DNA Microsatellite Markers for Swartzia glazioviana (Fabaceae), a Threatened Species from the Brazilian Atlantic Forest

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PRIMER NOTE

DNA MICROSATELLITE MARKERS FOR *SWARTZIA GLAZIOVIANA* (FABACEAE), A THREATENED SPECIES FROM THE BRAZILIAN ATLANTIC FOREST[1]

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- *Premise of the study:* Development and characterization of a set of DNA microsatellite markers for *Swartzia glazioviana* (Fabaceae), a naturally rare and threatened tree species, were carried out to investigate its conservation genetics.
- *Methods and Results:* Through an enriched genomic library procedure, 10 DNA microsatellite loci were isolated and characterized for the species. The mean expected heterozygosity was 0.776 (0.424–0.894). Cross-species amplifications of these loci were successfully tested for six congener taxa (*S. apetala* var. *apetala*, *S. flaemingii*, *S. langsdorffii*, *S. macrostachya*, *S. myrtifolia* var. *elegans*, and *S. simplex* var. *continentalis*).
- *Conclusions:* The 10 polymorphic microsatellite markers developed are quite informative and will provide a valuable resource to study the population and conservation genetics of *S. glazioviana* and other *Swartzia* species.

Key words: conservation; Fabaceae; population genetics; simple sequence repeat (SSR); *Swartzia glazioviana*; *Swartzia* spp.

Swartzia glazioviana (Taub.) Glaz. (Fabaceae) is an endemic legume tree species that occurs on the slopes of coastal hills with sandbanks in the Região dos Lagos, Rio de Janeiro, Brazil (Mansano and Lima, 2007; Bohrer et al., 2009). *Swartzia glazioviana* is considered a threatened species (Ministério do Meio Ambiente, 2014) due to its rare distribution and limited occurrence in very disturbed and fragmented habitats, which may compromise the viability of their populations as evolutionary units. To our knowledge, there are no detailed studies on the ecology, reproductive biology, and population genetics for this species that could contribute effectively to its conservation. Microsatellites (or simple sequence repeats [SSRs]) are considered the marker of choice for population genetic studies due to their codominant nature, high information content (polymorphisms), and reproducibility (Litt and Luty, 1989; Selkoe and Toonen, 2006). They are ideal markers to investigate the genetic diversity and structure, mating system, and gene flow of tree populations (Ashley, 2010), including those considered endangered species, such as *S. glazioviana*. Here we present the development and characterization of a set of highly polymorphic microsatellite markers developed for *S. glazioviana* for use in population genetic and conservation studies of this endemic species from the Brazilian Atlantic Forest.

METHODS AND RESULTS

For the library construction, total genomic DNA was extracted from dried leaves previously collected from a single adult of *S. glazioviana*. The genomic library was enriched for the repeat motifs CT and GT using biotinylated probes with streptavidin-coated magnetic beads according to Billotte et al. (1999). The total genomic DNA extraction followed a modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1990; Ferreira and Grattapaglia, 1998).

The total genomic DNA was cleaved using *Afa*I restriction enzyme (Invitrogen, Carlsbad, California, USA), and the fragments were ligated to Afa21 (5′-CTCTTGCTTACGCGTGGACTA-3′) and Afa25 (5′-TAGTCCACGC GTAAGCAAGAGCACA-3′) adapters. The linked fragments were amplified via PCR and purified using QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany). Selected fragments were reamplified using primer Afa21, cloned using pGEM-T Easy Vector (Promega Corporation, Madison, Wisconsin, USA), and transformed into *Escherichia coli* (XL1-Blue) competent cells by electroporation. The transformed cells were grown in Luria–Bertani medium containing ampicillin and IPTG/X-Gal and incubated. Recombinant-positive clones were selected and grown on Circlegrow (MP Biomedicals, Santa Ana, California, USA) and plasmid DNA extracted. Sequencing reactions used T7 or Sp6 universal primers and BigDye Terminator Cycle Sequencing Kit (version 3.1; Life Technologies, Grand Island, New York, USA) and sequencing was performed on an ABI 377XL sequencer (Applied Biosystems, Waltham, Massachusetts, USA) following the manufacturer's instructions. Primer pairs complementary to the flanking regions of the repeats were designed using Primer3 software (Rozen and Skaletsky, 1999). Of 96 positive

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TABLE 1. Characteristics of 10 microsatellite markers developed for *Swartzia glazioviana*.

