Reproducibility of the RAPD marker and its efficiency in coffee tree genotype grouping analysis

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ABSTRACT

The genetic diversity of *Coffea arabic* L. cultivars is relatively narrow and its assessment and increase is important for breeding. Fifty two arbitrary primers were used to evaluate the reproducibility and the influence of the number of RAPD (Random Amplified Polymorphic DNA) markers on the estimation of genetic distances among 40 genotypes of *Coffea* spp. The average number of polymorphic bands was 6.69 per primer among all genotypes, and 1.27 among arabica coffee genotypes. RAPD markers were efficient in estimating the genetic distances among the genotypes. The increase in RAPD loci number during grouping analysis did not affect the major groups' composition; however, it affected the composition of subgroups. Marker reproducibility was 76.88% and replicated data was recommended for distinguishing genotypes with the same genetic background.

KEY WORDS: *Coffea*, DNA markers, diversity, coffee breeding.

INTRODUCTION

The subgenus *Coffea* consists of approximately 100 taxa. *Coffea arabica* L. (arabica coffee) and *Coffea canephora* Pierre ex Froenher (robusta coffee) are the most economically important species, while other species have valuable gene stocks for breeding purposes. *C. arabica* represents approximately 70% of the world production and gives a superior quality product.

In Brazil, arabica coffee improved cultivars were 300% more productive than the Typica , which was the first cultivar introduced in the country (Medina-Filho et al., 1984). Typica was derived from a few introduced plants and variability was increased later by the introduction of the Bourbon and Sumatra cultivars, mutation events, and the genetic recombination resulting from natural outcrossings (Carvalho, 1993). The increase of the genetic diversity is important for crop improvement, especially the incorporation of genes from related species (Medina-Filho et al., 1984). A dissimilarity value can be obtained through a similarity complement, which is expected to represent the lineal relation between two samples evaluated by a common group of variables. Numerous similarity coefficients are proposed in the literature, and the choice of the most appropriate depends on the research objectives, the material to be studied, and the properties of each coefficient. The Jaccard's coefficient (Jaccard, 1901) is largely used in biological studies, since it gives no importance to either negative or positive concordances. Thus, the Jaccard's coefficient is appropriate to compare populations within the same species, where concordances are more frequent (Dias, 1998).

Plant genetic diversity has been analyzed by using phenotypic markers, which are limited in number and are affected by the environment. Isozyme markers have been used in many species, but they were not efficient for coffee diversity studies (Berthou and Trouslot, 1977). Montagnon and Bouharmont (1996) analyzed the phenotypic diversity among 148 genotypes of *C. arabica* based on 18 morphologic and agronomic markers, and suggested the use of molecular markers for further studies. Several genetic markers based on direct DNA analysis were lately developed to detect variability among genotypes in a large number of loci. RFLP (Restriction Fragment Length Polymorphisms) (Botstein et al., 1980) was the first molecular marker developed to explore DNA polymorphism. Its use, however, can be limited by cost and intensive labor demand. Recently several techniques were developed to detect DNA polymorphisms with the help of the DNA polymerase

chain reaction (PCR) technology. These techniques are based on the amplification of DNA fragments and the separation of these fragments by electrophoresis for polymorphism detection. RAPD (Random Amplified Polymorphic DNA), microsatellites and AFLPs (Amplified Fragment Length Polymorphism) markers have been succesfully used for plant diversity analysis such as soybean (Akkaya et al., 1992), barley (Sanchez et al., 1996; Struss and Plieske, 1998), maize (Pejic et al., 1998), rice (Fuentes et al., 1999), and coffee tree (Lashermes et al., 1993; 1995; 2000; Orozco-Castillo et al., 1994; 1996). RAPD markers (Williams et al., 1990) are easily detected using a small amount of DNA, and they do not require information on the DNA sequence to be amplified and on the design of specific primers. The automation of the process is also permitted. For these reasons RAPD markers are frequently used for genetic similarities studies (Thormann and Osborn, 1992). However, in some cases, the loss of reproducibility may occur due to the differences of the DNA purity and concentration, Mg^{++} concentration, and temperature. Reproducible RAPD markers require standardization of the method and allow data from different assays to be accumulated and compared.

In the present study, the reproducibility of coffee RAPD markers was evaluated, and the grouping analysis of coffee tree genotypes with different numbers of markers were compared.

MATERIAL AND METHODS

Genetic material

The genetic diversity of 40 coffee tree genotypes from UFV/Epamig germplasm was evaluated based on RAPD (Random Amplified Polymorphic DNA) markers (Williams et al., 1990). Each genotype (Table 1) was represented by one plant.

