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Transferability of SSR markers from related Uredinales species to the coffee rust *Hemileia vastatrix*

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ABSTRACT. The aim of the present research was to test the transferability of simple sequence repeat (SSR) markers developed in two Uredinales species to *Hemileia vastatrix*, coffee rust. The development of efficient techniques for the identification of *H. vastatrix* isolates is imperative, given the continuous development of new races. The transferability of 25 SSR markers developed in the related Uredinales species *Puccinia coronata f.* sp *lolli* and *Melampsora linii* to *H. vastatrix* was tested. A low level of transferability of SSRs was detected, and only 4 potential markers that can be used to fingerprint the coffee rust races were identified.

Key words: Coffee rust; Simple sequence repeats

INTRODUCTION

Coffee rust, caused by the fungus *Hemileia vastatrix*, is a major disease in countries where it occurs. The disease can cause losses of up to 30% in production in susceptible varieties with no chemical control. The main strategy that producing countries have adopted for the control of the fungus is the development of resistant varieties. After a breeding program that lasted over 20 years, CENICAFE developed the Colombia variety, a successful resistant variety that has been grown for almost 20 years and nowadays occupies over one-third of the planted coffee area of Colombia.

Despite this success, the rust has already developed into new races that can overcome the resistance of the commercial resistant varieties. New resistance genes are currently targeted to try to introgress them into new commercial varieties and to try to understand the dynamics of the rust - plant interactions. Also, specific fingerprints for the differentiation of *H. vastatrix* races are being developed by the use of molecular marker techniques.

By virtue of their extreme polymorphism, some molecular markers such as simple sequence repeat (SSR) loci are considered to be ideal markers for population genetics. SSRs, also known as microsatellites, comprise tandemly repeated genetic loci of 1 to 6 bp (Tautz and Renz, 1984). SSRs are highly abundant and exhibit extensive degrees of polymorphism in eukaryotic (Weber, 1990; Toth et al., 2000; Katti et al., 2001) and prokaryotic (Field and Wills, 1996; Gur-Arie et al., 2000) genomes. They are found in protein-coding and noncoding regions (Toth et al., 2000; Katti et al., 2001), with SSRs being more abundant in noncoding regions than in exons. DNA sequence knowledge is imperative in the design of appropriate primers for the assay.

The homology of flanking regions of SSRs allows the transferability of microsatellite loci between closely related species, besides the possibility of comparative map construction among them (Slate et al., 1998). Transferability may reduce costs, opening new perspectives for the development of population genetic studies. The high rate of transferability has already been reported for plant species (e.g., Dayanandan et al., 1997; White and Powell, 1997; Brondani et al., 1998; Collevatti et al., 1999) and among animals for the human and chimpanzee species (Deka et al., 1994), besides the dog and the fox (Fredholm and Wintero, 1995). The cross-species transferability of microsatellites is very variable among groups of animals, plants and fungi (Barbará et al., 2007). SSR frequency is low in most fungal species, compared to other groups of organisms (Karaoglu et al., 2005), and it is particularly low for ectomycorryzal fungus (Wadud et al., 2006). Additionally, long SSRs are very uncommon in fungi, and the short SSRs present exhibit less polymorphism than in other organisms (Dutech et al., 2007).

The first reported study about the diversity of coffee rust in Colombia was performed with internal transcribed spacer-rDNA (ITS) markers (Cristancho et al., 2007). Although these markers showed their potential for the study of this pathogen, it was not possible to develop race- or isolate-specific markers, and it is not easy to sequence ITS bands from each isolate. It is necessary to develop a robust molecular marker for the study of coffee rust diversity.

The single most parsimonious tree recovered from combined 28S and 18S sequence data showed that *H. vastatrix* is closely related to *Maravalia cryptostegiae*, *Blastospora smilacis*, and *Mikronegeria alba*, a pathogen of conifers. In this study, the genera

Melampsora and *Puccinia* were shown to belong to different clades while *Hemileia* was left out of these two clades (Aime, 2006).

