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Inheritance of coffee leaf rust resistance and identification of AFLP markers linked to the resistance gene

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Abstract The most important disease of *Coffea arabica* is coffee leaf rust caused by the fungus *Hemileia vastatrix*. The purpose of this study was to characterize the inheritance of coffee resistance gene(s) to race II of this pathogen and to identify and map molecular markers linked to this trait. Different populations were used: F_2 (160 plants), BCr (20), and BCs (135), derived from a cross between the resistant genotype Híbrido de Timor UFV 427-15 and the susceptible cultivar Catuaí Amarelo UFV 2143-236 (IAC 30). The segregation analysis showed that the resistance of Híbrido de Timor to race II of the *H. vastatrix* is conferred by a single dominant gene. The amplification of 176 AFLP (Amplified fragment length polymorphism) primer combinations using bulked segregant analysis (BSA) allowed the identification of three molecular markers linked to the resistance gene. Genetic mapping of these three markers in the F_2 population indicated that they are distributed on both sides, flanking the resistance gene. The markers E.CTC/M.TTT405 and E.CGT/M.TGT 300 were found linked to the resistance gene at 8.69 cM (LOD 18.91) and 25.10 cM (LOD 5.37), respectively, while E.CCT/M.TTC230 was localized on the other side of the gene, at 20.50 cM (LOD 6.15). These markers are the first rust resistance markers identified in Híbrido de Timor and can be useful for marker assisted selection in coffee breeding programs.

Keywords Coffee leaf rust · Resistance gene · AFLP marker · Bulked segregant analysis

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Introduction

Coffee leaf rust, caused by the biotrophic fungus *Hemileia vastatrix* Berk. et Br., is considered the main disease in commercial *Coffea arabica,* but *C. canephora* can be also most affected. Spreading out from Africa and Asia, the disease first reached Brazil in 1970. Nowadays, it's present in all coffee producing countries, except in Hawaii and Australia.

The importance of establishing durable resistance to this pathogen lies in the fact that the economic damage

caused by the yield reduction at the global level varies from one to two billion dollars annually, since the majority of the arabica coffee cultivar are susceptible (Van der Vossen 2001), and *C. canephora* can sometimes be affected as well. In Brazil, it is estimated that disease losses, in the absence of effective control measures, can amount to 30% of the production (Kushalappa and Eskes 1989; Zambolim et al. 1999). The main damages caused by the disease are premature defoliation, resulting in a reduced leaf area and withered lateral branches, leading to a gradual debilitation of the infected plant (Matiello et al. 2002). Chemical control with protective copper and/or systemic fungicides of the triazol group for this disease proved effective (Matiello et al. 2002; Zambolim et al. 2002). Although efficient, the effects on the environment and non-target organisms may lead to a population explosion of pests and or other coffee diseases. Moreover, the selection pressure exerted on the pathogen with this control strategy paves the way for the emergence of new fungus races resistant to the products applied (Zambolim et al. 2002). The most appropriate alternative to chemical control is the use of resistant cultivars, which can be obtained by conventional breeding, aided by molecular techniques (Fazuoli et al. 2002; Pereira et al. 2002; Sera et al. 2002; Fazuoli et al. 2005).

Several research groups from different regions have sought to achieve durable resistance to coffee leaf rust, resulting in initial success followed by disappointments because of the emergence of new virulent pathogen races able to infect the initially resistant plants (Van der Vossen 2001). The variability in pathogenicity of biotrophic fungi, including *H. vastatrix,* is normally great. To date, about 45 physiological races of this species have been identified (Várzea and Marques 2005). Thirteen races have been confirmed in Brazil and among these, race II with the virulence gene v5 predominates in commercial crops areas, since the main commercial cultivars planted worldwide in the different producing regions contain the factor S_H5 of rust resistance (Fazuoli et al. 2005). The use of genotypes derived from the Híbrido de Timor (hybrid of the natural crossing between *C. arabica* and *C. canephora*) containing the resistance genes S_H5, S_H6, S_H7, S_H8 and S_H9 (Wagner and Bettencourt 1965; Vishveshwara 1974; Bettencourt and Rodrigues 1988) is an interesting source for the use in breeding programs for coffee leaf rust resistance.