DNA extraction

The DNA was extracted from young leaves, following the Doyle and Doyle (1990) protocol with modifications, adding PVP-40 (soluble) to the extraction buffer. After extraction, the DNA was quantified with a spectrophotometer and stored at 4°C. For amplification, the DNA was diluted in TE (Tris HCl 10 mM, EDTA 1 mM, pH 8.0) to a final concentration of 10 ng/µl.

DNA amplification and electrophoresis analysis of the products

Two sets of primers (Operon Technologies Inc.) were used to generate amplified DNA fragments by PCR (Polymerase Chain Reaction) using a Perkin-Elmer 9600 thermocycler: twenty four primers in the first set, and 39 in the second set. The two sets shared eleven primers (Table 2). The 25 ml reaction volume was composed of 25 ng of genomic DNA, 1 U of Taq DNA polimerase, 0.1 mM dNTP, 0.2 mM *primer*, 50 mM KCl, 10 mM Tris HCl pH 8.3, 2 mM $MgCl_2$, and ultrapure water. The thermocycler was programmed to perform one step of initial denaturation (60" at 95°C), 39 cycles of amplification (15" at 94°C, 30" at 35°C, 60" at 72°C), and a final additional step of primer extension (7' at 72°C).

The amplified DNA fragments were separated by electrophoresis in 1.4% agarose gels, stained with ethidium bromide, exposed to UV light, and photographed. Only clear polymorphic bands were scored. RAPD alleles were scored according to the presence or absence of a band in each locus.

Data analysis

The data sheet was set up by a given value: 1 for the presence and 0 for absence of a band in each RAPD locus. Estimates of genetic similarities were expressed as Jaccard similarity coefficients (Jaccard, 1901) using the equation $SG_{ij} = a/(a+b+c)$, where SG_{ij} is the genetic similarity between the genotypes i and j, a is the number of bands present in both i and j genotypes, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in i. The conversion to genetic distance (GD) was performed by the equation $DG_{ij} = 1 - SG_{ij}$. The GENES program (Cruz, 1997) was used to calculate the genetic distances. The dendrogram based on the matrix of genetic distances was obtained by cluster analysis (STATISTICA program, 5.0 version) using the UPGMA method (unweighted pair-group method using arithmetic average).

RESULTS AND DISCUSSION

Polymorphism level and reproducibility of RAPD markers

Each coffee RAPD marker was studied for either the presence or the absence of a band in the same locus, as it is exemplified in the Figure 1.

Table 1. Description of 40 coffee tree genotypes.

Genotype [1/]	Description
01. *Coffea racemosa*	*Coffea racemosa*
02. *Coffea congensis*	*Coffea congensis*
03. *Coffea canephora* T 3755	*Coffea canephora*
04. UFV 557-3	*C. racemosa* x *C. arabica*
05. Timor Hybrid CIFC 4106	*C. arabica* x *C. canephora*
06. Timor Hybrid CIFC 2234	*C. arabica* x *C. canephora*
07. Typica UFV 2945 (China)	*Coffea arabica*
08. Typica UFV 536 (Portugal)	*Coffea arabica*
09. Catuaí Vermelho IAC 15	*C. arabica* (Caturra Amarelo x Mundo Novo)
10. Catuaí Vermelho IAC 44	*C. arabica* (Caturra Amarelo x Mundo Novo)
11. Catuaí Vermelho IAC 99	*C. arabica* (Caturra Amarelo x Mundo Novo)
12. Catuaí Vermelho IAC 81	*C. arabica* (Caturra Amarelo x Mundo Novo)
13. Catuaí Amarelo IAC 30	*C. arabica* (Caturra Amarelo x Mundo Novo)
14. Catuaí Amarelo IAC 86	*C. arabica* (Caturra Amarelo x Mundo Novo)
15. Catuaí Amarelo IAC 47	*C. arabica* (Caturra Amarelo x Mundo Novo)
16. Catuaí Amarelo IAC 113	*C. arabica* (Caturra Amarelo x Mundo Novo)
17. Mundo Novo IAC 464-18	*C. arabica* (Bourbon Vermelho x Sumatra)
18. Mundo Novo IAC 515-3	*C. arabica* (Bourbon Vermelho x Sumatra)
19. Mundo Novo IAC 376-4-32	*C. arabica* (Bourbon Vermelho x Sumatra)
20. Mundo Novo IAC 388-17-16	*C. arabica* (Bourbon Vermelho x Sumatra)
21. Mundo Novo IAC 376-4-22	*C. arabica* (Bourbon Vermelho x Sumatra)
22. Bourbon Amarelo UFV 535 (CENICAFE)	*Coffea arabica*
23. Bourbon UFV 2952	*Coffea arabica*
24. Bourbon UFV 2946 (China)	*Coffea arabica*
25. Bourbon UFV 2947 (China)	*Coffea arabica*
26. Caturra Vermelho CIFC 19/1	*C. arabica* (mutation in Bourbon Vermelho)
27. Catimor UFV 395-141 (F_3 CIFC HW 26/5)	*C. arabica* (Caturra Vermelho x Timor Hybrid)
28. Catimor UFV 386-19 (F_3 CIFC HW 26/5)	*C. arabica* (Caturra Vermelho x Timor Hybrid)
29. Catimor UFV 1310 (F_4 CIFC HW 26/5)	*C. arabica* (Caturra Vermelho x Timor Hybrid)
30. Catimor UFV 1359 (F_4 CIFC HW 26/5)	*C. arabica* (Caturra Vermelho x Timor Hybrid)
31. Catimor UFV 2983 (F_5 CIFC HW 26/5)	*C. arabica* (Caturra Vermelho x Timor Hybrid)
32. H 484-2	*C. arabica* (Mundo Novo x Timor Hybrid)
33. H 415-3	*C. arabica* (Catuaí Amarelo x Timor Hybrid)
34. Mundindu UFV 315-76	*C. arabica* (Mundo Novo x S 795)
35. Cachimor UFV 351-13	*C. arabica* [(Vila Sarchi x Timor H.) x Caturra]
36. Cavimor UFV 357-4	*C. arabica* [(Caturra x Timor H.) x Catuaí]
37. San Ramon UFV 3094	*C. arabica* (mutation in Typica)
38. Airi UFV 3095	*C. arabica* (selection in San Ramon)
39. Purpuracens UFV 4072	*C. arabica* (mutation in Catuaí Amarelo)
40. H 843	*C. arabica* (UFV 1359 x Maragogipe)