Currently CENICAFE maintains several differential coffee plants for the identification of new rust races. These plants have allowed the identification of races II and XXII and have suggested the presence of other races in Colombia, which have not been identified yet (Leguizamón et al., 1984; Gil and Ocampo, 1998). The identification of new races is underway and new differential plants are currently being analyzed for the purpose of dissecting the genetics of these races with fruitful collaboration with the CIFC Institute in Oeiras, Portugal.

In this study, we present the results of the transferability of microsatellite loci from two related species *Puccinia coronata f.* sp *lolii* and *Melampsora linii* to orange rust isolates (*Hemileia vastatrix*).

MATERIAL AND METHODS

Rust isolates

The DNA for microsatellite identification came from urediniospores on inoculated coffee plants from a greenhouse and field samples from the Colombian Central Coffee Zone. Urediniospores were collected on gel cartridges by scraping rust-infected coffee tissue and stored at -20°C. The origin of all samples is given in Table 1.

Table 1. Origin of rust samples used for simple sequence repeat amplification.

Number of isolate	Sample ID	Origin
1	HvCTAuto	*Coffea arabica* var. Caturra
2	HvCtLib	*Coffea arabica* var. Caturra
3	RII.5	Race II, sample 5
4	RII.6	Race II, sample 6
5	HvCtCed	*Coffea arabica* var. Caturra
6	HvInv2	Mixed samples from field plants
7	HvTipLib	*Coffea arabica* var. Typica

DNA extraction

Total genomic DNA of the urediniospores was extracted using the Goodwin and Lee (1993) protocol. Concentrations of DNA were estimated by fluorometry. Typically, 50-300 ng DNA was obtained per gel cartridge, which was confirmed by agarose gel electrophoresis, and stored at -20°C until use.

PCR protocols

Amplification was carried out in a 96-well thermal cycler (MJ Research Model PTC-100). *P. coronata f.* sp *lolli* (Dracatos et al., 2006b) and *M. linii* (Barrett and Brubaker,

2006) primers tested are detailed in Table 2. Polymerase chain reaction (PCR) was carried out in a final volume of 10 µL containing 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 1X PCR buffer, 0.25 µM of each primer, 3 U Go Taq Polymerase (Promega) and 30 ng extracted genomic DNA. The thermal cycler was programmed for an initial denaturation step at 95°C for 5 min, 35 cycles at 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, and then 72°C for 5 min followed by a holding step of 10°C. The amplified products were separated on 6% denaturing polyacrylamide gels, which were then stained with silver nitrate (Bassam et al., 1991).

Table 2. Simple sequence repeat primers tested with DNA samples from *Hemileia vastatrix* isolates.

	Puccinia coronata f. sp *lolli*[1]		*Melampsora linii*[2]
1	PCESTSSRB04K08	1	MI10
2	PCESTSSRB01D04	2	MI21
3	PCESTSSRB04K18	3	MI22
4	PCESTSSRB01L19	4	MI24
5	PCESTSSRB01F04	5	MI26
6	PCESTSSRB02O03	6	MI27
7	PCESTSSRB02G02	7	MI30
8	PCESTSSRB02I11	8	MI32
9	PCESTSSRB02A04	9	MI141
10	PCESTSSRB01B10	10	MI145
11	PCESTSSRB01E14	11	MI148
12	PCESTSSRB01B18		
13	PCESTSSRB01H08		
14	PCESTSSRB04M23		

[1]Dracatos et al., 2006b. [2]Barrett and Brubaker, 2006.

Gel analysis

Differences in fingerprinting patterns between the primers tested (Table 2) were assessed visually. For those primer pairs producing amplification products, seven rust samples were screened to evaluate their potential to detect polymorphism as well as transferability characteristics.

RESULTS AND DISCUSSION

Twenty-five primers were evaluated with DNA extracted from seven coffee rust isolates. The results of the amplifications are summarised in Table 3. Four primers did not show amplification products, 13 primers showed non-specific amplifications, exhibiting a large number of amplification products, and 4 primers generated readable amplification bands in some isolates, but not all (less than 50% of the isolates).