The resistance of coffee to this pathogen has been much explored in *C. arabica* and other species of the same genus. Components of fungus resistance, of monogenic as well as oligogenic and polygenic nature, have been identified. Within the genus *Coffea*, at least nine dominant genes (S_H1–S_H9) confer resistance to *H. vastatrix*. Breeding programs for rust resistance in coffee have focused on the accessions collected in the primary centers of diversity (Bettencourt and Rodrigues 1988). Among these resistance factors present in the genus *Coffea*, S_H1, S_H2, S_H4 and S_H5 were found in *C. arabica*. Other genes, such as S_H6, S_H7, S_H8 and S_H9, were introgressed from *C. canephora*, while S_H3 originated from *C. liberica* (Wagner and Bettencourt 1965; Vishveshwara 1974; Bettencourt and Rodrigues 1988).

It has been demonstrated that the genes present in *C. arabica*, alone or in combination, have not conferred durable resistance against many *H. vastatrix* races. However, the S_H3 factor, present in *C. liberica* has conferred durable resistance to commercial crops under field conditions (Srinivasan and Narasimhaswamy 1975; Prakash et al. 2005). This information suggests that a combined accumulation of these genes in other varieties that have agronomic traits of interest could establish the desired durable resistance (Kushalappa and Eskes 1989).

Although coffee can be considered one of the most studied and well-documented plant regarding its resistance to *H. vastatrix*, development of selection strategies involving molecular markers linked to resistance remains difficult. Hence, the identification of molecular markers closely linked to S_H genes and other conditioning genes of coffee rust resistance could be very helpful in the development of cultivars with durable resistance characteristics to this pathogen.

This paper reports three AFLP markers linked to the resistant gene in Híbrido de Timor UFV 427-15 by analyzing the F_2 population from artificial cross between this resistant parent and the susceptible genotype Catuaí Amarelo UFV 2143-236 (IAC 30).

Materials and methods

Plant material and crosses

A F_1 plant (H 421-4), derived from the cross between the resistant source Híbrido de Timor UFV 427-15

and the susceptible variety Catuaí Amarelo UFV 2143-236 (IAC 30), was selfed to generate the F_2 seeds and used for backcrossing with Catuaí Amarelo UFV 2143-236 (BCs) and the Híbrido de Timor UFV 427-15 (BCr). The UFV 427-15 is a rust resistant germplasm introduced from Centro de Investigação das Ferrugens do Cafeeiro (CIFC), Portugal, as a seed collected in coffee trees selected in the Estação Regional de Uige (ERU) of the Instituto de Café de Angola. The resultants 160 F_2, 135 BCs and 20 BCr plants were used to characterize the genetic resistance. To identify markers linked to the resistance gene, only the F_2 plants were used. All crosses were performed in Universidade Federal de Viçosa experimental station at Viçosa, Minas Gerais State, Brazil.

Disease evaluation

The resistance was characterized by inoculation of the abaxial leaves of parental genotypes (resistant and susceptible) and F_1, F_2, BCs and BCr plants, with 20 drops (5.0 µl each) of urediniospores suspension (2.0 mg ml^{-1}) of the *H. vastatrix*, race II. The inoculations were done in three replicates, and occasionally, additional ones were used in the case of unclear symptom. The inoculated leaves were transferred to an acrylic box (gerbox), containing a 1.0 cm thick foam, saturated with distilled water and covered with nylon mesh. The gerbox were kept at the dark for 48 h at 22 ± 2°C, and then, exposed to a photoperiod of 12 h light, 12 h dark at 22°C, at constant saturated humidity within the boxes. Symptoms were assessed 45 days after inoculation according to a 6-point scale of Tamayo et al. (1995), where 1—absence of symptoms; 2—small clorotic lesions; 3—median clorotic lesions, without spores formation; 4—clorotic lesions, with small urediniospores formation (urediniospores occupying <25% of the lesion area); 5—sporulation occupying among 25 and 50% of the lesion area; and 6—sporulation occupying >50% of the lesion area. The plants were classified in two phenotyping groups, the ones scored 1–3 (absence of urediniospores) were considered resistant and 4–6 susceptible (presence of urediniospores).The phenotypic segregations of the populations were analyzed by the χ^2 test, using the GENES software (Cruz 2006). χ^2 tests were used to estimate probabilities for segregation ratios of the predicted and observed at $P < 0.05$ probability.