[1/] CENICAFE: Centro National de Investigaciones del Café (Colombia); CIFC: Centro de Investigação das Ferrugens do Cafeeiro (Portugal); ERU: Estação Regional de Uige (Angola); IAC: Institute Agronômico de Campinas (Brazil); T: Turrialba (Costa Rica); UFV: Universidade Federal de Viçosa (Brazil); H: hybrid and HW: Wagner hybrid.

In the analysis of 40 coffee genotypes, the first set of primers (24 primers) generated 151 polymorphic bands (also called RAPD loci), whereas 22 (14.57%) bands were polymorphic among arabica coffee genotypes.

The second set of primers (39 primers) generated 314 polymorphic bands, whereas 64 (20.4%) bands were polymorphic for arabica coffee.

The total of 52 primers (11 primers were repeated and scored once) generated 348 polymorphic bands, with the average of 6.69 bands per primer, whereas 66 (18.97%) bands were polymorphic for arabica coffee, with the average of 1.27 polymorphic bands per primer. Therefore, the total number of polymorphic markers was

Table 2. List of *primers* used to evaluate the genetic distances among 40 coffee tree genotypes.

1st set of primers	*2st set of primers*		*Set of repeated primers* amplificações
01. OPA-01	01. OPA-01	25. OPB-19	01. OPA-01
02. OPA-02	02. OPA-02	26. OPB-20	02. OPA-02
03. OPA-04	03. OPA-05	27. OPC-09	03. OPA-05
04. OPA-05	04. OPA-07	28. OPE-01	04. OPA-10
05. OPA-10	05. OPA-08	29. OPE-08	05. OPA-18
06. OPA-12	06. OPA-09	30. OPE-15	06. OPA-20
07. OPA-18	07. OPA-10	31. OPE-19	07. OPB-01
08. OPA-20	08. OPA-11	32. OPF-03	08. OPB-07
09. OPB-01	00. OPA-13	33. OPF-06	09. OPB-11
10. OPB-07	10. OPA-17	34. OPF-14	10. OPB-12
11. OPB-11	11. OPA-18	35. OPG-09	11. OPC-09
12. OPB-12	12. OPA-19	36. OPG-16	
13. OPC-01	13. OPA-20	37. OPG-17	
14. OPC-02	14. OPB-01	38. OPH-16	
15. OPC-03	15. OPB-02	39. OPH-17	
16. OPC-04	16. OPB-03		
17. OPC-05	17. OPB-04		
18. OPC-08	18. OPB-05		
19. OPC-09	19. OPB-06		
20. OPC-10	20. OPB-07		
21. OPC-13	21. OPB-10		
22. OPC-15	22. OPB-11		
23. OPD-15	23. OPB-12		
24. OPD-20	24. OPB-16		

high; but the level of polymorphism among *C. arabica* genotypes was low.