Four of the candidate primers showed a robust amplification and fragments with the expected sizes, and were selected based on their consistency of amplification in all isolates tested (Table 4). Of these four primers, PCESTSSRB01E14 and PCESTSSRB01B18 showed a polymorphic profile in *H. vastatrix* isolates, but primers PCESTSSRB01D04 and MI30 were monomorphic in the seven isolates tested. The monomorphic primers still need to be tested in a larger set of isolates of *H. vastatrix*. Primers that showed non-specific amplification products were subjected to some optimization by varying the PCR extension temperature, but this optimization did not produce better results (data not shown).

Table 3. Characterization of amplification patterns of non-selected primers.

EST-SSR primer	Motif	Expected size (bp)	Observed amplification pattern
PCESTSSRB01F04	(TCACC)2	209	Non-specific amplification. Less than 50% of isolates amplified
PCESTSSRB01L19	(CGA)11	237	Non-specific amplification
PCESTSSRB02A04	(ATTGC)2	240	Non-specific amplification
PCESTSSRB02G02	(CAC)4	105	Non-amplification
PCESTSSRB02I11	(ACC)4	160	Non-specific amplification
PCESTSSRB02O03	(CAC)4	173	Non-specific amplification
PCESTSSRB04K08	(GGC)3GAC(GGC)3	334	Non-specific amplification
PCESTSSRB04K18	(CAT)11	253	Non-specific amplification. Less than 50% of isolates amplified
PCESTSSRB01B10	(ACC)4	277	Non-specific amplification
PCESTSSRB01H08	(CAG)5	238	Non-specific amplification
PCESTSSRB04M23	(AGA)4	277	Non-specific amplification
MI10	(GAA)8	221-224-227	Non-amplification
MI21	(AG)8	195-197-199-201-203	Less than 50% of isolates amplified
MI22	(GTA)8	239-242-250-253-259	Non-specific amplification. Less than 50% of isolates amplified
MI24	(AAG)9	193-211-214	Non-amplification
MI26	(AC)7	197-199-205	Less than 50% of isolates amplified
MI27	(GAAGAG)3(GAA)8	253-259	Less than 50% of isolates amplified
MI32	(AAG)4,6(TTGATT)3	135-138	Non-specific amplification
MI41	(TCT)6	199-202	Less than 50% of isolates amplified
MI45	(AGA)8	274-281	Non-amplification
MI48	(TTA)4(AGA)7	194-197	Non-specific amplification. Less than 50% of isolates amplified

EST-SSR = expressed sequence tag-simple sequence repeat.

Table 4. Characterization of the 4 microsatellite loci transferred to *Hemileia vastatrix*.

EST-SSR primer	Motif	Expected size (bp)	Observed size (bp)	Amplified products 1 2 3 4 5 6 7
PCESTSSRB01D04	(AAC)5	116	114-116	+ + + + + + +
PCESTSSRB01E14	(TTTA)2	337	123-125	+ + – – + – +
			98-102	+ + + + + + +
PCESTSSRB01B18	(ATCTC)2	299	136-137	+ + + + + + +
			299-300	+ + + + + + +
MI30	(TTTGTTATT)	(202-210-211)	130-131	+ + + + + + +

EST-SSR = expressed sequence tag-simple sequence repeat.

Assays of cross-amplification in other taxa with some of the SSR markers used in this study indicated a moderate level of transferability with genetically distant organisms (Dracatos et al., 2006a). The low rate of transferability observed in this study could be evidence of mutations in the flanking regions of the SSR, the possibility of interruptions within the repeat motif or the presence of introns in the amplified region, as evidenced by sequencing of amplification products of these EST-SSRs by Dracatos et al. (2006a). Additionally, it would be ideal to perform a further optimisation of PCR conditions for the markers designed in *H. vastatrix* isolates, which was performed in this study.

The low degree of polymorphism detected could be due to the origin of the samples,

given that they were collected from *C. arabica* varieties cultivated in the central coffee region of Colombia. The inclusion of a more diverse group of isolates, e.g., those from different coffee species and varieties and different races of the pathogen from the CIFC collection in Portugal, could give better evidence of the genetic polymorphism of *H. vastatrix* discriminated by the use of SSR markers. This report confirms the low intra-specific transferability of SSRs in fungi compared to other groups of organisms (Barbará et al., 2007).

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