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Characterization of differential coffee tree hosts for *Hemileia vastatrix* Berk. et Br. with RAPD markers

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ABSTRACT - *Eighteen clones of differential coffee tree hosts for* Hemileia vastatrix *Berk. et Br. were characterized with RAPD markers. The genetic distances were estimated and the genealogical origin of the clones compared to data of marker-based clusters. Thirty-five primers identified 158 polymorphic loci of RAPD markers. The cluster based on the matrix of genetic dissimilarity values was compatible with information on the genealogical origin cited in literature. Specific markers for a number of clones were identified, and a combination of 12 RAPD markers allowed the characterization of the studied clones.*

Key words: *Coffea*, RAPD, leaf rust, differential hosts, genealogical origin.

INTRODUCTION

The highly variable fungus *Hemileia vastatrix* Berk. et Br., causal agent of orange rust on coffee plants, presents a great number of described physiological races (Mayne 1932, Reyes 1957, D'Oliveira and Rodrigues 1960, Rodrigues Jr et al. 1975, Lopes and Godinho 1976), several of which are found in Brazil (Cardoso et al. 1981, Cardoso et al. 1988).

According to genetic studies into the behavior of *Coffea arabica* L plant progenies and interspecific hybrids, nine dominant genes, S_H 1,2,3,4,5,6,7,8, and 9, responsible for the control of resistance to this disease, were identified by inoculation with physiological races of *H. vastatrix* (Mayne 1936, Noronha-Wagner and Bettencourt 1967, Bettencourt and Noronha-Wagner 1971, Bettencourt et al. 1980; Bettencourt and Rodrigues 1988). Genes S_H 1,2,4, and 5 are present in the species *C. arabica* (Noronha-Wagner and Bettencourt 1967, Bettencourt and Noronha-Wagner 1971). Gene S_H 3 is merely found in Arabica coffees that stem from India and has, most likely, been derived from *C. liberica* through interspecific hybridization (Mayne 1936, Noronha-Wagner and Bettencourt 1967). Genes S_H 6,7,8, and 9 probably stem from *C. canephora* and, when associated to one or more unknown genes, present resistance to all known fungus races (Bettencourt and Rodrigues 1988). Different combinations of these genes are found in the populations derived from the 'Híbrido de Timor'. This hybrid, quite largely used in breeding programs as source of *Hemileia vastatrix* resistance, is probably result of a natural cross between *C. arabica* and *C. canephora* (Bettencourt 1973).

Varieties used for the identification of pathogen races are called differential hosts. For host-pathogen systems in which the gene-to-gene concept was confirmed or inferred, a group of differential hosts, in which every member has only one single resistance gene, was considered the most efficient and informative method for studies into pathogen variation (Flor 1971). The validity of the gene-to-gene theory was acknowledged by a host-pathogen interaction analysis for the compound *Coffea-Hemileia vastatrix*, and intense studies conducted at the Centro de Investigação das Ferrugens do Cafeeiro - CIFC, Portugal, selected a group of differentiating coffee plants (Bettencourt 1981).

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For the maintenance of the genetic identity of these materials, the characterization is crucial, and requisite for identification purposes of the physiological races of *H. vastatrix*. Molecular markers based on direct DNA analysis, potentially unlimited in number and uninfluenced by the environment, are indicated for such characterization studies (Sakiyama 2000).

RAPD (*Random Amplified Polymorphic DNA)* markers (Williams et al. 1990) have been used to study several species and proved efficient at the characterization of coffee genotypes (Lashermes et al. 1993, Orozco-Castillo et al. 1994, Lashermes et al. 1996, Orozco-Castillo et al. 1996, Silva et al. 2002, Teixeira-Cabral et al. 2002).

Main goals of our study were the molecular characterization of 18 clones of differential coffee tree hosts for physiological races of *Hemileia vastatrix*, the estimation of genetic distances, and a comparison of their genealogy.

MATERIAL AND METHODS

Plant material

Eighteen clones of *Coffea* sp differential hosts for physiological races of *Hemileia vastatrix* were used. These clones were originally obtained by vegetative propagation from the original collection of the CIFC (Table 1).