Eleven primers, which were present in both sets of primers, generated 73 polymorphic bands with consistent scores for all 40 genotypes. Since these 11 primers generated 97 polymorphic bands in the evaluation of the first set of primers, and 93 in the evaluation of the second set of primers, the reproducibility was 75,26% and 78,49%, respectively Therefore, the RAPD marker reproducibility for coffee was 76.88%, on average. This was compatible with the RAPD marker reproducibility of 76% obtained by Skroch and Nienhuis (1995) in *Phaseolus vulgaris* L.

Genetic distances based on 151 RAPD loci (24 primers)

The genetic distances among 40 coffee tree genotypes, based on 151 RAPD loci, expressed as arithmetic complements of Jaccard coefficients, ranged from 0% (e.g. genetic distance between the genotypes Mundo Novo IAC 515-3 and Mundo Novo IAC 388-17-16, and between Catuaí Vermelho IAC 81 and Catuaí Amarelo IAC 86) to 95% (between *C. racemosa* and *C. canephora*). The average genetic distance between *C. arabica* and *C. racemosa* was 89%. A 95% genetic distance between these two species was previously reported, based on RAPD markers (Lashermes et al.,1995). The lowest genetic distance for *C. racemosa* was obtained from UFV 557-3 (71.15%), a genotype derived from a cross of *C. racemosa* x *C. arabica*.

Six groups were obtained by cluster analysis based on Jaccard arithmetic complements, using the UPGMA method (Figure 2) with 19% genetic distance: group A (*C. racemosa*); group B (*C. canephora*), group C (UFV 557-3), group D (*C. congensis*), group E (Timor Hybrid in generation F_1 CIFC 4106), and group F (all other 35 genotypes). According to the genetic distances, the species were ordered as *C. arabica*, *C. congensis*, *C. canephora*, *and C. racemosa*, which is in accordance with previous publications (Lashermes et al., 1995; Raina et al., 1998).

At the 9.5% genetic distance limit, the F group was

subdivided into three subgroups: subgroup F1 with two genotypes (Bourbon Vermelho UFV 2947 and Bourbon Vermelho UFV 2946); subgroup F2 with nine genotypes (H 484-2, H 843, Catimor UFV 2983, Catimor UFV 1359, Cachimor UFV 351-13, Cavimor 357-4, Catimor UFV 386-19, H 415-3 and Catimor UFV 395-141); and subgroup F3 with 24 remaining genotypes. All genotypes from the F2 subgroup are "Timor Hybrid" descendants. 'Catimor' UFV 1310 (F_4 progeny of "Caturra Vermelho" CIFC 19/1 x "Timor Hybrid" CIFC 832/1 cross) was genetically closer to "Caturra Vermelho" than "Timor Hybrid".

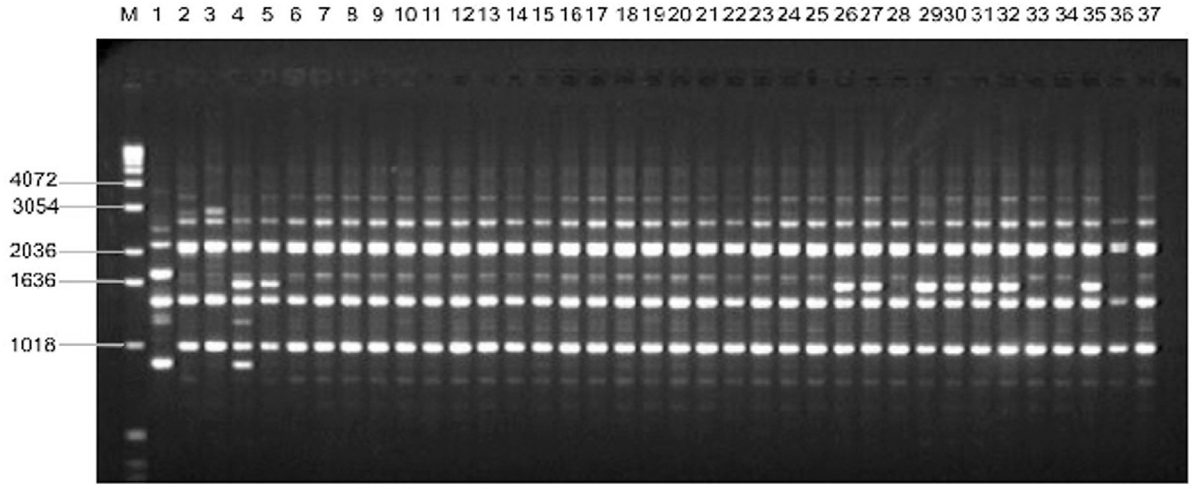

Figure 1. Electrophoresis profile of the coffee genotypes obtained from the *primer* OPC-09.

