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Genetic Diversity Among Forty Coffee Varieties Assessed by RAPD Markers Associated with Restriction Digestion

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ABSTRACT

The genetic variability of 40 accessions of C. arabica was evaluated using a combination of the random amplified polymorphic DNA (RAPD) technique and restriction digestion of genomic DNA. The genetic variability and the relatedness among all accessions were initially evaluated using 195 RAPD primers which revealed a very low level of genetic variation. To improve the efficiency in the detection of polymorphism, the genomic DNA of all accessions were submitted to digestion with restriction endonucleases prior to PCR amplification. A total of 24 primers combined with restriction digestion of DNA rendered 318 bands, of which 266 (83.65%) were polymorphic. The associations among genotypes were estimated using UPGMA-clustering analysis. The accessions were properly clustered according to pedigree and agronomic features. The ability to distinguish among coffee accessions was greater for RAPD plus restriction digestion than for RAPD alone, providing evidences that the combination of the techniques was very efficient for the estimation of genetic relationship among C. arabica genotypes.

Key words: *Coffea arabica*, genetic variability, RAPD, restriction endonucleases

INTRODUCTION

The main varieties of *Coffea arabica* L. cultivated in Brazil are derived from at least two botanical types; the Bourbon varieties, introduced from the Reunion Islands, and the Typica variety brought to Brazil from the French Guiana in 1727 (Smith, 1985; Berthaud and Charrier, 1988). Currently, coffee is the one of the most important Brazilian commodities. Located in southern Brazil, Paraná state is an important coffee producer. In this state, the Research Center named Instituto Agronômico do Paraná (IAPAR) maintains a *Coffea* germplasm collection that consist of seven species, several varieties and cultivars, and more than one thousand progenies of *C. arabica* and *C. canephora*. Despite its importance, this *Coffea* collection lacks information about genetic variability, mainly when it is concerning to DNA level. Until recently, genetic diversity among species or cultivars of *Coffea arabica* was determined using morphological or isozyme markers. However, these markers are unsuitable to measure genetic variation in arabica accessions (Berthou and Trouslot, 1977; Louarn, 1978).

In the last ten years, detection of variation at DNA level has been made possible by the advent of molecular markers. Molecular techniques such as RFLP (Restriction Fragment Length Polymorphism) (Sambrook et al., 1989), RAPD (Random Amplified Polymorphic DNA) (Welsh and McClelland, 1990; Williams et al., 1990) and AFLP (Amplified Fragment Length Polymorphism) (Vos et al., 1995) provide

powerful tools for study of genetic diversity. RFLP analysis of the chloroplast genome and the atp-rbc intergenic region were used to study the variation among different taxa of *Coffea* and two species of *Psilanthus*. The low sequence divergence suggested that *Coffea* is a young genus (Lashermes et al., 1996a). The AFLP technique was useful for the detection of introgression in *C. arabica* (Lashermes et al., 2000). AFLP and SSR markers were used to assess polymorphism between and within coffee accessions (Anthony et al., 2002).

RAPD technique provides a useful tool to identify and estimate genetic diversity in *Coffea*. Orozco-Castilho et al. (1994) detected polymorphism between 22 *C. arabica* accessions, one natural interspecific hybrid (Hibrido de Timor), three accessions of *C. canephora* and one accession of *C. liberica*. The study also showed that plants originated from Ethiopia and plants from the arabica sub-groups (*C. arabica* var Typica and *C. arabica* var Bourbon) were clearly distinguished. Analysis of genetic diversity using RAPD revealed a significant difference between cultivated (Arabica and Bourbon) and wild accessions of *C. arabica* from Kenia and Ethiopia (Lashermes et al., 1996b). Anthony et al. (2001) studied the genetic diversity among 19 *C. arabica* accessions from spontaneous and sub spontaneous trees of Ethiopia. The authors concluded that RAPD markers could be applied for DNA fingerprint of coffee accessions, providing information for the introgression of desirable traits and, therefore, increase the effectiveness of the breeding program. Sera et al. (2003) using RAPD markers associated with restriction digestion showed genetic divergence between arabica accessions of the same origin. Chaparro et al. (2004) used RAPD technique for the analysis of 50 wild and semi-wild accessions of *C. arabica* and concluded that a much larger polymorphism was present in the collection examined than previously reported in others coffee collections. In this study, we assessed the genetic relationship among arabica accessions from a germplasm collection from Brazil using RAPD associated with prior digestion of DNA with restriction enzymes.

MATERIALS AND METHODS

Plant material

Forty coffee genotypes were used in this study (Table 1). The accessions were obtained from the *Coffea* Germplasm Collection of the Instituto Agronômico do Paraná (IAPAR), Londrina, Brazil. Many of the genotypes represented elite cultivars that have been used in breeding programs.

DNA extraction, amplification, and gel electrophoresis

Genomic DNA was isolated from fresh leaves, obtained from at least five different plants of each accession following the CTAB method (Doyle and Doyle, 1987), except that CTAB was replaced by MATAB (Mixed Alyltrimethylammonium Bromide, Sigma) in the extraction buffer. DNA concentration was estimated using a fluorometer (DyNA Quant 200, Höefer-Pharmacia), adjusted to 10ng/µl and bulked by accessions (Michelmore et al., 1991). Amplification reactions were in a volume of 15 µl of a standard PCR and 20ng template DNA. For restriction digestion, genomic DNA was incubated for 1 h at 37º C with one of the following enzymes, *Bam* HI, *Eco* RI, or *Hae* III, just prior to the PCR reaction. DNA amplification was carried out using a PTC 100 (MJ Research) thermal cycler programmed with a 3 min at 94º C for initial DNA denaturation, followed by 48 cycles of 1 min at 94º C, 1 min 45 sec at 38º C, and 2 min at 72º C. The final cycle was followed by a 7 min extension at 72º C. Amplified products were resolved in 1.2 % agarose gel in 1x TAE buffer (40 mM Tris-acetate, 1 mM EDTA pH 8.0) and stained with ethidium bromide. The RAPD profiles were visualized under UV light and stored for further analysis in a PC computer.