Locus	Primer sequences (5′–3′)	Fluorescent label	Repeat motif	Allele size (bp)	T_a (°C)	GenBank accession no.[a]
Sgl01	F: CGCTCTCATTTCCTCCATTC R: TGTTGAGCACACTCTTGTCG	6-FAM	$(CT)_7T_2(CT)_9$	193	56	KR002083
Sgl05	F: AGATGCTCCTAACGCAGAGTG R: GAGGCAGCGGTTCAAACT	NED	$(TC)_6C(CT)_{14}$	156	63	KR002084
Sgl11	F: TGCTCCTGCCAAACTCATC R: TCTGGGCGTTTGTCTGAGT	6-FAM	$(GA)_{20}$	209	58	KR002086
Sgl12	F: ATGAGCCGTGACAGAGTTG R: TCATGGGGAACTGTCCTG	NED	$(AG)_{19}$	180	60	KR002087
Sgl15	F: TGCCATTCCACATATCAGC R: GACAACACAGCCACTAGAATCA	VIC	$(GA)_{15}$	185	57	KR002088
Sgl16	F: GTGTGCTGTGTGGATATGTGG R: CCTGTGATAGGGAATCTGCTG	6-FAM	$(AG)_{19}$	224	58	KR002089
Sgl17	F: AGAAGGGGAAATGGGTAACTG R: GAAAGTTTTGCTGGGATGCT	6-FAM	$(AG)_{16}$	226	63	KR002090
Sgl18	F: CAGCCACTGACTTCACATTTC R: AGGGCCTTAGTGGAATTTTTAC	6-FAM	$(CT)_{12}G(TC)_6$	162	56	KR002091
Sgl19	F: GAGTTGGGAGCTTGGTTGAG R: CTCTTTCTGGACGCGAAGA	VIC	$(CT)_{17}$	188	67	KR002092
Sgl20	F: CAGGTGGGCTGCATACAATA R: GGACCCTTTGAGACAGTTCG	VIC	$(CT)_{20}$	185	60	KR002093

Note: T_a = annealing temperature.
[a]GenBank sequences available in April 2016.

sequenced clones, 38 (~40%) showed repetitive elements of which ca. 70% were dinucleotide CT or GA. Of these, 20 (52.6%) sequences were appropriate for primer design. The loci were initially amplified using six DNA samples of *S. glazioviana* from the three populations. PCRs were performed in a final volume of 13.0 µL containing: 2.0 µL of ultrapure water, 1.4 µL of 10× reaction buffer (NH_4), 1.0 µL $MgCl_2$ (50 mM), 1.2 µL bovine serum albumin (BSA; 2.5 mg/mL), 0.8 µL dNTPs (250 mM), 4.3 µL of unlabeled primers (0.9 mM of each), 0.3 µL *Taq* Polymerase (5 U/µL), and 2.0 µL of DNA template (2.5 ng/µL) in a Veriti thermocycler (Life Technologies). The following PCR program was used: one cycle at 95°C for 5 min; 30 cycles at 94°C for 1 min, at primer annealing temperature for 1 min, and at 72°C for 1 min; and a final extension cycle at 72°C for 10 min. Ten out of 20 markers that showed specific and robust PCR products in agarose gels were chosen, and the forward primer of each pair was fluorescently labeled (6-FAM, VIC, and NED). The PCR mixture contained a final volume of 10.0 µL with: 1.6 µL of ultrapure water, 1.0 µL of 10× reaction buffer (NH_4), 2.0 µL of $MgCl_2$ (50 mM), 2.0 µL BSA (2.5 mg/mL), 0.8 µL dNTPs (250 mM), 0.3 µL of primers (10 mM), 0.3 µL of *Taq* DNA Polymerase (5 U/µL), and 2.0 µL of DNA template (2.5 ng/µL). The PCR program was as follows: one cycle at 94°C for 5 min; 30 cycles at 94°C for 1 min, at annealing temperature of each primer pair for 1 min, and at 72°C for 1 min; and a final cycle at 72°C for 60 min.