DNA extraction

To identify AFLP markers linked to the resistant gene, two leaves from the parents and each F_2 plant were collected, labeled and stored at −80°C for later DNA extraction. The DNA was extracted with CTAB (Diniz et al. 2005). The DNA concentration was estimated by spectrophotometry (Smart SpecTM 3000). The integrity was evaluated on agarose gel (1.0%) and visualized under UV light by ethidium-bromide-staining (10 mg ml^{-1}) and photographed (Eagle-eye IITM Stratagene). Based on the estimated concentration, the DNA was diluted to 50 ng μl^{-1} and stored at −20°C.

DNA reaction and amplification

The AFLP analysis was performed according to the methodology described by Vos et al. (1995), with some modifications. In our study 176 primer combinations were analyzed. Initially, 350 ng μl^{-1} DNA was digested, using a rare cutting (*Eco*RI) and a frequent cutting (*Mse*I) restriction enzyme. Adapters were ligated to the ends of the digested DNA fragments in a reaction containing T4 DNA ligase (Promega). The ligase reaction occurred at 20 ± 2°C for 3 h. Then the material was diluted 1:10 in TE pH 8.0 and stored at −20°C.

For the pre-selective amplification, the DNA fragments contained in the diluted solution of the ligase stage were amplified using primers complementary to adapters with one more selective nucleotide at the 3′ end. The reactions consisted of 1.50 ng μl^{-1} oligo *Eco*RI + N, 1.50 ng μl^{-1} oligo *Mse*I + N, 0.25 mM of each dNTP (Promega), 1× Taq buffer, 1.5 mM $MgCl_2$, 2.5 µl of ligase reaction diluted 1:10 and 2.5 units of *Taq* DNA polymerase (Phoneutria), amounting to a final volume of 25 µl. The amplification program consisted of 23 denaturation cycles at 94°C for 30 s, annealing at 56°C for 60 s and an extension step at 72°C for 60 s. In the end, the reaction product was diluted 1:40 in TE pH 8.0 and stored at −20°C.

In the selective amplification, primers were used containing three nucleotides added to the 3′ end. The reactions consisted of 1.25 ng μl^{-1} of *Eco*RI + NNN primer, 1.50 ng μl^{-1} of *Mse*I + NNN primer, 0.25 mM of each dNTP (Promega), 1× Taq buffer, 1.5 mM $MgCl_2$, 1.5 units of *Taq* DNA polymerase

(5 U.μl^{-1}, Phoneutria) and 5.0 μl diluted pre-selective amplification reaction, amounting to a final reaction volume of 20 μl. The selective amplification program consisted of 13 cycles denaturation at 94°C for 30 s, annealing at 65°C for 30 s (−0.7°C at each cycle), extension at 72°C for 60 s, followed by 23 cycles at 94°C for 30 s, at 56°C for 30 s and at 72°C for 60 s.

The amplified fragments were separated by electrophoresis in 6% polyacrylamide gel, at 80 W, for approximately 3 h. The gel was stained by the silver nitrate method (Creste et al. 2001), with some adjustments. After electrophoresis, the gel plate was immersed in a solution containing 10% absolute ethanol and 1% acetic acid under continuous slow stirring for 12 min. Subsequently, the gel was washed with 2 l ultrapure water under stirring for 1 min. The pre-treatment was carried out by immersion in the gel solution containing 1.5% nitric acid for 3 min. The gel was washed once more using 2 l ultrapure water for 1 min under stirring. Subsequently, it was stained with $AgNO_3$ solution (0.3%) for 25 min, and washed twice (35 s each) with ultrapure water under stirring. Then the gel was immersed in 1 l Na_2CO_3 (3%) and formaldehyde solution (0.02%) and continuously stirred until the first bands appeared. The solution was discarded and another 1 l of new solution added to the process. After immersion in 2 l blocking solution (5% acetic acid) for 5 min under slow agitation, the gel was washed in 2 l ultrapure water for 1 min.

Identification of the gene markers linked to resistance

The BSA (Bulk Segregant Analysis) technique (Michelmore et al. 1991), was used to identify AFLP markers linked to the resistance gene(s) present in Híbrido de Timor UFV 427-15. According to the authors, this methodology is a fast procedure to identify markers in specific genome regions. In our study, two contrasting bulked DNA sample were constructed, one containing DNA from five resistant F_2 plants and the other containing DNA from five susceptible F_2 plants.