From left to the right: DNA marker of molecular weight 1 kb Ladder (M), C. *racemosa* (1), C. *congensis* (2), C. *canephora* (3), UFV 557-3 (4), Timor Hybrid CIFC 4106 (5), Timor Hybrid CIFC 2234 (6), Typica UFV 2945 (7), Typica UFV 536 (8), Catuaí IAC 15 (9), Catuaí IAC 44 (10), Catuaí IAC 99 (11), Catuaí IAC 81 (12), Catuaí IAC 86 (13), Catuaí IAC 47 (14), Catuaí IAC 113 (15), Mundo Novo IAC 464-18 (16), Mundo Novo IAC 515-3 (17), Mundo Novo IAC 376-4-32 (18), Mundo Novo IAC 388-17-16 (19), Mundo Novo IAC 376-4-22 (20), Bourbon Amarelo UFV 535 (21), Bourbon UFV 2952 (22), Bourbon Vermelho UFV 2946 (23), Bourbon Vermelho UFV 2947 (24), Caturra CIFC 19/1 (25), Catimor UFV 395-141 (26), Catimor UFV 386-19 (27), Catimor UFV 1310 (28), Catimor UFV 1359 (29), Catimor UFV 2983 (30), H 484-2 (31), H 415-3 (32), Mundindu UFV 315-76 (33), Cachimor UFV 351-13 (34), Cavimor UFV 357-4 (35), San Ramon UFV 3094 (36) and Airi UFV 3095 (37).

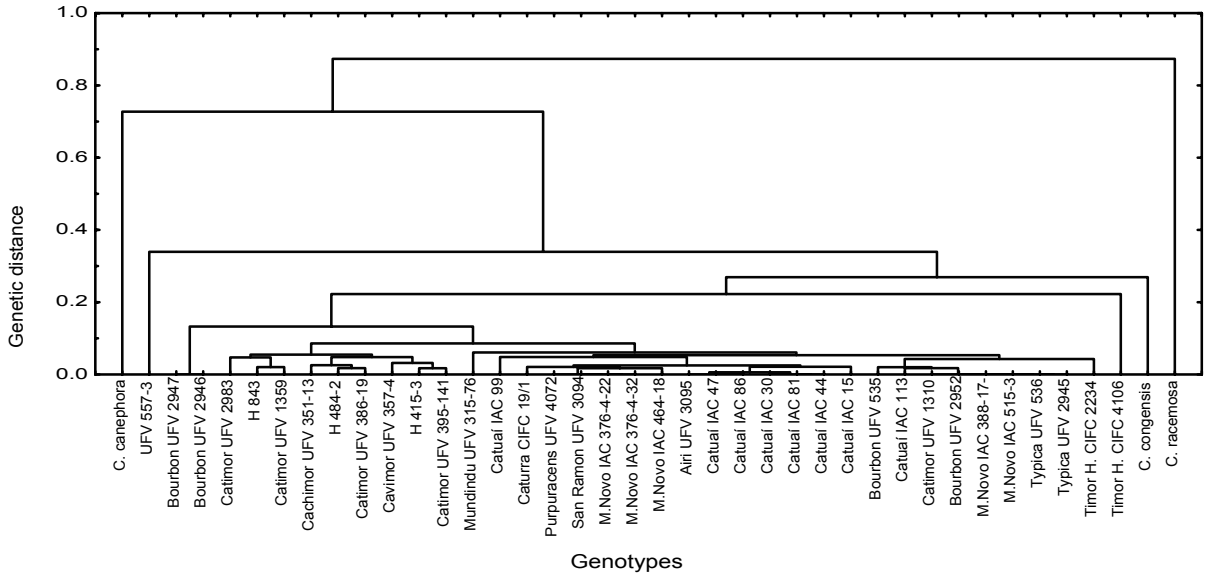

Figure 2. Dendrogram obtained from the genetic distances expressed in Jaccard's complements estimated among 40 coffee tree genotypes, based on 151 RAPD markers using the UPGMA method.

