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Research Note

Characterization of nine microsatellite loci for the tree species *Parapiptadenia rigida* (Fabaceae-Mimosoideae) and their transferability

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ABSTRACT. *Parapiptadenia rigida*, locally known as angico, is a tropical tree common in the semideciduous Brazilian forest. Its wood is naturally resistant to insect attack and is useful for construction. Extracts from the tree have medicinal properties. We characterized nine microsatellite loci for *P. rigida*. Thirty-five alleles were detected in a sample of 45 individuals from 3 different populations, with an average of 3.9 alleles per locus. The average polymorphic information content ranged from 0.099 to 0.640. Observed and expected heterozygosities varied from 0.111 to 0.489 and from 0.106 to 0.707, respectively. One locus exhibited significant deviation from Hardy-Weinberg equilibrium and four pairs of loci showed significant linkage disequilibrium. All nine primers were tested for cross-amplification in species from the Fabaceae-Mimosoidea family, yielding a transferability success rate of 7 loci in *Stryphnodendron adstringens* to 0 transferred loci in

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Pithecellobium incuriale and *Inga marginata*. These microsatellites will be valuable to study population genetics of this and other species where primer transferability was detected.

Key words: Microsatellite libraries; Genetic diversity; Fabaceae; Tree; SSR; Cross-amplification

INTRODUCTION

Parapiptadenia rigida (Benth.) Brenan. (Fabaceae, Mimosoideae) is a deciduous, heliophyte, allogamous, monoecious, early secondary tree that grows in various soil types and is recommended for the recovery of degraded forests, especially in areas of permanent preservation (Durigan and Nogueira, 1990; Vaccaro et al., 1999). This tree is found in the semideciduous forests of the Brazilian States of Minas Gerais, São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul, with the greatest occurrence in the last 3 States. The species grows 20-30 m tall and has a trunk diameter of 60-110 cm. It is characterized by a hard wood that is commonly used in construction, shipbuilding, carpentry and joinery, and the manufacture of coaches, stakes, light and telephone poles, and railway sleepers (Lorenzi, 2002). This species also has medicinal properties and is widely used in folk medicine for the treatment of sinusitis and cough (Franco and Fontana, 1997). Despite being a monoecious plant, *P. rigida* presents self-incompatibility (Ribas, 1999); its seeds are dispersed by wind, water, or barochory, and pollens are dispersed by small- and medium-sized bee species (Kageyama, 1992).

MATERIAL AND METHODS

Total genomic DNA was extracted from leaf tissue of 45 individuals from 3 different populations of *P. rigida* using a cetyltrimethylammonium bromide method (Doyle and Doyle, 1987). Microsatellites were isolated using a hybridization-based capture methodology following a protocol described by Billotte et al. (1999) with $(CT)_8$ and $(GT)_8$ probes in the enrichment step. Briefly, approximately 5 ng genomic DNA was digested with the restriction enzyme *Rsa*I, and the blunt-ended fragments were ligated to the adaptors *Rsa*I-21 and *Rsa*I-25 (Edwards et al., 1996). Fragments containing CT and GT repeats were selected through hybridization to biotinylated oligonucleotides complementary to the repetitive sequence and recovered with streptavidin-coated magnetic beads (Invitrogen Dynal A.S., Lillestrøm, Norway). Microsatellite-rich fragments were amplified using polymerase chain reaction (PCR) with *Rsa*I-21 primer (Peters et al., 2008), cloned into pGEM-T Easy Vector (Promega, Madison, WI, USA), and transformed into *Escherichia coli* XL1 Blue MRF' super-competent cells (Agilent Technologies Inc., Santa Clara, CA, USA).

Plasmids from individual colonies were isolated, and the sequence of the inserts was determined using an ABI PRISM terminator cycle sequencing kit in the ABI 377 automated sequencer (Applied Biosystems, Foster City, CA, USA). Sequenced fragments were screened for microsatellites using the Gramene program markers database (Ware et al., 2002). Fragments containing microsatellite regions of dinucleotides or trinucleotides repeated more than 4 times and surrounded by a flanking region suitable for primer design were chosen for further study. Although the $(CT)_8$ and $(GT)_8$ oligomers were used for pre-cloning enrichment, other

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repeat motifs were also found in the cloned products (Table 1). Primer pairs complementary to sequences flanking the repeat elements were designed using the PRIMER3 web interface software (Rozen and Skaletsky, 2000). PCR amplifications were carried out across 45 individuals from 3 populations (15 individuals from each region: Curiúva County, Paraná State, 24°01'S 50°26'W; Londrina County, Paraná State, 26°26'S 51°14'W, and Lages County, Santa Catarina State, 28°11'S 50°43'W). PCRs were performed in a volume of 0.025 cm^3 containing 1X PCR buffer, 1 U *Taq* DNA polymerase, 2.5 mM $MgCl_2$, 0.2 mM of each deoxyribonucleotide triphosphate, 8 pM forward and reverse primers, and 20-30 ng template DNA. Cycling conditions consisted of 2 min at 94°C followed by 35 cycles of 45 s at 94°C, 1 min at the locus-specific annealing temperature, and 2 min at 72°C, and a final extension of 5 min at 72°C. The amplified microsatellite products were visualized on a 5% acrylamide:bisacrylamide (29:1) gel. We used Cervus version 2.0 (Marshall et al., 1998) to estimate the number of alleles per locus, observed and expected heterozygosities, and polymorphism information content. Deviations from Hardy-Weinberg equilibrium and linkage disequilibrium were determined using Genepop version 1.2 (Raymond and Rousset, 1995).

