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Development and Characterization of Microsatellite Markers for *Piptadenia gonoacantha* (Fabaceae)

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*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 economically useful. 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 *Afl*I (Invitrogen, Carlsbad, California, USA) and enriched in microsatellite fragments using (CT)4 and (GT)4 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 MgCl₂, 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 microsatellite primers 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 fluorescent (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...
Microsatellite markers were developed 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 MgCl₂ (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 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). Genetic disequilibrium between pairs of loci and other statistics were estimated using GENEPOP (Raymond and Rouset, 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.
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