"Timor Hybrid" CIFC 2234 (UFV 376-52), was grouped with the commercial cultivars of *C. arabica*, which is in accordance with the results obtained by Fontes et al. (2000). Its phenotype is similar to that of "Mundo Novo". It is derived from Tanzania VCE 1587 selections ,and it is probably resistant to *Colletotrichum kahawae*, a coffee berry disease (CBD) causal agent.

Genetic distances based on 314 RAPD loci (39 primers)

The genetic distances based on 314 polymorphic bands ranged from 0% (e.g. between Mundo Novo IAC 515-3 and Mundo Novo IAC 388-17-16, and between Catuaí Vermelho IAC 81 and Catuaí Amarelo IAC 86) to 91% (between *C. racemosa* and *C. canephora*).

At the 19% genetic distance limit, six groups were formed with 314 RAPD loci (Figure 3), which were the same ones formed with 151 loci (Figure 2). By comparing these two figures, a switched position of *C. congensis* and "Timor Hybrid" CIFC 4106 was observed. In the grouping presented in Figure 3, *C. congensis* was closer to the arabica genotype group than "Timor Hybrid". The genetic distance between the arabica group and *C. congensis,* as well as distance between the arabica group and "Timor Hybrid", was approximately 30%, and different numbers of markers modified their positions.

At the 9.5% genetic distance limit , the F group was subdivided into seven subgroups: subgroup F1 with a genotype (H 484-2); subgroup F2 with two genotypes (Bourbon Vermelho UFV 2947 and Bourbon Vermelho UFV 2946); subgroup F3 with one genotype (Timor Hybrid CIFC 2234); subgroup F4 with one genotype (Cachimor UFV 351-13); subgroup F5 with one genotype (H 415-3); subgroup F6 with six genotypes (H 843, Catimor UFV 1359, Catimor UFV 2983, Catimor UFV 386-19, Cavimor UFV 357-4 and Catimor UFV 395-141); and subgroup F7 with 23 remaining genotypes. The increased number of polymorphic bands separated "Timor Hybrid" CIFC 2234 from the arabica cultivars group, forming the subgroups F1, F4, F5 and F6, all included (with 151 loci) in the same group (F2). All genotypes of the subgroup F6 were derived from the "Caturra Vermelho" CIFC 19/1 x "Timor Hybrid" CIFC 832/1 cross.

Genetic distances based on 348 RAPD loci (52 primers)

The genetic distances based on 348 RAPD markers ranged from 0% (e.g. between Mundo Novo IAC 515-3 and Mundo Novo IAC 388-17-16, and between Catuaí Vermelho IAC 81 and Catuaí Amarelo IAC 86) to 93% (between *C. racemosa* and *C. canephora*).

At the 19% genetic distance limit, six groups were formed with 348 RAPD loci (Figure 4), which were the same ones formed with 151 loci (Figure 2), or with 314 loci (Figure 3).

At the 9.5% genetic distance limit, group F was subdivided into five subgroups: subgroup F1 with two

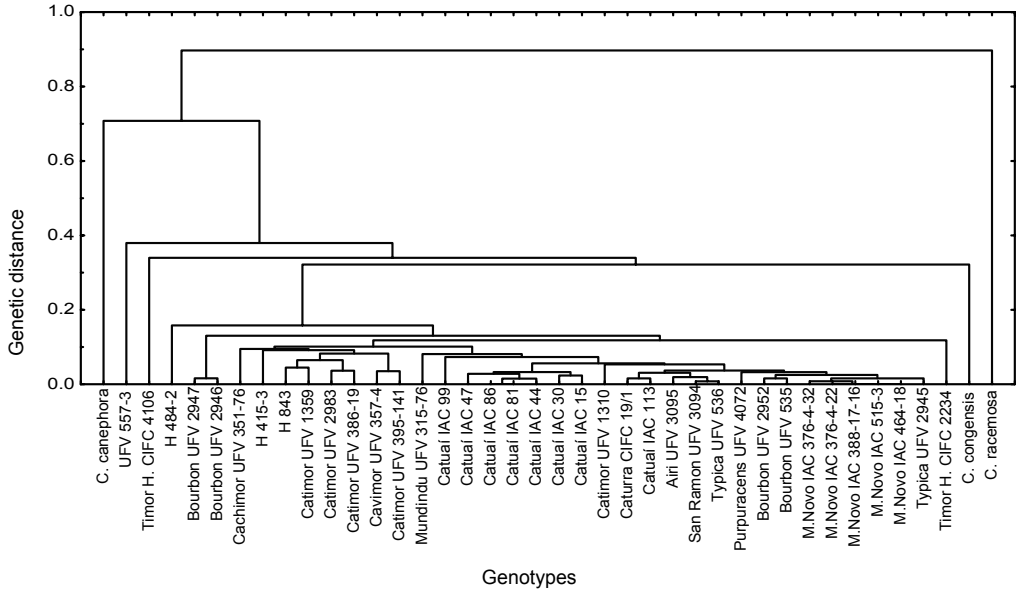

Figure 3. Dendrogram obtained from the genetic distances expressed in Jaccard's complements estimated among 40 coffee tree genotypes, based on 314 RAPD markers, using the UPGMA method.