DNA Extraction

The DNA of 18 genotypes was extracted from young leaves, according to the modified protocol of Doyle and Doyle (1990), under addition of soluble PVP-40 to the extraction buffer. After the extraction, the DNA was quantified in a spectrophotometer and stored at 4 °C. The DNA was diluted in TE (Tris HCl 10 mM, EDTA 1 mM, pH 8.0) to a final concentration of 10 ng/µl for the amplification.

DNA Amplification and electrophoretic product analysis

Thirty-five primers of ten bases (Operon Technologies) were used to amplify the DNA of each one of the 18 genotypes. The amplification was carried out in a Perkin-Elmer 9600 thermocycler, every reaction with a total volume of 25 µl and the following components: 25 ng of genomic DNA, 1 unit of Taq DNA polymerase, 0.1 mM of each dNTP, 0.2 µM primer, 50 mM KCl, 10 mM Tris HCl pH 8.3, and 2 mM $MgCl_2$, and completed up to the final volume with pure water. The following program was run: one denaturation cycle (95 °C for 1 min), 39 amplification cycles (15 sec at 94 °C, 30 sec at 35 °C, 60 sec at 72 °C) and, in a final step, 7 min at 72 °C. The amplification reaction products were separated by electrophoresis in 1.4% agarose gel, stained with ethidium bromide, visualized under UV, and photo-documented. RAPDs were registered as presence or absence of bands. Only polymorphisms observed in sharply defined bands were taken into consideration.

Data analysis

The data were scored as value 1 representing presence and 0 absence of band in the same locus. Estimates of genetic similarities were expressed as similarity coefficients of Jaccard (Jaccard 1901), given by the equation $GS_{ij} = a/(a + b + c)$, where Gs_{ij} is the genetic similarity between genotypes *i* and *j*, a is the

Table 1. Clones of coffee tree differential hosts for *Hemileia vastatrix* and respective origin

Code	Description	Origin	Group of resistance	Genes of resistance
1. CIFC 110/5	S 4 Agaro	Ethiopia	J	S_H4,5
2. CIFC 128/2	Dilla and Alghe	Kenya	α	S_H1
3. CIFC 87/1	Geisha	Tanzania	C	S_H1,5
4. CIFC 635/3	S 12 Kaffa	Ethiopia	W	S_H1,4,5
5. CIFC 1006/10	KP 532 (Kent) plant 31	Tanzania	L	S_H1,2,5
6. CIFC 134/4	S 12 Kaffa	Ethiopia	I	S_H1,4
7. CIFC 32/1	DK 1/6	India	D	S_H2,5
8. CIFC HW 17/12	CIFC 35/2 x CIFC 134/4	Portugal	O	S_H1,2,4,5
9. CIFC H 152/3	CIFC 32/1 x CIFC 110/5	Portugal	γ	S_H2,4,5
10. CIFC 644/18	Kawisari Hybrid	Indonesia	M	S_H?
11. CIFC 33/1	S 288-23	India	G	S_H3,5
12. CIFC 147/1	CIFC 34/13 x CIFC 110/5	Portugal	T	S_H1,3,4,5
13. CIFC H 153/2	CIFC 87/1 x CIFC 33/1	Portugal	Z	S_H1,3,5
14. CIFC 4106	Híbrido de Timor	Timor	A	S_H5,6,7,8,9,?
15. CIFC 1343/269	Híbrido de Timor	Timor	R	S_H6
16. CIFC 832/1	Híbrido de Timor	Timor	A	S_H5,6,7,8,9,?
17. CIFC H 419/20	MN 1535/33 x HW 26/13	Portugal	3	S_H5,6,9
18. CIFC H 420/10	MN 1535/33 x HW 26/14	Portugal	1	S_H5,6,7,9

Source: Adapted from Bettencourt (1981).
Clones 1 through 9 = *C. arabica*; Kawisari Hybrid = *C arabica* x *C. liberica*; S 288-23 = *C arabica* x *C. liberica*; CIFC 34/13 = S 353 4/5 = *C arabica* x *C. liberica*; Híbrido de Timor = *C. arabica* x *C. canephora;* CIFC HW 26 = Caturra Vermelho CIFC 19/1 x Híbrido de Timor CIFC 832/1; CIFC – Centro de Investigação das Ferrugens do Cafeeiro (Oeiras - Portugal); MN = Mundo Novo; H = hybrid.

number of bands present in both *i* and *j*, b is the number of bands present in *i* and absent in *j*, and c the number of bands present in *j* and absent in *i*. The conversion to the genetic distance (GD) was given by the equation $GD_{ij} = 1 - GS_{ij}$, calculated by software GENES (Cruz 1997). The dendogram, based on the matrix of genetic distances, was obtained by means of the cluster analysis with software STATISTICA version 5.0 (StatSoft 1997), using the method UPGMA (unweighted pair-group method based on arithmetic averages). Differentiating molecular patterns were sought for a characterization of the clones by comparing the electrophoretic profiles of the amplified products.

