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Development of microsatellite markers for the neotropical tree species *Dipteryx alata* (Fabaceae)[1]

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- *Premise of the study:* Microsatellite markers were developed for the population genetic analyses of the neotropical tree *Dipteryx alata* (Fabaceae).
- *Methods and Results:* Microsatellites were developed from a genomic shotgun library. Polymorphism at each microsatellite loci was analyzed based on 94 individuals from three populations. Eight loci amplified successfully and presented one to 10 alleles, and expected heterozygosities ranged from 0.097 to 0.862. Four loci also amplified in *Pterodon emarginatus* and presented similar polymorphism.
- *Conclusion:* The eight microsatellite primer pairs are potentially suitable for population genetic studies and successfully amplified in another Fabaceae species.

Key words: *Dipteryx alata*; Fabaceae; genetic diversity; neotropical savannas; neotropical tree; *Pterodon emarginatus*; shotgun library.

Dipteryx alata Vogel (Fabaceae) is a neotropical tree species that is widely distributed in eutrophic and drained soils of seasonal savannas (cerradão) in the cerrado biome, central Brazil. The seeds have a very woody endocarp with an edible nut that is eaten and dispersed by mammals, such as bats, tapirs, and monkeys (Sano et al., 2004). The seeds are also consumed by humans raw or toasted and are a source of raw material for small and midsized food industries, playing an important role in the economy of the local population of central Brazil. The species is threatened because of the high levels of disturbance caused by fire during the dry season and habitat fragmentation due to agricultural expansion (Collevatti et al., 2010). Despite its high ecological and economic importance, studies on population genetics of *D. alata* are still scarce due to the lack of codominant markers, such as microsatellites (but see Collevatti et al., 2010). Here we report the development and characterization of eight microsatellite loci for *D. alata* aimed at the study of population genetic structure and patterns of gene flow and mating system.

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METHODS AND RESULTS

We developed a genomic shotgun library for microsatellite isolation and primer design. The DNA from one individual of *D. alata* was extracted following the cetyltrimethylammonium bromide (CTAB) 2% protocol (Doyle and Doyle, 1987). The DNA was sheared (2.0 μg) using a sonicator at 120 W for 1:45 h to obtain fragments of 200 bp to 1.0 kb. Fragments were recovered and cloned into pMOSBlue dephosphorylated blunt vector using the Blunt-ended PCR Cloning Kit (GE HealthCare, Uppsala, Sweden). DNA inserts were sequenced on a 3100 automated DNA sequencer (Applied Biosystems, Foster City, California, USA) using the U19 primer with the DYEnamic ET Terminator Kit (GE Healthcare), according to the manufacturer's instructions. We sequenced 1031 clones from the genomic library. Sequences were screened for microsatellites using WEBSAT software (Martins et al., 2009), and oligonucleotides complementary to the repeats were designed using Primer3 (Rozen and Skaletsky, 2000). Fifty-eight fragments presented microsatellites but primers could be designed for 28 microsatellite loci. For primer design, some stringent criteria were applied: (i) maximum primer melting temperature (T_m) of 68°C; (ii) maximum of 3°C difference in T_m between primer pair; (iii) GC content ranging from 40% to 60%; (iv) maximum of two dimers between primers; and (v) absence of hairpins.

To characterize microsatellite loci polymorphisms, we then genotyped 94 individuals from three localities, Alcinópolis (ALC), Mato Grosso do Sul (18.26800°S, 53.92600°W); Alvorada (ALV), Tocantins (12.44900°S, 49.11500°W); and Cáceres (CAC), Mato Grosso (15.85303°S, 56.82217°W). The vouchers of the sampled populations were deposited in the herbarium of Universidade Federal de Goiás (ALC, UFG40998; ALV, UFG41010; CAC, UFG40992).