In total, 96 individuals were sampled (32 per population) from three populations of *S. glazioviana* located in Arraial do Cabo (22°58′07″S, 42°01′00″W) and Cabo Frio (22°52′21″S, 41°58′53″W; 22°51′20″S, 41°59′38″W), Rio de Janeiro, Brazil (voucher numbers in Appendix 1). A batch of 96 adult individuals (32 per population) of *S. glazioviana* was used for SSR loci screening (Table 1). The products were analyzed in a 3500XL DNA sequencer (Life Technologies) following the manufacturer's instructions. Alleles were sized using GeneScan 600 LIZ Size Standard (Life Technologies) and GeneMapper 4.0 software (Life Technologies). We estimated total and average number of alleles per locus (A) and observed (H_o) and expected (H_e) heterozygosities according to the proportions of Hardy–Weinberg equilibrium, for each and overall loci. Inbreeding was quantified by estimating the coefficient of inbreeding (F), and the statistical significance of the F values was tested by Monte Carlo permutation resampling alleles among individuals. We also tested the occurrence of genotypic disequilibrium between pairwise loci using a Bonferroni correction. All estimates were performed using FSTAT (Goudet, 1995). We

TABLE 2. Genetic diversity and coefficient of inbreeding estimated for three populations of *Swartzia glazioviana* in the central coast of Rio de Janeiro, Brazil, based on the analysis of 10 microsatellite loci.

	Trilha do Forno population (n = 32)					Praia das Conchas population (n = 32)					Encosta Rua Vitória population (n = 32)				
Locus	A	H_o	H_e	F	P value	A	H_o	H_e	F	P value	A	H_o	H_e	F	P value
Sgl01	8	0.876	0.709	–0.235	0.011	9	0.656	0.858	0.235	0.002	7	0.581	0.848	0.315*	0.001
Sgl05	10	0.531	0.838	0.366*	0.001	10	0.290	0.798	0.636*	0.001	6	0.549	0.687	0.201	0.044
Sgl11	8	0.781	0.727	–0.074	0.310	7	0.813	0.704	–0.155	0.966	4	0.406	0.424	0.042	0.452
Sgl12	12	0.969	0.830	–0.167	0.017	11	0.781	0.870	0.102	0.120	9	0.625	0.838	0.254	0.002
Sgl15	5	0.438	0.539	0.188	0.076	11	0.501	0.847	0.409*	0.001	5	0.562	0.553	–0.017	0.550
Sgl16	12	0.875	0.894	0.021	0.486	12	0.719	0.840	0.144	0.044	9	0.688	0.731	0.059	0.301
Sgl17	8	0.906	0.749	–0.210	0.012	7	0.656	0.726	0.096	0.212	5	0.531	0.613	0.133	0.169
Sgl18	6	0.625	0.816	0.234	0.010	8	0.594	0.826	0.281*	0.001	7	0.843	0.803	–0.050	0.405
Sgl19	12	0.500	0.878	0.431*	0.001	10	0.750	0.826	0.092	0.162	6	0.688	0.776	0.114	0.149
Sgl20	11	0.613	0.839	0.269	0.038	11	0.407	0.828	0.509*	0.001	9	0.500	0.775	0.355*	0.001
Mean	9.2	0.712	0.782	0.090	0.002	9.6	0.617	0.812	0.241*	0.001	6.7	0.598	0.705	0.152*	0.001
SD	2.6	0.192	0.105	0.247		1.8	0.171	0.055	0.232		1.8	0.121	0.137	0.138	
Total	92	—	—	—		96	—	—	—		67	—	—	—	

Note: A = number of alleles; F = coefficient of inbreeding; H_e = expected heterozygosity; H_o = observed heterozygosity.
*Significant departure from Hardy–Weinberg equilibrium, after Bonferroni correction (P = 0.0001).

TABLE 3. Cross-species amplification analysis for 10 microsatellite markers developed for *Swartzia glazioviana* and tested in six congeneric species.[a]

Locus	*S. apetala* (*n* = 3)	*S. flaemengii* (*n* = 2)	*S. langsdorffii* (*n* = 3)	*S. macrostachya* (*n* = 3)	*S. myrtifolia* var. *elegans* (*n* = 5)	*S. simplex* var. *continentalis* (*n* = 2)
Sgl01	+/4	+/2	+/3	—	+/6	+/2
Sgl05	+/3	+/?	+/2	+/4	+/3	+/m
Sgl11	+/3	+/4	+/4	+/5	+/5	+/3
Sgl12	+/4	—	+/2	+/4	+/m	+/m
Sgl15	+/3	+/2	—	+/4	—	—
Sgl16	+/4	+/4	+/2	+/3	+/5	+/3
Sgl17	+/2	+/3	+/m	+/5	—	—
Sgl18	+/4	+/?	+/2	+/?	+/2	+/2
Sgl19	—	+/?	+/3	+/4	+/m	+/m
Sgl20	+/3	+/2	+/3	+/2	+/4	+/m

Note: + = positive amplification; — = no amplification; m = monomorphic; *n* = number of individuals.
[a] Shown for each locus is amplification/number of alleles.

also inferred the discriminatory power of the SSR loci by estimating the probabilities of genetic identity (*I*) for each locus and combined (*IC*) overall loci according to Taberlet and Luikart (1999), and the probabilities of paternity exclusion (*Q*) for each locus and the combined probability (*QC*) for all loci (Weir, 1996).