Statistical analysis

The segregation of the heteromorphic fragments and resistance gene was evaluated by the χ^2 test, using GENES software (Cruz 2006). The order of the markers and their distances to the coffee leaf rust resistance gene were determined using the recombination frequency calculation and GQMOL software (Cruz and Schuster 2001), expressed in centiMorgan (cM). It was considered 30% of the maximum recombination rate between the marker and the gene ($r_{max} = 0.3$) and minimum LOD score of 3.0 ($LOD_{min} = 3$). The recombination percentage was obtained by the graphic procedure from maximum likelihood (Liu 1998). The algorithm used to define the best marker order was Rapid Chain Delineation—RCD (Doerge 1996).

Results

Disease resistance inheritance

The resistant parent Híbrido de Timor UFV 427-15 was completely free of any disease symptoms (score 1 in the disease evaluation) in all assessments and replications, while the susceptible parent Catuaí Amarelo UFV 2143-236 (IAC 30) was infected (usually score 6), as evidenced by the abundant sporulation.

Among the 160 plants examined in the F_2 population, resistance was detected in 124 plants (77%) while 36 were considered susceptible (23%). These data indicated a segregation ratio of 3:1, as expected for a single dominant gene ($\chi^2 = 0.5336$, $P = 0.4652$; Table 1). The resistance/susceptibility segregation observed in BCr and BCs populations confirms the monogenic dominant inheritance of the Híbrido de Timor (Table 1).

Identification of AFLP markers associated with resistance gene

In this study, the AFLP technique proved to be suitable to identify molecular markers associated with coffee leaf rust resistance. A total of 176 AFLP primer combinations and about 11,000 bands were analyzed for ligation (Table 2). Depending on the primer combination used in the reaction, between 18 and 165 amplified DNA fragments were detected.

Of the combinations primers used, 57% (101) were polymorphic between the two parents. These primers resulted in 278 clearly polymorphic fragments, present in Híbrido de Timor and absent in Catuaí. Only 5.75% (16) were also polymorphic between the two

Table 1 Segregation analysis for resistance to *H. vastatrix* race II in populations derived from cross between the Híbrido de Timor UFV 427-15 and the Catuaí Amarelo UFV 2143-236 (IAC 30)

Population[a]	No. of plants		Observed ratio[b]	Expected ratio[b]	χ^2	Probability (%)
	R	S	R:S	R:S		
UFV 427-15	20	0	1:0	1:0	–	–
UFV 2143-236	0	20	0:1	0:1	–	–
F_1	20	0	1:0	1:0	–	–
F_2	124	36	3.40:1	3:1	0.5336	46.5208
BCs	63	72	0.87:1	1:1	0.3684	54.3866
BCr	20	0	1:0	1:0	–	–

[a] Population derived from a cross between the Híbrido de Timor UFV 427-15 and Catuaí Amarelo UFV 2143-236 (IAC 30)

[b] Observed and expected ratio for susceptible (S) and resistant (R)

Table 2 Number of AFLP primer combinations tested and polymorphisms observed among the resistant (Híbrido de Timor UFV 427-15) and the susceptible (Catuaí Amarelo UFV 2143-236) parent and DNA bulks

	E-CNN/ *M*-TNN[a]
Total of AFLP primer combinations	176
Polymorphic primers between parents	101
Polymorphic bands present in UFV 427-15	278
Polymorphic bands present in UFV 2143-236	142
Polymorphic primers between parents and bulks	09
Polymorphic bands present in the resistant bulk	16
Polymorphic bands present in the susceptible bulk	03

[a] "*E*" and "*M*" designate the primers *Eco*RI and *Mse*I, respectively, and "N" stands for each additional nucleotide in the primers

contrasting DNA bulks and three of them were present in all resistant individuals of the resistant bulk and absent in all susceptible ones. The candidate markers (E.CTC/M.TTT_{405}), (E.CCT/M.TTC_{230}) and (E.CGT/M.TGT_{300}) correspond to DNA fragment of 405, 230 and 300 bp, respectively (Fig. 1).

The three candidate markers were confirmed to be linkage to the coffee leaf rust gene by screening 160 individuals of the segregating F_2 population. The segregation of the rust resistance gene and the three markers identified are shown in Table 3.

