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Primer Note

Development and characterization of microsatellite markers for *Piptadenia gonoacantha* (Fabaceae)[1]

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- *Premise of the study:* Microsatellite primers were designed for *Piptadenia gonoacantha* (Fabaceae) and characterized to estimate genetic diversity parameters. The species is a native tree from the Atlantic Forest biome commonly used in forest restoration; it has medicinal potential and the wood is economically useful.
- *Methods and Results:* Twenty-eight microsatellite loci were identified from an enriched genomic library. Fifteen loci resulted in successful amplifications and were characterized in a natural population of 94 individuals. Twelve loci were polymorphic, with allele numbers ranging from three to 15 per locus, and expected and observed heterozygosities ranging from 0.2142 to 0.8325 and 0.190 to 0.769, respectively.
- *Conclusions:* The developed markers will be used in further studies of population genetics of *P. gonoacantha*, aimed at conservation and management of the species in natural populations and in forest restoration projects.

Key words: Atlantic Forest; conservation genetics; Fabaceae; forest restoration; microsatellites; *Piptadenia gonoacantha*.

Piptadenia gonoacantha (Mart.) J. F. Macbr. (Fabaceae: Mimosoideae) is a native tree species from the Brazilian semideciduous Atlantic Forest; it is mainly used in reforestation projects due to its fast growth and resilience, playing the role of an early secondary species in the ecological succession process (Leite and Takaki, 1994). The species also has medicinal potential related to the flavonoids it produces, and its wood is extensively used as firewood and charcoal (Carvalho et al., 2010). Because of these features, *P. gonoacantha* has been used in several forest restoration efforts. However, because early Brazilian restoration projects did not take genetic variation into account (Rodrigues et al., 2009), it is desirable to estimate the genetic diversity to develop more effective strategies for conservation and management purposes. Here we report the identification and characterization of 12 microsatellites for *P. gonoacantha*, as a tool to estimate population genetic parameters.

METHODS AND RESULTS

The genomic DNA was extracted from leaves of *P. gonoacantha* following the protocol developed by Cavallari et al. (2014). A microsatellite-enriched library was obtained using the protocol adapted from Billotte et al. (1999). Genomic DNA from one individual of *P. gonoacantha* was digested with *Afa*I (Invitrogen, Carlsbad, California, USA) and enriched in microsatellite fragments using $(CT)_8$ and $(GT)_8$ motifs. Microsatellite-enriched DNA fragments were ligated to pGEM-T Easy Vector (Promega Corporation, Madison, Wisconsin, USA) and used to transform Epicurean Coli XL1-Blue *Escherichia coli* competent cells (Promega Corporation). Positive clones were selected using β-galactosidase gene expression and grown on a selective medium with ampicillin. The sequencing reactions (10 µL) contained 200 ng of plasmid DNA, 0.5 pmol of SP6 primer, 0.4 µL of BigDye Terminator mix (version 3.1; Applied Biosystems, Foster City, California, USA), 1 mM $MgCl_2$, and 40 mM Tris-HCl (pH 9.0).

Ninety-six clones were sequenced on an ABI 3700 automated sequencer (Applied Biosystems), and the sequence of 84 clones exhibited good quality. Microsatellites were identified in 47 sequences, resulting in an enrichment index of 55.95%. Twenty-eight primer pairs were designed using Primer3 software (Rozen and Skaletsky, 1999). The parameters were set to obtain final amplification products in the range of 150 to 250 bp, GC percentage of at least 50% and maximum 60%, primer annealing temperatures varying from 55°C to 70°C, and the difference in annealing temperature between primer pairs of 3°C at most. Their 5′ forward ends were labeled with M13 fluorescence (5′-CACGACGTTGTAAAACGAC-3′).

Six individuals of *P. gonoacantha* were screened during primer testing, resulting in amplicons for 15 primer pairs (Table 1). These primers were used

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TABLE 1. Characteristics of the 15 microsatellite markers developed for *Piptadenia gonoacantha*.

Locus	Primer sequences (5′–3′)[a]	Repeat motif	Allele size range (bp)	T_a (°C)	GenBank accession no.
Pgo01	F: CACCGAGGAGGTTCATCTCTG R: ACCCCCAAATAAGGAGGAAG	$(TA)_8$	224–240	50	KM877492
Pgo02	F: CTGGATCGAAACAAAATGGAAG R: TGGTTGATCTTTCCAAGATGG	$(GT)_8$	106–140	56	KM877493
Pgo03	F: CTTGTGTCCCCTGCTATCTG R: TGAAAAGACTGCATGGTGC	$(TC)_{13}$	238–264	56	KM877494
Pgo04	F: CGGAGGATGAGGATCGACG R: GAACCACACAAGACGTTAGG	$(GT)_5$	210–228	50	KM877495
Pgo05	F: CTCCCCTTCAACAACCTCATT R: GGTCCTTCGTGACATGGTC	$(AC)_9$	252–288	50	KM877496
Pgo06	F: CTCTTAACCCACCCTCCATTT R: CGGCATTAACCTAACAATCA	$(GT)_{13}$	240–282	54	KM877497
Pgo07	F: CTGCTGGTGCAGAAGAAGAGA R: CCAACAACAAGCAAGAGCTG	$(GT)_9(GA)_6$	180–190	50	KM877498
Pgo08	F: CAGGGGAAGGATGAAGATACA R: GCTACGAAATGAACAAGCAG	$(AT)_7(CG)_5$	224–250	50	KM877499
Pgo09	F: CGCAGCATCAACAAGAAAACA R: GGATTTTGAGTTTCCACAGG	$(GT)_8(TC)_8$	261–293	50	KM877500
Pgo10	F: CGGTTCACACACTCCACAGGA R: AACCTGCCATAAGCGTGAGT	$(AC)_8$	236–268	50	KM877501
Pgo11	F: CGACCGATCAACAGGGATTGA R: AACAATAAGGCCATCCGTTC	$(GT)_{11}$	180–218	50	KM877502
Pgo12	F: CCCAATCCCGTTGTTGTCTTT R: CGGGAACAGTAATTTCCTCA	$(TC)_{10}$	209–237	50	KM877503
Pgo13	F: CCCAATTCCAAGTCCTACCA R: GCGTAAGGCTAACAAGAATCAA	$(GT)_7$	195	50	KP324793
Pgo14	F: GGATAATCCGAAGATGCATTG R: AGGAAGGATTAAGAGAAGAAAAACA	$(GT)_7$	228	50	KP324794
Pgo15	F: TCAACAAAGGCTGCAAAAGA R: TCGTATGGCACAGCACTTC	$(ATT)_5$	215	50	KP324795

