHIA NACIONAL DE ABASTECIMENTO – **Estimativas da safra brasileira de café 20006/2007.** Brasília: 2007. 13 p.

Conagin CHTM and Mendes AJT (1961) Pesquisas citológicas e genéticas em três espécies de *Coffea*. Auto-incompatibilidade em *Coffea canephora* Pierre ex Frohner. **Bragantia 20:** 787-804.

Cruz RP and Milach SCK (1998) Análise de RAPD. In: Milach SCK (ed.). **Marcadores moleculares em plantas.** Porto Alegre, p. 107-116.

Cruz CD (2001) **Programa Genes:** versão Windows. Aplicativo computacional em genética e estatística. Editora UFV, Viçosa, 648p.

Cruz CD and Carneiro PCS (2003) **Modelos biométricos aplicados ao melhoramento genético.** v.2. Editora UFV, Viçosa, 585p.

Diniz LEC, Ruas PM, Ruas CF and Sera T (2001) O uso de RAPD associado a enzimas de restrição para detectar variabilidade genética no Banco de Germoplasma de *Coffea arabica* L. do IAPAR. **II Simpósio de Pesquisa dos Cafés do Brasil**, p. 550-556.

Doyle JJ and Doyle JL (1990) Isolation of plant DNA from fresh tissue. **Focus 12:** 13-15.

Duarte JM, Santos JB and Melo LC (1999) Genetic divergence among common bean cultivars from different races based on RAPD markers. **Genetics and Molecular Biology 22:** 419-426.

Ferrão MAG, Fonseca AFA, Ferrão RG, Oliveira MA, Barbosa WM, Silva MF and Barbosa RP (2007) **Técnicas moleculares e biotecnológicas aplicadas ao café.** In: Ferrão RG, Fonseca AFA, Bragança SM, Ferrão MAG and DeMunner LH. (Org.). Café Conilon. 1 ed. Vitória,1: 170-201.

Ferrão RG, Fonseca AFA, Silveira JSM, Ferrão MAG and Bragança SM (2000a) Emcapa 8141 – Robustão Capixaba, variedade clonal de café conilon tolerante a seca, desenvolvida para o Estado do Espírito Santo. **Revista Ceres 47:** 555-559.

Ferrão RG, Fonseca AFA, Ferrão MAG and Bragança SM (2000b) **Emcaper 8151 – Robusta Tropical: primeira variedade de café conilon propagada por semente no Espírito Santo.** Vitória, ES: EMCAPER. (EMCAPER, Documento 103).

Ferrão RG, Fonseca AFA, Ferrão MAG, De Muner LH, Verdin Filho AC, Volpi PS, Marques EMG and Zucateli F (2004) **Café conilon: técnicas de produção com variedades melhoradas.** Vitória, ES: Incaper, 60p. (Circular Técnica, 03-I)

Ferreira ME and Grattapaglia D (1998) **Introdução ao uso de marcadores moleculares em análise genética.** 3.ed. Editora EMBRAPA-CENARGEN, Brasília, 220p.

Fonseca AFA (1999) **Análises biométricas em café conillon (*Coffea canephora* Pierre).** Tese de Doutorado, Universidade Federal de Viçosa, Viçosa, 121p.

Fonseca AFA, Ferrão MAG, Ferrão RG, Verdin Filho AC, Volpi S and Zucateli F (2004) 'Conilon Vitória – Incaper 8142': improved *Coffea canephora* var. Kouillou clone cultivar for the state of Espírito Santo. **Crop Breeding and Applied Biotechnology 4:** 503-505.

Fontes JRM, Sakiyama NS, Cardoso AA, Zambolim L and Pereira AA (2000) Avaliação de híbridos F_1 de café (*Coffea arabica* L.) e respectivos genitores, com marcadores RAPD. **I Simpósio de Pesquisa dos Cafés do Brasil.** p.160-163.

Lanza MA, Guimarães CT and Schuster I (2000) Aplicação de marcadores moleculares no melhoramento genético. **Informe Agropecuário 1:** 97-108.

Lashermes P, Trouslot P, Anthony F, Combes MC and Charrier A (1996) Genetic diversity for RAPD markers between cultivated and wild accessions of *Coffea Arabica*. **Euphytica 87:** 59-64.

Maluf MP, Silvestrini M, Ruggiero LM, Guerreiro Filho O and Colombo CA (2005) Genetic diversity of cultivated *Coffea arábica* inbred lines assessed by RAPD, AFLP and SSR marker systems. **Scientia Agricola 62:** 366-373.

Orozco-Castillo C, Chalmers KJ, Waugh R and Powell W (1994) Detection of genetic diversity and selective gene introgression in coffee using RAPD markers. **Theoretical and Applied Genetics 87:** 934-940.

Ruas PM, Sera T, Ruas CF, Diniz LC, Torres FM, Carvalho VP, Rampim L, Ruas EA and Silveira SR (2001) Diferenças genéticas entre seleções de café (*Coffea arábica* L.) da mesma origem avaliadas por RAPD com digestão prévia com enzima de restrição. **II Simpósio de Pesquisa dos Cafés do Brasil.** p.329-337.

Silveira SR, Ruas PM, Ruas CF, Sera T, Carvalho VP and Coelho ASG (2003) Assessment of genetic variability within and among coffee progenies and cultivars using RAPD markers. **Genetics and Molecular Biology 26:** 329-336.

Teixeira-Cabral TA, Sakiyama NS, Zambolim L, Pereira AA and Schuster I (2004) Single-locus inheritance and partial linkage map of *Coffea arabica* L. **Crop Breeding and Applied Biotechonology 4:** 416-421.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA and Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. **Nucleic Acids Research 18:** 6531-6535.