Co-segregation was observed between the resistance locus analyzed and the candidate markers (Table 3). The recombination analysis showed that marker E.CTC/M.TTT_{405} is located at a distance of 8.69 cM from the resistance gene, with a LOD score value of 18.91. Marker E.CCT/M.TTC_{230} is located at a distance of 20.50 cM from the gene, with a LOD score value of 6.15 and a third marker, E.CGT/M.TGT_{300}, is located at 25.10 cM with a LOD score value of 5.37.

The map containing the coffee leaf rust resistance gene and the AFLP markers is shown in Fig. 2. Genetic mapping showed that the markers flanks both sides of the gene. These markers are linked, in the coupling phase, to the gene of coffee rust resistance.

Discussion

Disease resistance inheritance

The inheritance study of the Híbrido de Timor UFV-427-15 to *H. vastatrix* race II, conducted in this study with three coffee populations (F_2, BCr and BCs), confirmed that the resistance is monogenic and dominant. However, this result does not exclude the possibility that the loci under study correspond to a complex grouping of several resistance genes arranged in tandem and segregating as a single loci as reported for other species of plants (Richly et al. 2002; Town et al. 2006).

The accessions of Híbrido de Timor possess five known dominant genes, named S_H5, S_H6, S_H7, S_H8 and S_H9 (Bettencourt et al. 1980, 1992). The resistance spectrum conferred by these genes can be totally or partially annulled by the combination of virulence genes (*v5–v9*) present in different races of the fungus (Bettencourt and Rodrigues 1988; Pereira 1995). The race II of *H. vastatrix* used in this study