RESULTS AND DISCUSSION

The electrophoretic profiles, obtained for coffee tree with the RAPD marker technique, presented clear polymorphic bands, as presented in Figure 1, obtained with primer OPB-18. Thirty-five primers brought forth 158 polymorphic bands, on average 4.5 polymorphic bands per primer.

The genetic distances obtained for the 18 clones of coffee tree, based on 158 RAPD bands, varied from 4% (between clones CIFC 128/2 and CIFC 87/1) to 91% (between clones CIFC 644/18 and CIFC 1343/269). The cluster analysis based on the UPGMA method at a level of 39% genetic distance (Figure 2), defined three groups: group A, with one clone (Kawisari Hybrid CIFC 644/18); group B, five clones Híbrido de Timor CIFC 1343/269, Híbrido de Timor CIFC 4106, Híbrido de Timor CIFC 832/1, CIFC H 419/20, and CIFC H 420/10); and group C with the other 12 clones. The most divergent clone was Kawisari Hybrid CIFC 644/18, which is, most likely, a natural tetraploid hybrid between *C. arabica* and *C. liberica*, susceptible only to race XIII (Chaves 1976). Group B assembled three Híbrido de Timor progenies and two hybrids derived from crosses involving Híbrido de Timor CIFC 832/1. Genes S_H 6, S_H 7, S_H 8, and S_H 9, found exclusively in Híbrido de Timor derivates, are present in this genotype group (Table 1).

Clone CIFC 4106 is the plant selected on Timor Island, supposedly Híbrido de Timor in generation F_1, reproduced via vegetative propagation, and introduced at the Centro de Investigação das Ferrugens do Cafeeiro (Pereira et al. 2002). The other two accesses, CIFC 1343/269 and CIFC 832/1, are clones derived from Híbrido de Timor and introduced in the CIFC via seeds. These clones were also introduced at the UFV via vegetative propagation, under the registers UFV 516, UFV 305, and UFV 529, respectively. Group C contains nine materials of Arabica and three of Arabica with *C. liberica* introgression.

Assuming a limit of 25% of genetic distance, groups B and C were subdivided in three subgroups: subgroup B1, with one clone (Híbrido de Timor CIFC 1343/269); subgroup B2, one clone (CIFC 4106); subgroup B3, three clones (CIFC 832/1, CIFC H 419/20, and CIFC H 420/10); subgroup C1, two clones (CIFC H 147/1 and CIFC 33/1); subgroup C2, one clone (CIFC H 153/2); and subgroup C3 with nine clones (CIFC 87/1, CIFC 128/2, CIFC 110/5, CIFC 1006/10, CIFC 635/3, CIFC 32/1, CIFC H 152/3, CIFC 134/4, and CIFC HW 17/12). The three accesses of Híbrido de Timor (CIFC 4106, CIFC 1343/269, and CIFC 832/1) were placed in different subgroups, demonstrating that, although Híbrido de Timor is derived from a single plant (Bettencourt 1973), the variability in populations and genotypes derived of this same Híbrido de Timor is considerable (Lashermes et al. 2000). The formation of subgroup B3 (CIFC 832/1, H 419/20, and H 420/10) is coherent, since Híbrido de Timor CIFC 832/1 is one of the genitors of hybrids H 419/20 and H 420/10.