Data analysis

DNA markers were scored for the presence (1) and absence (0) of homologous amplified products. The genetic similarity among accessions was estimated using the Dice coefficient of the NTSYS package (Numerical Taxonomy and Multivariate Analysis for personal computer), version 2.1 (Rohlf, 2000). A dendrogram was constructed using the UPGMA (unweighted pair-group method using arithmetic averages) method. The matrix of

genetic similarity was also used in a principal coordinate analysis (PCOORD) to resolve the patterns of variation among the genotypes. The cophenetic coefficient between the matrix and the dendrogram was measured using the appropriate routine of the NTSYS package. The bootstrap method was employed to evaluate the reliability of tree topology. The calculations were performed with the BOOD software, version 3.0 (Coelho, 2001). The significance of the cophenetic correlation was estimated with the Mantel correspondence test (Mantel, 1967).

RESULTS AND DISCUSSION

The RAPD technique combined with a prior digestion of genomic DNA with restriction enzymes allowed for the detection of polymorphism among 40 accessions of coffee. From 195 RAPD primers initially screened, only 24 (12.3%) revealed polymorphism. Low percentage of RAPD primers showing informative bands in *Coffea* were also reported by Lashermes et al. (1996a) and Anthony et al. (2001). The authors attributed the results to the very narrow genetic basis of *C. arabica*. To increase the number of informative bands, the total DNA from each of the accessions studied were treated with restriction enzymes prior PCR reaction with the 24 primers screened. Only three (*Bam* HI, *Eco* RI, and *Hae* III) out of seven enzymes tested modified the amplification patterns obtained with the primers alone, resulting in 27 primers/enzyme combinations (Table 2). The PCR amplification of genomic DNA from all accessions yielded a total of 318 bands of which 266 (83.6%) were polymorphic. The mean coefficient of variation (CV = 2.6%), estimated using the bootstrap procedure (Coelho, 2001) initiated to stabilize at about 100 markers and the rate of decrease was minimal beyond 200 markers (not shown). These data suggested that 318 markers were adequate for the estimation of genetic relationship among the arabica accessions. DNA polymorphism generated with and without restriction digestion was similar, however, the informative bands obtained per primer increased significantly, often including different amplified products (Table 2, Fig. 1). In wheat genotypes, Koebner (1995) observed that digestion of template DNA prior to PCR amplification allowed for more efficiency of primer annealing along shorter DNA fragment, where a simplified secondary structure was less likely to interfere with the process. Similar results were obtained by Sera et al. (2003) in 14 accessions of arabica coffee and by Silveira et al. (2003) in a study of genetic variability within and among Sarchimor progenies.

Phylogenetic relationship

The dendrogram and the PCO (Principal Coordinate Analysis) estimated from the similarity matrix (Table 3) for the 40 *Coffea* accessions are shown in Figs. 2 and 3, respectively. The high value of cophenetic correlation (r = 0,81) between the similarity matrix and the dendrogram indicated the extent to which the clustering of genotypes accurately represented the estimates of genetic similarities among the accessions studied.

The primary trend in the dendrogram was to separate the genotypes into two main groups (Figs 2 and 3), one comprising the cultivars of the arabica and the Bourbon type coffee and the other including the cultivars derived from genotypes containing genes of the Hibrido de Timor.

The first cluster showed that *C. arabica* var *typica* was highly related to the Bourbon Vermelho cultivar (similarity of 0.94). This result was supported by the origin of Bourbon Vermelho as a spontaneous mutation found in Reunion Island in a field of *C. arabica* var *arabica* from Ethiopia (Rothfos, 1980). Both, Arabica typica and Bourbon Vermelho were close to Bourbon amarelo (mean similarity of 0.90) with a bootstrap support of 91% (Figs. 2, 3 and Table 3). High values of genetic similarity between *C. arabica typica* and the Bourbon group were also reported by Anthony et al. (2001). The authors concluded that even though Bourbon and Typica varieties had accumulated small differences over three centuries of selections, they still presented little divergence with wild material from Ethiopia.

The first cluster also grouped the cultivars Catuaí Vermelho IAC 81, Catuaí Amarelo IAC 17, Catuaí Semperflorens, and Acaiá IAC 474-19. Catuaí Vermelho IAC 81 and Catuaí Amarelo IAC 17 (similarity of 0.92) were artificial hybrids (Caturra Amarelo IAC 476 x Mundo Novo) whose progenies with red and yellow fruits, selected in F_3 populations were named Catuaí Vermelho IAC 81 and Catuaí Amarelo IAC 17, respectively (Fazuoli, 1986).

Table 1 - Accessions name, type, and origin of the *C. arabica* germplasm studied.