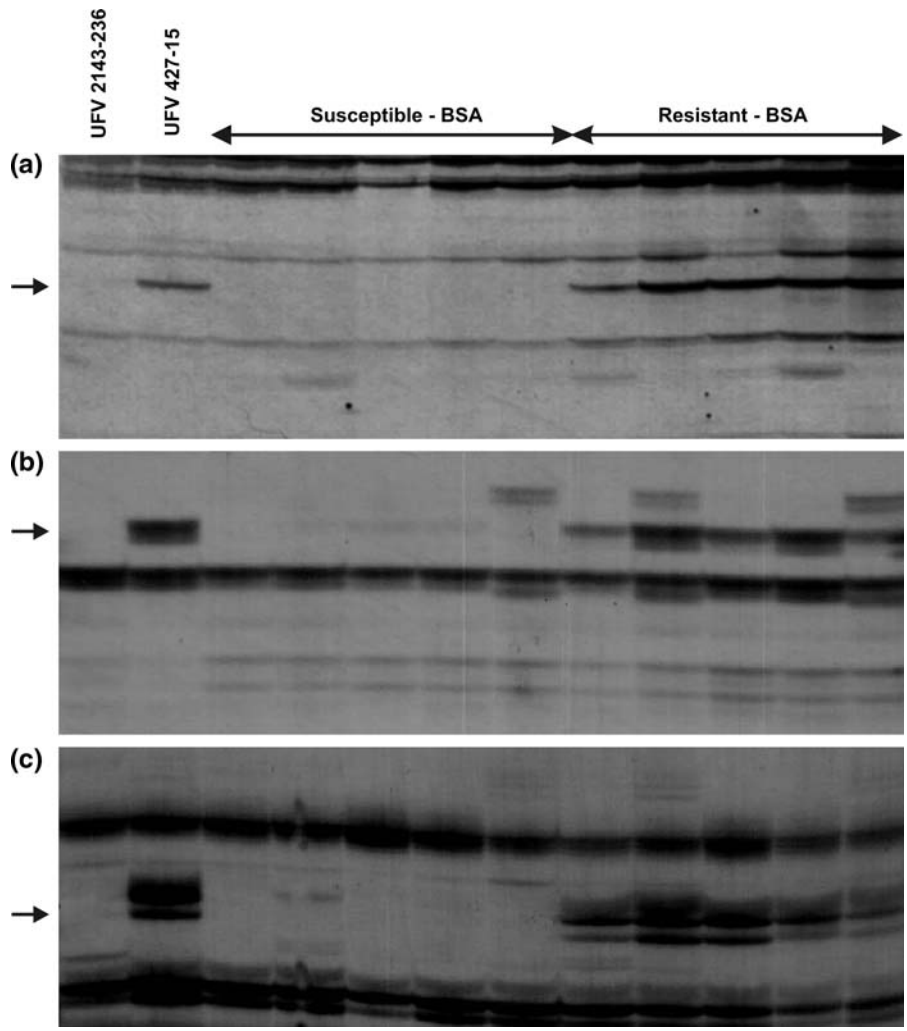

Fig. 1 AFLP marker candidate of 405 bp amplified by the primer combination E.CTC/M.TTT (**a**). A second (E.CCT/M.TTC$_{230}$) amplified a fragment of 230 bp (**b**) and a third (E.CGT/M.TGT$_{300}$) amplified a fragment of 300 bp (**c**). From the *left to the right* the susceptible parent UFV 2143-236 and the resistant UFV 427-15. The *arrows* along the upper end of the gel indicate the five individuals of the susceptible bulk and the five individuals of the resistant bulk. The *arrow on the left* indicates the position of marker

Table 3 Linkage analysis of the resistance gene and the three markers identified in the F_2 population from a cross between the Híbrido de Timor UFV 427-15 and Catuaí Amarelo UFV 2143-236

Locus tested	Expected ratio	Observed ratio	χ^2	Probability (%)	Distance (cM)[c]
R	3:1[a]	124:36	0.5336	46.5208	–
E.CTC/M.TTT$_{405}$	3:1	124:36	0.30	58.3882	–
E.CCT/M.TTC$_{230}$	3:1	133:27	5.6333	1.7622	–
E.CGT/M.TGT$_{300}$	3:1	118:42	0.1333	71.5000	–
R/E.CTC/M.TTT$_{405}$	9:3:3:1[b]	118:6:6:30	85.1111	0.0000	8.69
R/E.CCT/M.TTC$_{230}$	9:3:3:1	118:6:15:21	47.5111	0.0000	20.50
R/E.CGT/M.TGT$_{300}$	9:3:3:1	103:21:15:21	24.1777	0.0022	25.10

[a] Expected ratio for the monogenic and dominant inheritance in the F_2 population (3 resistant, R_, or band presence : 1 susceptible, rr, or band absence)

[b] Expected ratio for segregation of two independent loci in the F_2 progeny (R_/+; R_/−; rr/+; rr/−)

[c] Genetic distances in centiMorgans

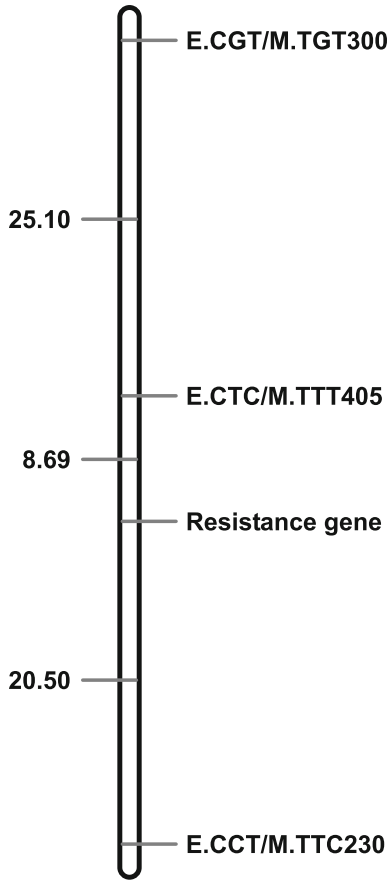

Fig. 2 Linkage map of the positions of the AFLP markers identified and the distances (cM) between them and to the coffee leaf rust resistance gene

has only the virulence allele *v5*, that is able to annul S_H5 resistant gene (Bettencourt and Noronha-Wagner 1971). In this case, the resistance of the Híbrido de Timor UFV-427-15 to the race II must be conferred by another gene than S_H5. Considering the fitting of the *Coffea–Hemileia* (Bettencourt and Rodrigues 1988) to the gene-for-gene model (Flor 1971), the resistance gene analyzed in this study differ also from S_H6. This is support by previous study where was showed that the Híbrido de Timor UFV-427-15 is resistant to the race XXV (Pereira 1995), which has, in addition to *v2* and *v5*, the virulence allele *v6* (Bettencourt and Rodrigues 1988). Therefore, the resistance gene identified in this study is most likely related to one of the genes S_H7, S_H8 or S_H9 that were previously identified in Híbrido de Timor derivative (Bettencourt et al. 1980, 1992), or another unknown gene.

