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Development and characterization of new microsatellites for *Eugenia dysenterica* DC (Myrtaceae)

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ABSTRACT. Microsatellite markers were developed for population genetic analyses of the Neotropical tree *Eugenia dysenterica* DC (Myrtaceae), after construction of a shotgun genomic library for microsatellite discovery. Nine primers were designed, of which 5 yielded amplified product. These primers were polymorphic for 97 individuals collected in 3 distinct localities. The number of alleles per locus (primer) ranged from 3 to 11 and expected heterozygosities varied from 0.309 to 0.884. The probability of locus identity was ~1.88 x 10^{-4} and the probability of paternity exclusion was ~0.9367. The 5 microsatellite primer pairs may be suitable for population genetic studies such as parentage and fine-scale genetic analyses of this species.

Key words: Cagaita; Cerrado; Genetic diversity; Shotgun library; Microsatellite

INTRODUCTION

Eugenia dysenterica (Myrtaceae) is a Neotropical tree widely distributed in the Brazilian savannas of the Cerrado Biome, Central Brazil. The fruit is enjoyed for the edible and refreshing mesocarp that is consumed *in natura* or as a source of raw material for small and middle-sized food industries, and plays an important role in the local economy of Central Brazil (Sano et al., 1995). Previous population genetic studies using different molecular markers indicated low genetic diversity and high differentiation among populations of *E. dysenterica* (Telles et al., 2003; Zucchi et al., 2005). However, despite its high ecological and economic importance, the only 7 polymorphic microsatellites available for this species were transferred from *Eucalyptus* (Zucchi et al., 2002). Thus, the development of more specific molecular markers is important to clarify the evolutionary mechanisms of genetic variability in this species.

This study is part of a larger project to characterize genetic variability and evaluate ecological and evolutionary processes in Cerrado tree species, and the development and characterization of microsatellite loci is an important step toward this goal (see Telles et al., 2011; Soares et al., 2012). Here we report the development and characterization of microsatellite loci for *E. dysenterica* and demonstrate their suitability for further studies in population genetic structure and gene flow.

MATERIAL AND METHODS

We developed a genomic shotgun library for microsatellite isolation and primer design. DNA from an individual *E. dysenterica* was extracted by the 2% CTAB protocol (Doyle and Doyle, 1987) and sheared (2.0 µg) using a sonicator at 120 W for 1 h and 45 min. Fragments from 200 bp to 1.0 kb were recovered, cloned into dephosphorylated pMOSBlue blunt vector using the Blunt-ended PCR Cloning Kit® (GE HealthCare, Uppsala, Sweden), and sequenced on an Applied Biosystems 3100 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). For sequencing, we used the U19 primer with the DYEnamicET terminator kit (GE Healthcare), according to manufacturer instructions. Sequences were screened for microsatellites using the WEBSAT software (Martins et al., 2009) and primers were designed using Primer3 (Rozen and Skaletsky, 2000). The following stringent criteria were applied for primer design: i) maximum primer Tm (melting temperature) 68°C; ii) maximum 3°C difference in Tm between primers; iii) GC content ranging from 40 to 60%; iv) maximum of 2 dimers between primers; v) absence of hairpins.

We then genotyped 97 individuals from 3 localities (local populations) widely scattered throughout the species' geographic range, Balneário Santo Antonio (S15°59.52', W50° 6.695') in Goiás State, Roda Velha (S12°58.343', W45°59.392') in Bahia State, and Porto Nacional (S10°42.892', W48°46.840') in Tocantins State.

Genotyping was performed in a 15-µL reaction volume containing 15 ng template DNA, 2.60 µM of each primer, 1 U *Taq* DNA polymerase (Phoneutria, Brazil), 210 µM of each dNTP, 2.16 mg bovine serum albumin, and 1X reaction buffer (10 mm Tris-HCl, pH 8.3, 50 mm KCl, 1.5 mm $MgCl_2$), with the following conditions: 95°C for 5 min (1 cycle); 94°C for 1 min, 52° to 64°C (see Table 1) for 1 min, 72°C for 1 min (30 cycles); 72°C for 7 min (1 cycle). PCR fragments were electrophoresed on 6% denaturing polyacrylamide gels stained with silver nitrate (Creste et al., 2001) and sized by comparison to a 10-bp DNA ladder (Invitrogen).

RESULTS

We sequenced 1632 clones from the genomic library and 713 cloned inserts contained microsatellites. Of these, 683 were mononucleotide (95.79%), 25 dinucleotide (3.51%), 2 trinucleotide (0.28%), and 3 tetranucleotide (0.42%). However, primers could be designed for only 9 microsatellite loci and 5 of these amplified clearly interpretable products in a single PCR protocol (Table 1).

Table 1. Microsatellite primers developed for *Eugenia dysenterica* DC.

Locus	Sequence (5'- 3')	Repeat	Size range (bp)	Ta (°C)
Ed03	F: GTAAGTATGCAGTTGCCTCA R: AAATCATAAATGGGTTTACAA	GAA(7)	224-230	52
Ed04	F: ATCTGACCCTCAGTCATTGT R: AATTAAGCATCTCTTGACTGG	GA(14)	222-270	64
Ed05	F: CTAGCCATTGCTACATTGAA R: CAACCAAACTCAACAATCAG	GA(16)	208-246	61
Ed08	F: AAGACAGATTGGAAAAGCAT R: ACCTTCCAGACAAAAGTCAA	AG(10)	164-204	60
Ed09	F: ACTTCACTCGTGCTCCTAAT R: AGCAAATAAATTCCCACCTA	AG(10)	205-231	62

Data reported for 97 individuals from three local populations. Ta = annealing temperature.

E. dysenterica presented similar levels of polymorphism as other plant species from the Brazilian Cerrado (e.g., Telles et al., 2011; Soares et al., 2012), with 3 to 11 alleles per locus and expected heterozygosities ranging from 0.309 to 0.884 (Table 2). The polymorphism level was also similar to the one obtained using microsatellites transferred from *Eucalyptus* (Zucchi et al., 2002). All pairs of loci were in linkage equilibrium ($P > 0.05$), when analyses were performed with the FSTAT 2.9.3.2 software (Goudet, 2002). The 5 loci presented relatively low probability of identity for all local populations (1.8752×10^{-4}) and high (0.936650404) probability of paternity exclusion.

Table 2. Genetic characterization of five microsatellite loci in three populations of *Eugenia dysenterica* DC.

Locus	BSAGO local population				RVBA local population				PNTO local population			
	N	N_A	H_E	H_O	N	N_A	H_E	H_O	N	N_A	H_E	H_O
Ed03	32	3	0.309	0.094	32	3	0.678	0.344	33	3	0.600	0.212
Ed04	32	8	0.483	0.406	32	8	0.437	0.000	33	11	0.511	0.296
Ed05	32	7	0.714	0.313	32	7	0.557	0.485	33	10	0.884	0.697
Ed08	32	4	0.830	0.406	32	6	0.769	0.812	33	6	0.835	0.787
Ed09	32	4	0.682	0.625	32	6	0.807	0.682	33	7	0.794	0.666

BSAGO = Balneário Santo Antonio; RVBA = Roda Velha; PNTO = Porto Nacional. N = number of individuals genotyped; N_A = number of alleles; H_E = expected heterozygosity; H_O = observed heterozygosity.

Thus, the 5 microsatellite loci developed in this study may be suitable for parentage analysis and fine-scale genetic structure and present a new opportunity for the generation of genetic data for *E. dysenterica*.

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