Nº	Accessions	Type	Origin
1	Arabica typica	cultivar of *C. arabica*	Ethiopia
2	Bourbon Amarelo	cultivar (Bourbon x Amarelo de Botucatu)	Brazil
3	Bourbon Vermelho	cultivar of *C. arabica*	Reunion Island
4	Acaiá IAC 474 -19	cultivar (Bourbon x Sumatra)	Brazil
5	Mundo Novo IAC[1] 376-4	cultivar (Bourbon x Sumatra)	Brazil
6	Mundo Novo Semperflorens	mutant of Acaiá	Brazil
7	Catuaí Semperflorens	mutant of Catuaí Amarelo	Brazil
8	Catuaí Amarelo IAC 17	cultivar (Mundo Novo x Caturra)	Brazil
9	Catuaí Vermelho IAC 81	cultivar (Mundo Novo x Caturra)	Brazil
10	Rubi - MG 1192	Cultivar (Catuaí x Mundo Novo)	Brazil
11	IAPAR 77.055	progeny (Icatu x Caturra)	Brazil
12	Catuaí SH_2 SH_3	F_5 progeny SH_2 SH_3	Brazil
13	Caturra Vermelho IAC 477	mutant variety of Bourbon	Brazil
14	Caturra Amarelo IAC 476	mutant variety of Bourbon	Brazil
15	Superprecoce	germplasm of *C. arabica*	Ethiopia (FAO, 1968)
16	Icatu Precoce IAC 3282	cultivar of Icatu	Brazil
17	Icatu Amarelo IAC 2944	cultivar of Icatu	Brazil
18	Icatu Vermelho IAC 2945	cultivar of Icatu	Brazil
19	Mokka	mutant of Bourbon variety	Brazil
20	Laurina IAC 870	mutant of Bourbon variety	Brazil
21	Villa Lobos	mutant of Bourbon variety	Costa Rica
22	San Bernardo	mutant of Bourbon variety	Costa Rica
23	Villa Sarchi	mutant of Bourbon variety	Costa Rica
24	Colômbia Amarelo	cultivar of Catimor	Colombia
25	IAPAR 77.028	F_4 progeny of Sarchimor	Brazil
26	IAPAR 59 (75.163-22)	F_4 cultivar of Sarchimor	Brazil
27	Tupi IAC 1669-33	F_5 cultivar of Sarchimor	Brazil
28	IAPAR 75.163-21-10	F_5 progeny of Sarchimor	Brazil
29	IAPAR 75.163-12	F_4 progeny of Sarchimor	Brazil
30	Kattimor	progeny of Sarchimor	Brazil
31	Mundo Novo x IAPAR 59 F_1	F_1 hybrid	Brazil
32	Mundo Novo x IAPAR 59 F_2	F_2 hybrid	Brazil
33	Semi-erecta	mutant of Bourbon	Brazil/Ethiopia (FAO, 1968)
34	Catuaí Erecta	genotype of Catuaí Amarelo	Brazil
35	Goiaba	mutant of Bourbon	Brazil
36	Maragogipe	Cultivar	Brazil
37	Cera	mutant of Bourbon	Brazil
38	Geisha	cultivar of *C. arabica*	Brazil/Ethiopia
39	Cioicie	cultivar of *C. arabica*	Brazil/Ethiopia
40	Purpurascens	mutant of Bourbon	Brazil

[1] IAC - Instituto Agronômico de Campinas (Campinas, SP)

Table 2 - RAPD primers (Operon Technologies) used alone for PCR or in combination with restriction enzymes for the analysis of 40 *Coffea* accessions.

Primer	Number of bands	Number of polymorphic bands	Primer	Number of bands	Number of polymorphic bands
OPAD 01 + *Hae* III	9	8	OPP 20	13	12
OPAD 06	8	4	OPAE 20	4	2
OPAD 06 + *Hae* III	13	10	OPAE 20 + *Eco* R1	6	5
OPAD 06 + *Bam* HI	10	10	OPAE 20 + *Hae* III	6	4
OPAD 07	14	14	OPA 17	8	3
OPAD 07 + *Eco* RI	8	8	OPAT 12	4	3
OPAD 07 + *Hae* III	13	13	OPAT 12 + *Eco* RI	3	2
OPAD 11	9	8	OPA 01	4	2
OPAD 11 + *Eco* RI	8	6	OPA 01 + *Bam* HI	2	2
OPAD 11 + *Hae* III	5	5	OPA 01 + *Eco* RI	2	1
OPAD 11 + *Bam* HI	6	6	OPAE 01	3	2
OPAD 14 + *Eco* RI	5	3	OPN 08	3	3
OPAD 15 + *Bam* HI	11	8	OPN 08 + *Bam* HI	7	4
OPAD 19 + *Bam* HI	7	7	OPN 08 + *Eco* RI	4	2
OPO 9	13	13	OPN 07	4	2
OPO 9 + *Bam* HI	11	10	OPN 07 + *Hae* III	7	6
OPO 10	9	9	OPN 07 + *Eco* RI	7	7
OPO 10 + *Bam* HI	11	11	OPAV 09 + *Hae* III	6	4
OPO 13	8	8	OPP 03 + *Eco* RI	3	1
OPO 14	16	15	OPY 20 + *Eco* RI	4	2
OPP 18	8	7	OPY 20 + *Hae* III	7	5
OPP 18 + *Hae* III	7	7	OPY 20 + *Bam* HI	2	1
			Total	**318**	**266**