The number of alleles per locus ranged from four to 12 (mean 8.5). Mean H_o was 0.642 (0.290–0.969) and H_e ranged from 0.424 to 0.894 (mean 0.776). Mean *F* was significantly greater than zero for two of the populations (Table 2). *QC* was 0.999995 (0.213–0.477), and *IC* was 2.245×10^{-14} (0.022–0.120), across the 10 loci, clearly showing the high individual discrimination power of the loci (Table 2). After Bonferroni corrections, significant genotypic disequilibrium was detected between only two pairwise loci (Sgl15xSgl19 and Sgl15xSgl20).

Amplification of the 10 SSR loci developed for *S. glazioviana* was tested for six congeners: *S. apetala* Raddi var. *apetala*, *S. flaemingii* Raddi, *S. langsdorffii* Raddi, *S. macrostachya* Benth., *S. myrtifolia* Sm. var. *elegans* (Schott) R. S. Cowan, and *S. simplex* (Sw.) Spreng. var. *continentalis* Urb. We found a high cross-species transportability rate (68%) of the 10 loci across the six *Swartzia* species. The success amplification rates of the loci for each species were as follows: *S. apetala* var. *apetala* (90%), *S. flaemingii* (45%), *S. simplex* var. *continentalis* (40%), *S. langsdorffii* and *S. macrostachya* (80%), and *S. myrtifolia* var. *elegans* (60%) (Table 3).

CONCLUSIONS

The 10 SSR loci isolated for *S. glazioviana* showed high levels of genetic diversity. The *IC* and *QC* estimates also indicate the highly informative and discriminatory power of these loci, which are important for their use in parentage analysis. The results also highlight the potential of the developed microsatellite markers for estimating population genetic parameters in six other *Swartzia* species.

The 10 polymorphic markers reported here will be useful tools for estimating pollen dispersal, mating system, genetic diversity, and population genetic structure and will provide relevant information for the effective population preservation of this threatened tree species.

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Appendix 1. Voucher numbers for *Swartzia glazioviana* populations and other *Swartzia* species sampled in the current study.

Species	Voucher accession no.[a]	Collection locality[b]	Geographic coordinates
S. glazioviana (Taub.) Glaz.	RB 623221	Trilha do Forno, Arraial do Cabo, Rio de Janeiro, RJ, Brazil	22°58′07″S, 42°01′00″W
S. glazioviana	RB 623226	Praia das Conchas, Cabo Frio, Rio de Janeiro, RJ, Brazil	22°52′21″S, 41°58′53″W
S. glazioviana	RB 623228	Encosta Rua Vitória, Cabo Frio, Rio de Janeiro, RJ, Brazil	22°51′20″S, 41°59′38″W
S. apetala Raddi	RB 439025	Santa Cruz, Vila de Itaparica, Espírito Santo, Brazil	19°58′21″S, 40°08′52″W
S. flaemingii Raddi	RB 436845	Machadinha, Rio de Janeiro, RJ, Brazil	22°01′29″S, 41°27′17″W
S. langsdorffii Raddi	RB 466380	Jardim Botânico do Rio de Janeiro	22°58′05″S, 43°13′28″W
S. macrostachya Benth.	RB 452769	Espírito Santo, Brazil	19°04′59″S, 39°53′06″W
S. myrtifolia Sm. var. *elegans* (Schott) R. S. Cowan	RB 452764	Linhares, Espírito Santo, Brazil	19°48′54″S, 39°13′23″W
S. simplex (Sw.) Spreng. var. *continentalis* Urb.	RB 452762	Espírito Santo, Brazil	NA

Note: NA = not available.

[a]Voucher deposited at Jardim Botânico do Rio de Janeiro herbarium (RB), Rio de Janeiro, Brazil.

[b]Locality, Brazilian province, state, country.