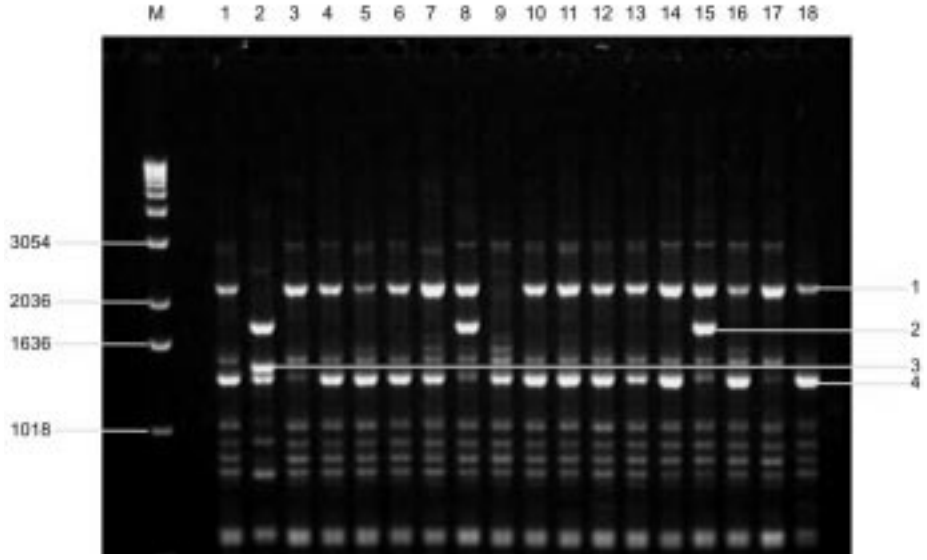

Figure 1. Electrophoretic pattern of DNA obtained with primer OPB-18 for the 18 clones of coffee tree differential hosts for *Hemileia vastatrix*. From left to right: pattern ëHindIII, white, CIFC HW 17/12, CIFC 644/18, CIFC 110/5, CIFC 128/2, CIFC H 420/10, CIFC 87/1, CIFC 4106, CIFC 33/1, CIFC 832/1, CIFC 635/3, CIFC 1343/269, CIFC 1006/10, CIFC H 152/3, CIFC 134/4, CIFC 147/1, CIFC H 419/20, CIFC H 153/2 and CIFC 32/1.

Clone CIFC 33/1 of subgroup C1 is based on the interspecific cross between *C. arabica* and *C. liberica*, selected at the Estação Experimental de Balehonnur (India). This selection (CIFC 33/1), as well as S 353 4/5 (CIFC 34/13), originated from India, were used at the CIFC, Portugal, in crosses for the achievement of CIFC H 147/1 hybrids of subgroup C1 and CIFC H 153/2 of subgroup C2 (Bettencourt 1981). Subgroup C3 consists of pure Arabica genotypes (cultivars, selections, or hybrids). Clone H 153/2 was set in-between subgroup C1 of the interspecific clones *C. arabica* x *C. liberica* and subgroup C3 of pure Arabicas. This position is in agreement with the genealogy, as it is a hybrid that stems from a cross between clones CIFC 87/1 and CIC 33/1 (Figure 2). The organization of the clones within subgroup C3 is also in line with the genealogical origin; for example, CIFC 134/4 is one of the genitors of hybrid HW 17/12, and CIFC 32/1 is one of the genitors of hybrid CIFC H 152/3. We verified, therefore, that the cluster obtained with base on the RAPD markers and the genealogical origins of the clones were consistent. The agreement between the clusters, based on molecular markers, and the origin of the genotypes of the genus *Coffea* was also observed by other researchers (Orozco-Castillo et al. 1994, 1996, Lashermes et al. 1997).

Sixty (38%) out of 158 RAPD markers were specific to Kawisari Hybrid CIFC 644/18 clone, ten (6.3%) specific to clone Híbrido de Timor CIFC 4106 and six (3.8%) specific to clone Híbrido de Timor CIFC 1343/269. A smaller number of specific markers were obtained for other clones, varying from one (for clone CIFC 87/1) to four markers (for clone CIFC 419/20). The comparison of the electrophoretic profiles of the amplified products permitted the selection of 12 RAPD markers, whose combination allowed the identification of the 18 clones (Table 2). Markers OPA08-1225 and OPC08-1692 are specific to clones with introgression of *C. liberica* genes, while markers OPA01-1066 and OPA07-1565 are specific to clones with introgression of *C. canephora* genes, and the