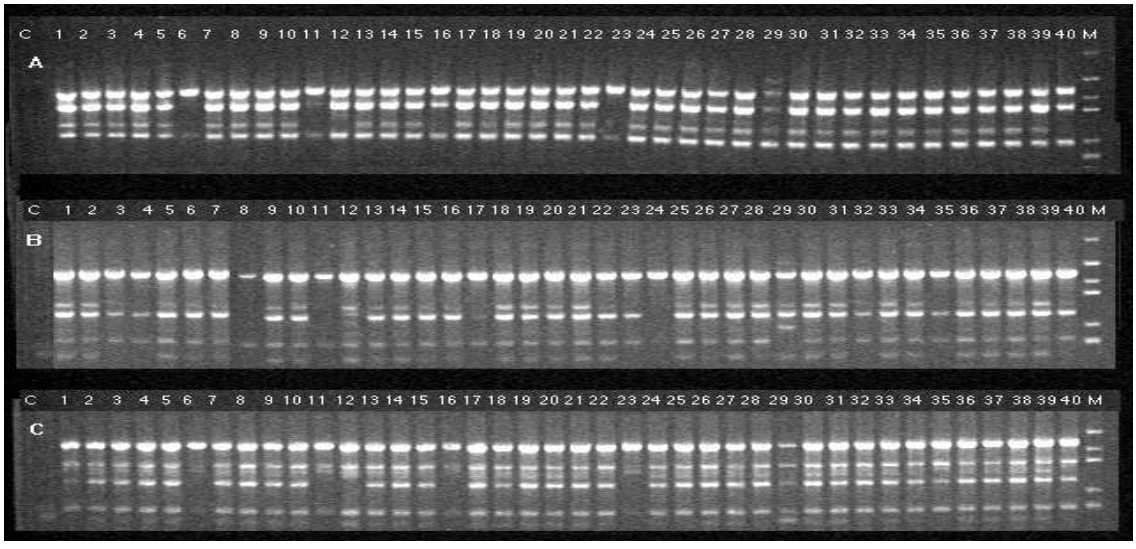

Figure 1 - Electrophoresis pattern generated with primer OPAE-20. Amplification products obtained without restriction digestion (A) and after DNA digestion with *Eco* R1 (B) and *Hae* III (C) enzymes.

Table 3 - Matrix of genetic similarities among 40 accessions of *Coffea arabica* using RAPD markers.