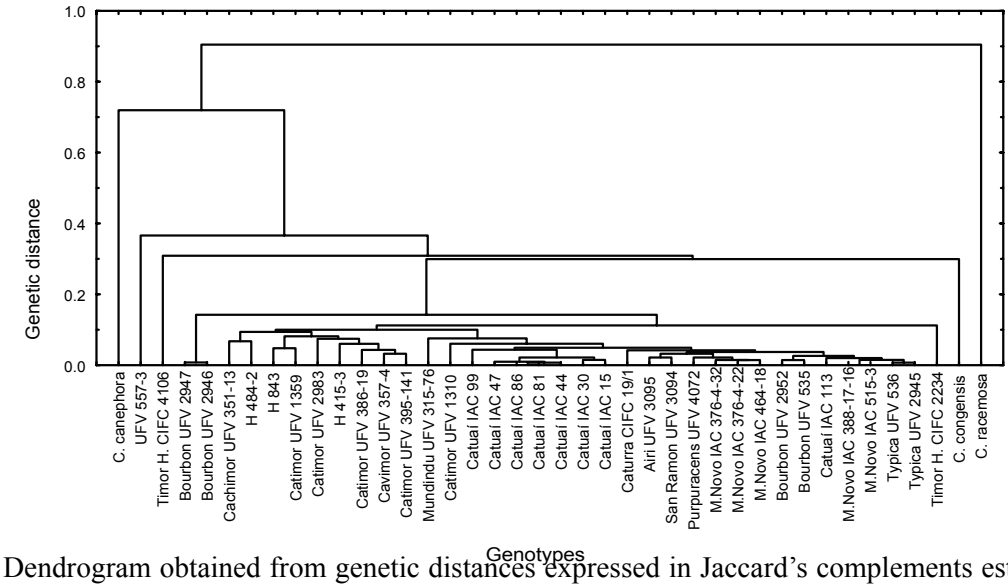

Figure 4. Dendrogram obtained from genetic distances expressed in Jaccard's complements estimated among 40 coffee tree genotypes, based on 348 RAPD markers, using the UPMGA method.

genotypes (Bourbon Vermelho UFV 2947 and Bourbon Vermelho UFV 2946); subgroup F2 with one genotype (Timor Hybrid CIFC 2234); subgroup F3 with two genotypes (Cachimor UFV 351-13 and H 484-2); subgroup F4 with seven genotypes (H 843, Catimor UFV 1359, Catimor UFV 2983, H 415-3, Catimor UFV 386-19, Cavimor UFV 357-4 and Catimor UFV 395-141); and subgroup F5 with the 23 remaining genotypes. The hybrid H 415-3 was again grouped with the "Timor Hybrid" derived genotypes in the subgroup F4. Cachimor UFV 351-13 and H 484-2, which formed the group F3 were also derived from "Timor Hybrid". The genotypes were coherently ordered within the F5 subgroup (arabica coffee plants); however, they did not form subgroups since they are genetically related. For example, the genotype Airi, a short plant, is a selection of 'San Ramon', which segregates for plant size. The cultivar Catuaí Amarelo IAC 113 was closer to "Mundo Novo" than to any other "Catuaí" cultivars. All Catuaí cultivars were derived from Mundo Novo, and the Catuaí Amarelo IAC 113 were selected from plant number 12 (H2077-2-12), while the other Catuaí cultivars were selected from plant number 5 (H2077-2-5). The subgroup F5 containing 23 genotypes of *C. arabica* supports the hypothesis of low genetic variability within this species, previously reported by Lashermes et al. (1993; 1995) and Orozco-Castillo