RESULTS AND DISCUSSION

Of 240 clones sequenced, 28 (11.66%) contained microsatellites, but only 21 were suitable for primer design. Of the original 21 primers developed, only nine presented polymorphic information and are described herein (see Table 1). The 9 polymorphic loci produced a total of 35 alleles, ranging from two (Prig 28) to five (Prig 26, Prig 42, and Prig 87) with an average of 3.9 alleles per locus. The average polymorphic information content was 0. 4126 and the values of observed and expected heterozygosity varied from 0.111 to 0.489 and from 0.106 to 0.707, respectively (see Table 1). One locus (Prig 26) deviated from expectations of Hardy-

Table 1. Characterization of nine polymorphic microsatellite loci genotyped in 45 individuals from three populations of *Parapiptadenia rigida*.

Locus/GenBank accession No.	Primer sequence (5'-3')	Repeat motif	Allele size (bp)	Ta	N_A	PIC	H_O	H_E
Prig 21/JQ042252	F: GGTGGGGATCGGATTATTTT R: CTATTGATGCGCATGTGAGT	$(AC)_{10}$	179	56	3	0.441	0.333	0.543
Prig 26/JQ042253	F: GGCAGAGCTGTCAGGATCA R: GACCTCTTCCACCCTTTTCC	$(AG)_{19}$	218	60	5	0.640	0.489	0.707*
Prig 28/JQ042254	F: CATTGAACCTATTCCCCTGA R: GGGACAAATAACATGTCTGACG	$(TAT)_3…(GA)_4$	168	54	2	0.099	0.111	0.106
Prig 29/JQ042255	F: ACCCCCGGTATTTCCATAAC R: GGAGGGGTCATGTCTTTGAA	$(CA)_4…(GT)_{11}$	200	58	4	0.409	0.378	0.450
Prig 33/JQ042256	F: TTTGCTTTTGCCATTGAAGA R: TTGGTGGGCTTAGGCAAATA	$(AC)_9$	206	56	3	0.294	0.356	0.335
Prig 38/JQ042257	F: TGGTCCTTCTTCTGCAGGTT R: CTAGCTTCATGGGCTTTTGG	$(TG)_5…(CT)_8AT$ $(GT)_7GA(GT)_3$	194	55	4	0.347	0.133	0.378
Prig 42/JQ042258	F: AGCATGCTTGAAAGAATTGAA R: TTCATTGAAAACTTTGGAGTGACTA	$(TG)_3…(AT)_5…$ $(GT)_6…(AT)_3$	233	53	5	0.433	0.311	0.469
Prig 62/JQ042259	F: GTTCAAAGCTTTTGCGTGGT R: CAGGGAAGACAAACCTGGAA	$(TG)_8$	244	57	4	0.285	0.333	0.311
Prig 87/JQ042260	F: CCTGCTTCCCTTTACGACAC R: TGTGATGAGCTAGGGCATTG	$(AC)_9$	192	59	5	0.621	0.422	0.671

Ta = highest annealing temperature (°C); allele size indicates the range of observed alleles in bp; N_A = number of alleles; PIC = polymorphic information content; H_O and H_E = observed and expected heterozygosities; F and R = forward and reverse, respectively. *P ≤ 0.001.

Weinberg equilibrium ($P \leq 0.001$) after Bonferroni's correction for multiple comparisons (see Table 1), whereas 4 pairs of loci (Table 2) exhibited significant linkage disequilibrium ($P \leq 0.01$): Prig21-Prig26, Prig26-Prig29, Prig26-Prig62, and Prig42-Prig62. Cross-amplification tests comprised 5 individuals from each of the 12 Fabaceae-Mimosoideae species studied, and we observed primer transferability success ranging from zero in 3 species (*Inga marginata*, *Mimosa bimucronata*, and *Pithecellobium incuriale*) to 7 in *Stryphnodendron adstringens* (see Table 2). Therefore, we demonstrated that these microsatellite loci can be effective tools for the detection of genetic structure and variability in *P. rigida*, contributing new information about this species of considerable ecological significance in Brazil.

Table 2. Cross-amplification test of nine microsatellite loci throughout nine species of the Fabaceae-Mimosoideae family.

Species	Loci								
	21	26	28	29	33	38	42	62	87
Anadenthera colubrina (Vell.) Brenan.	-	+	-	-	-	+	-	-	-
Anadenanthera macrocarpa (Benth.) Brenan.	-	+	+	-	-	+	-	-	-
Enterolobium contortisiliquum (Vell.) Morong.	-	+	-	-	-	+	-	-	-
Inga marginata Willd.	-	-	-	-	-	-	-	-	-
Inga sessilis (Vell.) Mart.	-	-	-	-	-	-	+	+	-
Mimosa bimucronata (DC.) O. Kuntze.	-	-	-	-	-	-	-	-	-
Mimosa pudica L.	-	-	-	-	-	+	-	-	-
Mimosa scabrella Benth.	-	-	-	+	-	+	-	-	-
Piptadenia gonoacantha (Mart.) Macbr.	-	+	+	-	-	+	+	-	-
Pithecellobium incuriale (Vell.) Benth.	-	-	-	-	-	-	-	-	-
Senegalia polyphylla (DC.) Britton & Rose.	-	-	-	+	-	+	-	-	-
Stryphnodendron adstringens (Mart.) Coville.	-	+	+	+	-	+	+	+	+

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