Accessions[1]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. Arabica typical	1.00																		
2. Bourbon Amarelo	0.92	1.00																	
3. Bourbon Vermelho	0.94	0.87	1.00																
4. Acaiá IAC 474 -19	0.85	0.85	0.89	1.00															
5. Mundo Novo IAC[1] 376-4	0.83	0.79	0.83	0.83	1.00														
6. Mundo Novo Semperflorens	0.77	0.75	0.80	0.80	0.81	1.00													
7. Catuaí Amarelo Semperflorens	0.85	0.85	0.88	0.87	0.84	0.82	1.00												
8. Catuaí Amarelo IAC 17	0.86	0.83	0.89	0.89	0.85	0.81	0.91	1.00											
9. Catuaí Vermelho IAC 81	0.83	0.82	0.86	0.85	0.82	0.81	0.92	0.92	1.00										
10. Rubi - MG 1192	0.79	0.76	0.83	0.81	0.80	0.75	0.82	0.84	0.85	1.00									
11. Icatuaí IAPAR 77.055	0.74	0.71	0.79	0.77	0.77	0.78	0.82	0.83	0.84	0.82	1.00								
12. Catuaí SH_2 SH_3	0.75	0.71	0.75	0.76	0.71	0.66	0.74	0.75	0.73	0.81	0.73	1.00							
13. Caturra Vermelho IAC 477	0.81	0.78	0.83	0.84	0.81	0.77	0.86	0.86	0.87	0.84	0.84	0.80	1.00						
14. Caturra Amarelo IAC 476	0.80	0.77	0.82	0.83	0.77	0.74	0.86	0.85	0.83	0.85	0.82	0.83	0.86	1.00					
15. Arabica Superprecoce	0.74	0.74	0.78	0.79	0.80	0.72	0.81	0.80	0.80	0.77	0.75	0.72	0.83	0.82	1.00				
16. Icatu Precoce IAC 3282	0.81	0.80	0.83	0.83	0.80	0.77	0.88	0.86	0.88	0.82	0.83	0.71	0.90	0.84	0.81	1.00			
17. Icatu Amarelo IAC 2944	0.82	0.81	0.85	0.85	0.81	0.75	0.88	0.87	0.86	0.84	0.81	0.77	0.89	0.88	0.85	0.90	1.00		
18. Icatu Vermelho IAC 2945	0.82	0.80	0.84	0.85	0.79	0.74	0.87	0.85	0.85	0.80	0.79	0.77	0.88	0.86	0.82	0.90	0.92	1.00	
19. Mokka	0.83	0.82	0.85	0.86	0.79	0.74	0.88	0.87	0.87	0.81	0.81	0.77	0.91	0.88	0.83	0.89	0.92	0.93	1.00
20. Laurina IAC 870	0.81	0.80	0.82	0.85	0.78	0.74	0.86	0.84	0.84	0.80	0.80	0.78	0.87	0.87	0.81	0.87	0.91	0.92	0.91
21. Villa Lobos	0.82	0.80	0.82	0.81	0.75	0.74	0.83	0.81	0.82	0.76	0.73	0.73	0.81	0.79	0.79	0.81	0.84	0.85	0.84
22. San Bernardo	0.82	0.81	0.83	0.81	0.76	0.74	0.85	0.82	0.82	0.79	0.74	0.74	0.85	0.81	0.77	0.84	0.85	0.86	0.86
23. Villa Sarchi	0.79	0.76	0.81	0.79	0.74	0.77	0.81	0.79	0.78	0.76	0.76	0.72	0.78	0.80	0.75	0.79	0.84	0.83	0.82
24. Colômbia Amarelo	0.80	0.79	0.80	0.78	0.75	0.70	0.83	0.78	0.80	0.76	0.72	0.72	0.81	0.78	0.77	0.80	0.84	0.85	0.84
25. IAPAR 77.028	0.81	0.79	0.81	0.80	0.74	0.72	0.82	0.79	0.81	0.76	0.75	0.74	0.82	0.79	0.77	0.81	0.82	0.83	0.83
26. IAPAR 59 (75.163-22)	0.82	0.80	0.83	0.80	0.76	0.73	0.82	0.81	0.81	0.80	0.73	0.75	0.82	0.82	0.80	0.80	0.84	0.83	0.83
27. Tupi (IAC 1669-33)	0.84	0.82	0.83	0.81	0.78	0.74	0.84	0.82	0.82	0.77	0.72	0.75	0.80	0.80	0.78	0.80	0.84	0.83	0.84
28. IAPAR 75.163-21-10	0.81	0.80	0.82	0.80	0.76	0.72	0.83	0.80	0.81	0.78	0.72	0.74	0.82	0.80	0.79	0.81	0.86	0.85	0.86
29. IAPAR 75.163-12	0.79	0.78	0.79	0.77	0.71	0.68	0.80	0.79	0.81	0.77	0.72	0.72	0.81	0.79	0.77	0.79	0.83	0.80	0.84
30. Kattimor	0.80	0.77	0.80	0.79	0.75	0.72	0.83	0.80	0.81	0.79	0.76	0.76	0.83	0.85	0.80	0.81	0.84	0.82	0.84
31. F_1 Mundo Novo x IAPAR 59	0.81	0.79	0.80	0.78	0.74	0.69	0.81	0.81	0.82	0.76	0.74	0.74	0.81	0.81	0.78	0.80	0.83	0.84	0.84
32. F_2 Mundo Novo x IAPAR 59	0.75	0.73	0.74	0.71	0.70	0.68	0.75	0.73	0.75	0.78	0.70	0.79	0.77	0.80	0.75	0.74	0.78	0.77	0.79
33. Semi-erecta	0.81	0.80	0.83	0.80	0.78	0.71	0.82	0.81	0.83	0.80	0.77	0.75	0.84	0.80	0.81	0.81	0.85	0.85	0.86
34. Catuaí Amarelo Erecta	0.82	0.80	0.81	0.79	0.77	0.70	0.82	0.80	0.81	0.78	0.75	0.74	0.82	0.81	0.80	0.82	0.85	0.83	0.86
35. Goiaba	0.81	0.78	0.81	0.79	0.78	0.73	0.81	0.79	0.78	0.77	0.75	0.74	0.81	0.81	0.80	0.80	0.84	0.82	0.86
36. Maragogipe	0.79	0.76	0.81	0.78	0.76	0.70	0.80	0.78	0.81	0.79	0.75	0.77	0.82	0.80	0.80	0.79	0.83	0.82	0.84
37. Cera	0.78	0.75	0.78	0.75	0.74	0.67	0.77	0.76	0.78	0.74	0.71	0.74	0.80	0.78	0.79	0.77	0.80	0.79	0.82
38. Geisha	0.77	0.75	0.78	0.76	0.73	0.65	0.78	0.78	0.78	0.76	0.72	0.76	0.81	0.79	0.77	0.80	0.83	0.82	0.85
39. Cioiccie	0.81	0.78	0.81	0.79	0.76	0.69	0.80	0.79	0.80	0.79	0.75	0.78	0.83	0.81	0.78	0.80	0.84	0.84	0.85
40. Purpurascens	0.79	0.78	0.80	0.79	0.77	0.71	0.82	0.78	0.79	0.76	0.74	0.74	0.83	0.82	0.82	0.83	0.83	0.84	0.85

[1] accessions are numbered according to Table 1.

Table 3 - Continuing...

Accessions[1]	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1. Arabica typical																					
2. Bourbon Amarelo																					
3. Bourbon Vermelho																					
4. Acaiá IAC 474 -19																					
5. Mundo Novo IAC[1] 376-4																					
6. Mundo Novo Semperflorens																					
7. Catuaí Amarelo Semperflorens																					
8. Catuaí Amarelo IAC 17																					
9. Catuaí Vermelho IAC 81																					
10. Rubi - MG 1192																					
11. Icatuaí IAPAR 77.055																					
12. Catuaí SH_2 SH_3																					
13. Caturra Vermelho IAC 477																					
14. Caturra Amarelo IAC 476																					

Cont. ...