The analysis carried out with others accessions of Híbrido de Timor by our group revealed the presence of more than one gene in the resistance to *H. vastatrix* (Capucho et al. 2009). However, the finder of our study is particularly interesting for the development of a physical resistance mapping in order to do chromosomal landing and cloning of rust resistance gene in coffee. Furthermore, the existence of only one dominant gene, with a simple inheritance pattern, determining the resistance of coffee to *H. vastatrix*, would of course facilitate its transfer to susceptible cultivars.

Identification of AFLP markers associated with the resistance gene

The BSA methodology was efficient to identify the AFLP markers linked to the resistance gene to coffee leaf rust. In most studies using this method, the contrasting bulks are constructed with DNA of eight individuals (Ferreira et al. 2000; Caixeta et al. 2003; Lukens et al. 2006). According to Michelmore et al. (1991), the use of bulks with fewer plants can be results in false positives. In the contrary, in this study, the identification of linked markers was improved by the reduction to five individuals per bulk without increasing the number of false positives. Our results is in agreement with others obtained elsewhere (Muylle 2003; Muylle et al. 2005). The probability of a false positive in a F_2 population using dominant markers and five-plant bulks is 1.8×10^{-6} and therefore virtually negligible (Muylle 2003).

The AFLP technique also proved to be efficient in the generation of markers linked to the rust resistance gene in coffee (Lashermes et al. 2000; Prakash et al. 2004). In our study, the two contrasting parents for rust resistance showed approximately 1.10 polymorphic AFLP bands per primer combination. These values are lower than those found by Lashermes et al. (2000), who compared accessions of *C. arabica* with genotypes derived from the Híbrido de Timor and found a rate of approximately 2.50 polymorphic AFLP bands amplified by a primer combination. This variation may be related to a lower genetic variability of the parents used in this study.

Three markers linked to the gene for rust resistance in the accession of Híbrido de Timor UFV 427-15 were identified in the coupling phase, two of which flanking the resistance gene. The first marker

(E.CTC/M.TTT$_{405}$) is located at a distance of 8.69 cM, and the second (E.CCT/M.TTC$_{230}$) at 20.50 cM from the resistance gene. The finding of markers flanking genes of interest could be useful for assisted breeding since the selection efficiency is greater when flanking markers are used, compared to the separate use (Corrêa et al. 2001). The increase of the efficiency can be attributed to the lower expected frequency of double recombinants in plants selected or discarded erroneously (Griffths et al. 1998).

The number of markers found here was significantly smaller than the one found by Prakash et al. (2004). These authors screened 80 AFLP primer combinations and identified 21 AFLP markers linked to the SH_3 gene. We used a much larger number of primer combinations (176) and just three markers were found. This difference may be related to the lower genetic variability in the parents used in present study. The resistant parent Híbrido de Timor UFV 427-15 is a hybrid that originated from a cross between *C. arabica* and *C. canephora* underwent successive natural backcrossings with *C. arabica*, which makes it, in terms of genetic diversity, very similar to the susceptible parent Catuaí Amarelo UFV 2143-236. This successive backcrossings could be higher in this accession under study, since is one accession that have lost other resistance genes, and the lower rate of polymorphism linked to resistance gene found here could be linked to a smaller presence of *C. canephora*. On the other hand, the F_2 population used by Prakash et al. (2004) was originated by the cross between *C.arabica*, and *C. liberica*, two genetically distant coffee species.

Considering the high genetic variability of the *H. vastatrix* and the time consumed to have accurately disease evaluation in the field, coffee breeders would benefit from the ability to select for resistance to leaf rust using genetic markers, which is still not a reality in Brazilian coffee breeding. Implementation of a marker assisted selection strategy in coffee would dramatically increase the efficiency of breeding programs (Lashermes et al. 1997; Van der Vossen 2005).

In conclusion, this is the first report on molecular markers flanking the rust resistance gene in Híbrido de Timor. The information is the basic step in the construction of a denser genetic map, and will helpful to find closer markers to be converted into SCAR (Sequence Characterized Amplified Regions), for direct use in marker assisted coffee breeding for rust resistance in Brazil. A new population recently generated by our group using these parents, confirmed the linkage of these three markers to the resistance gene, allowing the identification of new markers a little closer to the resistance gene. Genetic mapping are under progress to make these markers useful to develop a physical map to clone this resistance gene in near future.

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