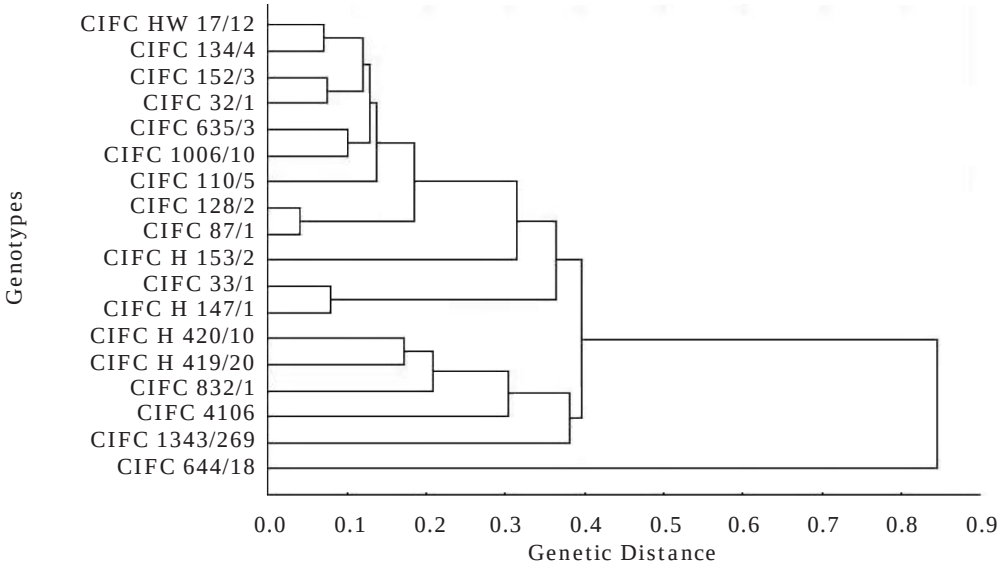

Figure 2. UPGMA dendrogram for 18 clones of coffee tree.

Table 2. Molecular characterization obtained with 12 RAPD markers for 18 clones of coffee tree differential hosts for *Hemileia vastatrix*

Marker	Clones																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
OPA01-1066	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+
OPA01-1532	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+
OPA07-0925	+	+	+	+	+	+	-	+	+	-	+	+	+	-	-	-	-	-
OPA07-1565	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-
OPA08-1225	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-
OPA09-1520	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OPA18-1162	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
OPA20-0867	-	-	-	-	+	+	+	+	+	-	-	-	-	+	-	-	-	+
OPB07-1764	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-
OPB12-2624	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
OPC03-1018	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
OPC08-1692	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-

Clones: 1 - CIFC 110/5; 2 - CIFC 128/2; 3 - CIFC 87/1; 4 - CIFC 635/3; 5 - CIFC 1006/10; 6 - CIFC 134/4; 7 - CIFC 32/1; 8 - CIFC HW 17/12; 9 - CIFC H 152/3; 10 - CIFC 644/18; 11 - CIFC 33/1; 12 - CIFC 147/1; 13 - CIFC H 153/2; 14 - CIFC 4106; 15 - CIFC 1343/269; 16 - CIFC 832/1; 17 - CIFC H 419/20; 18 - CIFC H 420/10. Data: + band presence; - band absence.

markers OPB07-1764, OPA09-1520, and OPA18-1162 are specific to pure Arabic clones, without any recent introgressions of genes of other species. The obtained molecular pattern can be used to identify the studied genotypes, which will help maintain the genetic identity of clones of coffee tree differential hosts.

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Caracterização de clones de cafeeiro diferenciadores de *Hemileia vastatrix* Berk. et Br. com marcadores RAPD

RESUMO - *Dezoito clones de cafeeiros diferenciadores para* Hemileia vastatrix *Berk. et Br. foram caracterizados com marcadores RAPD. As distâncias genéticas foram estimadas e a origem genealógica dos clones foi comparada com os dados de agrupamento com base nos marcadores. Utilizaram-se 35* primers *que identificaram 158 locos polimórficos de marcadores RAPD. O agrupamento com base na matriz de valores de dissimilaridades genéticas foi compatível com as informações disponíveis na literatura sobre a origem genealógica. Foram identificados marcadores específicos para vários clones, e a combinação de 12 marcadores RAPD permitiu a identificação dos clones estudados.*

Palavras-chave: *Coffea*, RAPD, ferrugem, clones diferenciadores, genealogia.

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