(Cont. Table 3)

15. Arabica Superprecoce	
16. Icatu Precoce IAC 3282	
17. Icatu Amarelo IAC 2944	
18. Icatu Vermelho IAC 2945	
19. Mokka	
20. Laurina IAC 870	1.00
21. Villa Lobos	0.85 1.00
22. San Bernardo	0.86 0.93 1.00
23. Villa Sarchi	0.83 0.88 0.87 1.00
24. Colômbia Amarelo	0.84 0.86 0.88 0.82 1.00
25. IAPAR 77.028	0.85 0.86 0.87 0.85 0.86 1.00
26. IAPAR 59 (75.163-22)	0.81 0.85 0.85 0.84 0.88 0.87 1.00
27. Tupi (IAC 1669-33)	0.82 0.86 0.86 0.86 0.87 0.89 0.91 1.00
28. IAPAR 75.163-21-10	0.84 0.85 0.85 0.85 0.89 0.88 0.92 0.93 1.00
29. IAPAR 75.163-12	0.80 0.81 0.82 0.79 0.84 0.86 0.88 0.86 0.90 1.00
30. Kattimor	0.81 0.82 0.82 0.83 0.86 0.86 0.89 0.87 0.89 0.89 1.00
31. F_1 Mundo Novo x IAPAR 59	0.82 0.82 0.82 0.81 0.86 0.86 0.89 0.88 0.89 0.90 0.89 1.00
32. F_2 Mundo Novo x IAPAR 59	0.75 0.79 0.79 0.78 0.83 0.81 0.87 0.85 0.87 0.82 0.84 0.82 1.00
33. Semi-erecta	0.83 0.83 0.84 0.80 0.87 0.85 0.87 0.85 0.89 0.88 0.88 0.91 0.83 1.00
34. Catuaí Amarelo Erecta	0.82 0.82 0.84 0.80 0.87 0.83 0.87 0.85 0.89 0.87 0.88 0.89 0.82 0.94 1.00
35. Goiaba	0.81 0.84 0.86 0.82 0.85 0.81 0.86 0.86 0.87 0.83 0.84 0.83 0.82 0.89 0.91 1.00
36. Maragogipe	0.81 0.82 0.85 0.81 0.87 0.86 0.86 0.85 0.89 0.87 0.87 0.87 0.85 0.92 0.91 0.90 1.00
37. Cera	0.77 0.78 0.80 0.76 0.84 0.81 0.82 0.81 0.85 0.86 0.84 0.85 0.82 0.88 0.89 0.87 0.92 1.00
38. Geisha	0.80 0.81 0.85 0.80 0.85 0.80 0.82 0.82 0.85 0.86 0.83 0.85 0.81 0.87 0.91 0.87 0.9 0.9 1.00
39. Cioiccie	0.83 0.83 0.85 0.83 0.86 0.83 0.85 0.84 0.87 0.87 0.85 0.87 0.83 0.91 0.91 0.90 0.9 0.9 0.93 1.00
40. Purpurascens	0.82 0.84 0.85 0.82 0.86 0.85 0.86 0.85 0.88 0.87 0.87 0.84 0.82 0.90 0.91 0.92 0.9 0.9 0.89 0.92 1.00

[1] accessions are numbered according to Table 1.

Catuaí Semperflorens was a mutant of Catuaí Amarelo IAC 17, thus justifying the close similarities with Catuaí Amarelo IAC 17 (0.91) and Caturra Vermelho IAC 477 (0.92).

Acaiá IAC 474 -19 was associated with the Catuai cultivars showing a mean similarity of 0.87 with a bootstrap support of 94%. The Acaiá genotype was derived by selection from progenies of the Mundo Novo germplasm, which arose from natural hybridization between Sumatra and Bourbon cultivars (Fazuoli, 1986; Carvalho et al., 1989). Caturra Vermelho IAC 477, Icatu precoce IAC 3282, Icatu Amarelo IAC 2944, Icatu Vermelho IAC 2945, Mokka, and Laurina IAC 870 were grouped together (mean similarity of 0.89). The variety Icatu was obtained after artificial crossing between *C. canephora* var *robusta* (4x) and *C. arabica* var Bourbon Vermelho. The F1 was crossed with Mundo Novo and selected for precocity giving rise to Icatu precoce IAC 3282. The predominance of genes from Bourbon Vermelho in both, Caturra Vermelho IAC 477 and Icatu Precoce IAC 3282 gave support to the high genetic similarities observed. The cultivar Icatu Amarelo was tightly associated in this group showing a mean similarity of 0.90 with Icatu Vermelho, Mokka, and Laurina IAC 870 (Figs 2, 3). Icatu Amarelo was obtained by selection of plants derived from natural crossing between Icatu Vermelho and Bourbon Amarelo (Fazuoli, 1981). Laurina IAC 870 was derived from the Bourbon Vermelho gene pool in Reunion Island, sharing most of its morphological characters with the Mokka cultivar (Rothfos, 1989; Krug et al., 1950). Therefore, the molecular data were fully supported by pedigree information of these cultivars.

The cultivars Rubi-MG 1192 and IAPAR- 77055 were clustered with a similarity 0.82 (Table 3 and Figs. 2, 3). Rubi MG 1192 was a cultivar derived from crossing between Catuai Vermelho IAC 81 and Mundo Novo while IAPAR-77055 was obtained from Icatu x Catuai Vermelho IAC 81 crossing after selection for plant size.

The accessions associated in the second group were clustered into three subgroups (Figs 2, 3). In the first subgroup, the varieties Villa Lobos and San Bernardo were tightly clustered with a similarity of 0.93 and a bootstrap support of. 99%. These varieties associated to Villa Sarchi with a mean similarity coefficient of 0.88 and a bootstrap of 63%. Villa Lobos, San Bernardo, and Villa Sarchi have the same origin (Bourbon Vermelho germplasm) giving support to the high similarities

observed. All but one (Colombia Amarelo of the Catimor gene pool) of the accessions associated in the second subgroup belonged to the Sarchimor germplasm (Table 1). IAPAR-59 and the accessions IAPAR 75163-21-10 and IAPAR 75163-12 were obtained from an open pollinated field of IAPAR 75163, which was a F_3 progeny from Villa Sarchi (CIFC 971/10) x Híbrido de Timor (CIFC 832/2) (Silveira et al., 2003).