Note: T_a = annealing temperature.
[a]The 5′ forward end was labeled with M13 fluorescence (5′-CACGACGTTGTAAAACGAC-3′).

to characterize 94 individuals of *P. gonoacantha*, randomly sampled from Mata Santa Genebra Reserve (22°49′20″S, 47°06′40″W), a 241.55-ha urban forestry fragment located in Campinas, São Paulo, Brazil. We deposited a voucher specimen collected in this conservation unit (22°49′38″S, 47°6′19″W) in the Herbarium of the Universidade Estadual de Campinas (voucher no. UEC-182,226).

PCR was performed in 10-μL reaction mixtures containing 2.5 ng of DNA, 0.2 μL of forward primer (10 μM), 0.15 μL of reverse primer (10 μM), 0.2 μL of fluorochrome-labeled primer (10 μM), 1 μL of dNTP mix (2.5 mM), 0.2 μL of 1× PCR buffer (50 mM KCl, 10 mM Tris-HCl [pH 8.9]), 0.5 μL of bovine serum albumin (BSA, 2.5 μM), 2 μL of $MgCl_2$ (25 mM), and 1 unit of *Taq* DNA polymerase (Fermentas, Thermo Fisher Scientific, Pittsburgh, Pennsylvania, USA). A touchdown cycling program was used before normal cycling: 94°C for 5 min followed by 10 cycles of 94°C for 1 min, 60°C decreasing to 50°C at 1°C per cycle for 40 s, and 72°C for 1 min. Subsequently, 30 cycles of 94°C for 40 s, annealing temperature of each primer for 40 s, and 72°C for 1 min were performed prior to a final extension at 72°C for 10 min. The amplification products were separated under denaturing conditions on 5% (v/v) polyacrylamide gel in an automatic sequencer (LI-COR 4300S DNA Analysis System; LI-COR Biosciences, Lincoln, Nebraska, USA). The loci were genotyped using Saga software (LI-COR Biosciences).

Twelve of the investigated loci were polymorphic. In the *P. gonoacantha* population, the number of alleles per locus in the 12 polymorphic loci ranged from three to 15, and the mean number of alleles per locus was 6.75, whereas observed and expected heterozygosities varied from 0.190 to 0.769 and from 0.2142 to 0.8325, respectively (Table 2). Gametic disequilibrium between pairs of loci and other statistics were estimated using GENEPOP (Raymond and Rousset, 1995). The sequential Bonferroni correction was used to correct multiple applications of the same test (Weir, 1996). No linkage disequilibrium was detected between pairs of loci after Bonferroni correction for multiple tests.

CONCLUSIONS

We developed the first set of microsatellite markers for *P. gonoacantha*. These molecular tools will be useful to estimate genetic diversity parameters for the development of more efficient management strategies in natural and reforested areas that not only consider conservation purposes but also permit the use of *P. gonoacantha* as a source of wood and pharmacological products.

TABLE 2. Estimates of the genetic diversity of *Piptadenia gonoacantha* population based on 12 polymorphic microsatellite markers.

Locus	*A*	H_o	H_e	*P*
Pgo01	8	0.500	0.7011	0.000*
Pgo02	5	0.427	0.3425	0.738
Pgo03	8	0.769	0.7229	0.000*
Pgo04	3	0.284	0.2369	1.000
Pgo05	15	0.419	0.8325	0.000*
Pgo06	7	0.313	0.4176	0.000*
Pgo07	3	0.286	0.2636	0.780
Pgo08	6	0.231	0.2142	0.089
Pgo09	6	0.190	0.4962	0.000*
Pgo10	7	0.317	0.4831	0.000*
Pgo11	8	0.387	0.3482	0.340
Pgo12	5	0.364	0.3892	0.000*

Note: *A* = number of alleles per locus; H_e = expected heterozygosity; H_o = observed heterozygosity; *P* = probability of Hardy–Weinberg equilibrium.
*Departs significantly from Hardy–Weinberg equilibrium after Bonferroni correction.

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