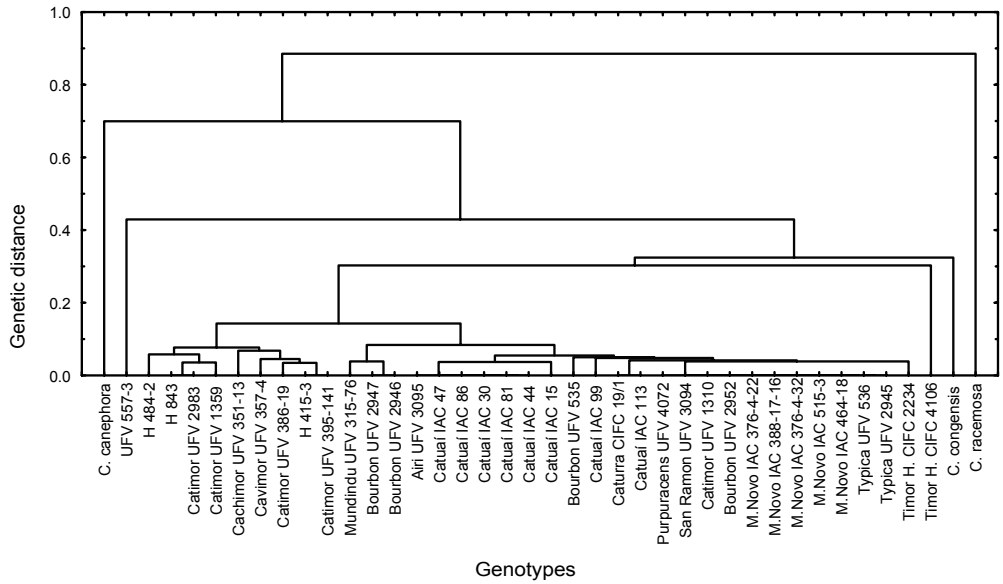

Figure 5. Dendrogram obtained from genetic distances expressed in Jaccard's complements estimated among 40 coffee tree genotypes, based on 73 replicated RAPD markers ,by UPGMA method.

et al. (1994; 1996). A high interspecies genetic diversity was observed in *Coffea*, as well as a relatively high diversity among the genotypes derived from "Timor Hybrid". Both results had been previously reported by Lashermes et al. (2000) , and they are considered important for breeding.

Genetic distances based on 73 RAPD loci (11 primers)

The genetic distances based on 73 polymorphic bands, which had consistent scores for all 40 genotypes in two amplifications, ranged from 0% (e.g. between Mundo Novo IAC 515-3 and Mundo Novo IAC 388-17-16, and between 'Catuaí Vermelho' IAC 81 and 'Catuaí Amarelo' IAC 86) to 95% (between *C. racemosa* and *C. canephora*).

At the 19% of genetic distance limit, the same six groups were formed with 73 RAPD loci (Figure 5), as they were with 151, 314, and 348 loci.

At the 9.5% genetic distance limit, group F was subdivided into two subgroups: the subgroup F1 with nine genotypes (H 484-2, H 843, Catimor UFV 2983, Catimor UFV 1359, Cachimor UFV 351-13, Cavimor 357-4, Catimor UFV 386-19, H 415-3, and Catimor UFV 395-141), and the subgroup F2 with the 26 remaining genotypes. Cultivars "Catuaí" and "Mundo Novo" were distinguished, but the genotypes within each of these two cultivars could not be separated. By comparing the results of subgroup F2 obtained from 73 repeated markers and the results of subgroup F3 obtained from 151 bands, it was observed that the genotypes' order was more coherent with the genealogy in the first case.

CONCLUSIONS

The evaluation of 40 *Coffea* genotypes with different numbers of RAPD markers did not affect the cluster analysis to separate the six major groups according to the genetic distances, but it affected the subgroups' composition and the genotypes ordering in the subgroups.

The marker reproducibility was 76.88% and there was no need for replicated data to distinguish major groups according to the genetic distances. However, it is recommended for distinguishing genotypes with the same genetic background, especially if a low number of polymorphic loci is considered.

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RESUMO

Reprodutibilidade de marcador RAPD e sua eficiência em análise de grupos de genótipos de árvores de café

A diversidade genética de cultivares de *Coffea arabica* L. é relativamente pequena e a sua avaliação e ampliação é importante para o melhoramento genético. Cinquenta e dois *primers* arbitrários foram utilizados para avaliar a reprodutibilidade e a influência do número de marcadores RAPD (*Random Amplified Polymorphic DNA*) na estimação de distâncias genéticas entre 40 genótipos de *Coffea* spp. O número médio de bandas polimórficas entre todos os genótipos foi de 6,69 por primer, e de 1,27 entre genótipos de café arábica. Marcadores RAPD foram eficientes para a estimação de distâncias genéticas entre os genótipos. O aumento do número de *loci* de RAPD não alterou a formação dos principais grupos na análise de agrupamento, mas influenciou na formação de subgrupos. A reprodutibilidade dos marcadores foi de 76,88% e a utilização de dados replicados foi recomendada para distinguir genótipos relacionados.

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