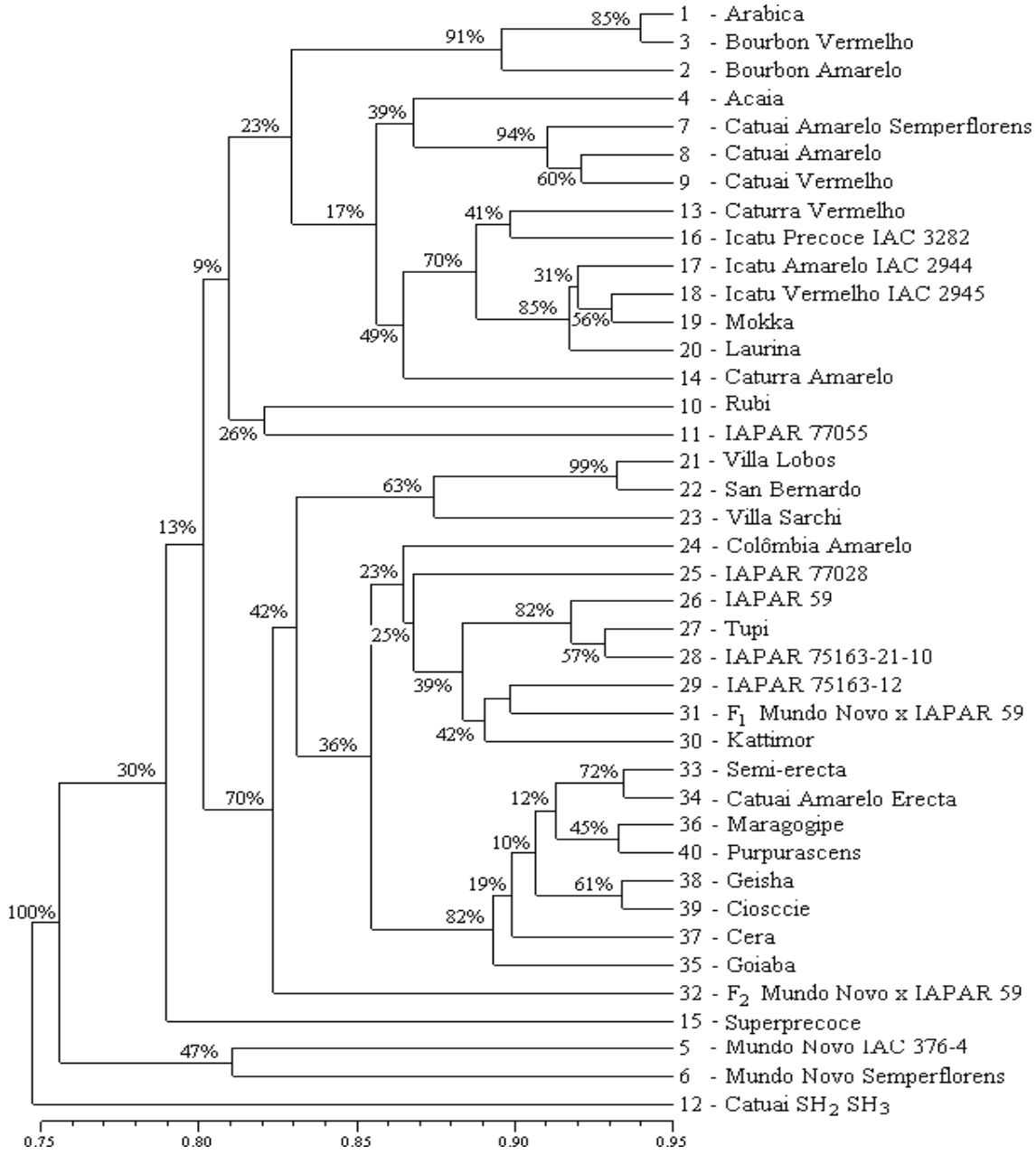

Figure 2 - UPGMA dendrogram of 40 *C. arabica* accessions based on Dice genetic similarity. Numbers at branches are bootstrap value (%) generated after 1000 permutations (Coelho, 2001).

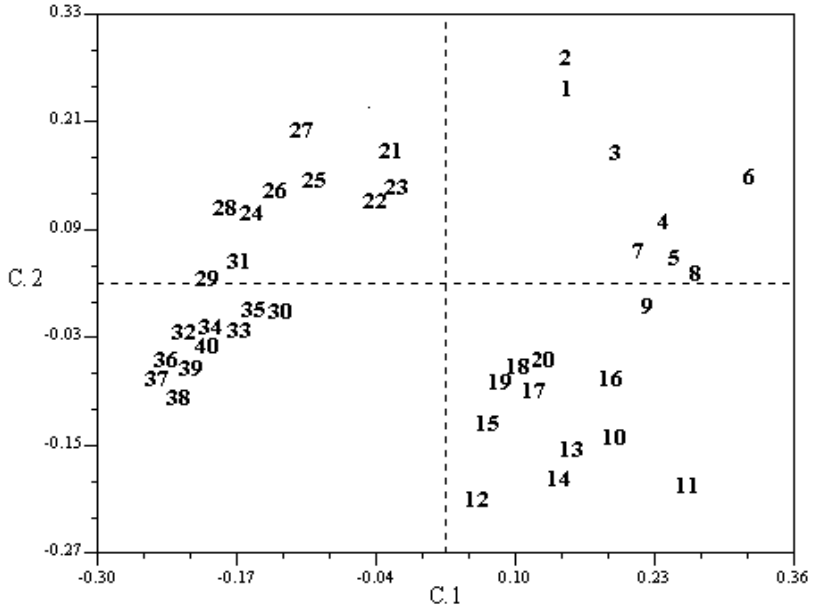

Figure 3 - Principal Coordinate Analysis of the 40 accessions of *C. arabica.* The numbers correspond to the accessions as listed in Table 1.]

