DEVELOPMENT AND CHARACTERIZATION OF MICROSATELLITE MARKERS FOR PIPTADENIA GONOACANTHA (FABACEAE)\textsuperscript{1}

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\begin{itemize}
  \item \textbf{Premise of the study:} Microsatellite primers were designed for \textit{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.

  \item \textbf{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.

  \item \textbf{Conclusions:} The developed markers will be used in further studies of population genetics of \textit{P. gonoacantha}, aimed at conservation and management of the species in natural populations and in forest restoration projects.
\end{itemize}

\textbf{Key words:} Atlantic Forest; conservation genetics; Fabaceae; forest restoration; microsatellites; \textit{Piptadenia gonoacantha}.

\textit{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 Takagi, 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, \textit{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 \textit{P. gonoacantha}, as a tool to estimate population genetic parameters.

\textbf{METHODS AND RESULTS}

The genomic DNA was extracted from leaves of \textit{P. gonoacantha} following the protocol developed by Cavalieri et al. (2014). A microsatellite-enriched library was obtained using the protocol adapted from Billotte et al. (1999). Genomic DNA from one individual of \textit{P. gonoacantha} was digested with \textit{A}fl\textit{i} (Invitrogen, Carlsbad, California, USA) and enriched in microsatellite fragments using (CT), and (GT), 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 \textit{Escherichia coli} competent cells (Promega Corporation). Positive clones were selected using \textbeta-galactosidase gene expression and grown on a selective medium with ampicillin. The sequencing reactions (10 \textmuL) contained 200 ng of plasmid DNA, 0.5 pmol of SP6 primer, 0.4 \textmuL of BigDye Terminator mix (version 3.1; Applied Biosystems, Foster City, California, USA), 1 mM MgCl\textsubscript{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′-CACGACGTTGATACGACGAC-3′).

Six individuals of \textit{P. gonoacantha} were screened during primer testing, resulting in amplification for 15 primer pairs (Table 1). These primers were used...