The high similarity coefficients (0.86, on average) obtained with RAPD data were correlated with characteristics such as rust resistance, architecture and vegetative vigor shared by these cultivars. Even so these varieties display some differences in canopy diameter and grain size. A more comprehensive study about the relationships among the Sarchimor varieties and some Bourbon varieties has been described by Sera et al. (2003). The third subgroup associated eight accessions (Semi-erecta, Catuaí Amarelo Erecta, Maragogipe, Purpurascens, Geisha, Cioiccie, Cera, and Goiaba). Semi Erecta clustered with Catuaí Amarelo Erecta with a similarity coefficient of 0.94. The Semi-erecta genotype was a presumed mutant of Bourbon Vermelho sharing characteristics such as plant architecture, productivity, and grain size similar to the Bourbon-type coffee. Although Semi Erecta and Bourbon Vermelho were included in different clusters, the similarity coefficient between them was 0.83 giving support to the morphological relationship. The accession of Catuaí Amarelo Erecta used in this assay was also of uncertain origin. It was found in a field of Catuaí Amarelo IAC 17, possibly derived from hybridization between plants of the Arabica Erecta gene pool and Catuaí genotypes. Catuaí Amarelo Erecta revealed a mean genetic similarity of 81% with Catuaí Vermelho IAC 81 and Catuaí Amarelo IAC 17.

The cultivars Maragogipe, Purpurascens, Cera, Goiaba, Geisha, and Cioicie showed a mean genetic similarity of 0.90. According to Clifford and Willson (1985), Purpurascens, Maragogipe, and Goiaba were mutants of Bourbon Vermelho. Carvalho et al. (1989) considered that Geisha and Cera were mutant versions of Bourbon Vermelho. All these varieties showed a mean genetic similarity of 80% with Bourbon Vermelho. The F_2 hybrid Mundo Novo x IAPAR-59 was associated to the second main group with a mean similarity coefficient of 0.83. This hybrid was from a segregant population (Sera et al., 2003) and showed only 0.70 of genetic similarity with Mundo Novo IAC-376 and 0.87 with IAPAR-59. These results were in agreement with morphological characters, suggesting that this segregant carried more genes from IAPAR-59 than from Mundo Novo IAC-376 (Sera et al., 2003).

The remaining cultivars, Superprecoce, Mundo Novo IAC-376, Mundo Novo Semperflorens (a spontaneous mutation of Mundo Novo), and Catuaí SH_2 SH_3, appeared isolated in the dendrogram. These cultivars were also spread over the PCA plot suggesting that they were less related

to the other cultivars. For instance, Mundo Novo IAC-376 and Mundo Novo Semperflorens showed a mean genetic similarity of only 0.75, with the other cultivars studied. Similarly, the cultivar Superprecoce showed a mean genetic similarity coefficient of 0.77 with the other accessions. This cultivar was obtained from accessions of *C. arabica* collected by FAO in 1968 in Ethiopia. The differences at DNA level reproduce the morphological divergence observed between Superprecoce and the other accessions studied.

The cultivar Catuaí SH_2 SH_3 was the most divergent genotype among all accessions studied. The range of the similarity coefficient was from 0.66 with Mundo Novo Semperflorens to 0.81 with the cultivar Caturra Amarelo IAC 476. Catuaí SH_2 SH_3 was a product of crossing between genotypes of Catuaí Amarelo IAC 17 and Catuaí SH_2. The F_1 hybrid was then crossed to Catuaí SH_3, a genotype derived by crossing between Catuaí and the interespecific hybrid *C. arabica* SH_3 (*C. liberica* x *C. arabica*), followed by backcrossing with the parental Arabica (Bettencourt, 1981). The grouping of *C. arabica* genotypes based on molecular markers was consistent with taxonomy and pedigree information described for this coffee collection. The combination of the RAPD technique with the prior digestion of template DNA with restriction endonucleases offered a reliable and effective method of accessing genetic variation in *C. arabica* accessions.

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RESUMO

A variabilidade genética de 40 acessos de cafeeiros de fenótipo arabica foi obtida usando a técnica de RAPD associada a uma digestão prévia do DNA genômico com endonucleases. A variabilidade genética e a relação entre os accessos foram inicialmente avaliadas pela amplificação de 195 primers. Para incrementar a eficiência na detecção de polimorfismo, o DNA genômico de cada acessos foi submetido a digestão com endonucleases antes da PCR. Um total de 24 primers combinados com restrição do DNA gerou 318 bandas, das quais 266 (83,65%) foram polimórficas. A associação entre os 40 acessos foi estimada pelo método de clusters UPGMA, sendo os acessos agrupados de acordo com seu pedigree e aspectos agronômicos. Os resultados mostraram que o uso de enzimas de restrição antes da reação de amplificação pode ser considerada uma ferramenta eficiente para incrementar o número de bandas informativas, possibilitando a diferenciação entre os 40 acessos de *C. arabica*.

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