Amplifications were performed in 15 μL reaction volumes containing 12 ng of template DNA, 3.8 μM of each primer, 1 U *Taq* DNA polymerase (Phoneutria, Belo Horizonte, Brazil), 250 μM of each dNTP, 0.25 μg bovine serum albumin (BSA), and 1× reaction buffer (10 mm Tris-HCl pH 8.3, 50 mm KCl, 1.5 mm $MgCl_2$), with the following conditions: 94°C for 5 min (one cycle); 94°C for 1 min, 56–66°C (see Table 1) for 1 min, 72°C for 1 min (30 cycles); and 72°C for 7 min (one cycle). Polymorphisms were detected in 6% denaturing polyacrylamide gels stained with silver nitrate (Creste et al., 2001) and sized by comparison to a 10 bp DNA ladder standard (Invitrogen, Carlsbad, California, USA). Statistical analyses

TABLE 1. Microsatellite primers developed for *Dipteryx alata* based on the genotyping of 94 individuals from three populations.

Locus	Primer sequences (5′–3′)	Repeat motif	Size range (bp)	T_a (°C)	GenBank accession no.
DaE06	F: TGCAGCATAAAAATTGCGAA R: TTACCCCAAAGCCTCAAGAA	$(AAAT)_4$	212–220	60	JN602068
DaE12	F: CCTTCTATGCGCTCTCTGCT R: TACTTCAACGCCAGCTTCCT	$(ATTTTT)_3$	216–222	64	JN602069
DaE20	F: AATAGCAGGGCACCATTCAC R: ACGTTTTTGCCGACATTCAT	$(AG)_8$	146–158	60	JN602070
DaE34	F: ATCCTCTGCGTGCATTCTTT R: CGCTTGCTGCTATTCCTTTC	$(GA)_{15}$	104–124	64	JN602071
DaE41	F: GCCTCCTCCTCCGGTATCTA R: CAGCAGTGGGAGTGTCAGAA	$(CA)_5$	118–148	66	JN602072
DaE63	F: TGAAATTGAGGAAGCAAGGG R: TCCTTTCAATCCTTTTAGAATTTG	$(TC)_6$	208–214	60	JN602073
DaE67	F: CAGACGGACCTGAGAGAAGG R: AATTGAGGCTGATGTTTGGG	$(GATACA)_4$	170–176	66	JN602074
DaE46	F: GCCTATGCGTCCTTCAGATT R: TTTTGCCACATGCTTCTTTG	$(TTA)_5$	247–253	56	JN602075

Note: T_a = annealing temperature.

TABLE 2. Genetic characterization of eight microsatellite loci in three populations of *Dipteryx alata*.

	ALC population				ALV population				CAC population			
Locus	n	N_a	H_e	H_o	n	N_a	H_e	H_o	n	N_a	H_e	H_o
DaE06	32	3	0.429	0.250	32	2	0.426	0.281	30	1	—	—
DaE12	32	5	0.376	0.406	32	2	0.222	0.250	30	2	0.364	0.333
DaE20	31	3	0.406	0.290	31	2	0.505	0.387	30	4	0.469	0.166
DaE34	32	7	0.802	0.156	32	5	0.613	0.468	30	2	0.476	0.267
DaE41	32	8	0.794	0.375	32	6	0.743	0.218	30	10	0.862	0.534
DaE63	32	3	0.176	0.187	31	2	0.432	0.419	29	2	0.483	0.276
DaE67	32	2	0.146	0.156	32	2	0.495	0.531	30	2	0.097	0.100
DaE46	32	3	0.521	0.718	32	3	0.627	0.688	30	3	0.414	0.167

Note: ALC = Alcinópolis; ALV = Alvorada; CAC = Cáceres; H_e = expected heterozygosity; H_o = observed heterozygosity; n = number of individuals genotyped; N_a = number of alleles.

were performed with FSTAT 2.9.3.2 software (Goudet, 2002). We also analyzed cross species amplification of these loci in 12 individuals from one population of *Pterodon emarginatus* Vogel (Fabaceae), a highly important cerrado tree species used as a source of raw material for traditional medicinal recipes.

Eight microsatellite loci amplified clearly interpretable products using a single PCR protocol (Table 1). *Dipteryx alata* presented similar levels of polymorphism to other neotropical tree species of Fabaceae (e.g., Dayanandan et al., 1997; Dick and Hamilton, 1999), with one to 10 alleles per locus and expected heterozygosities ranging from 0.097 to 0.862 (Table 2). Four loci amplified in *P. emarginatus* and presented the following number of alleles and expected heterozygosities, respectively: 5, 0.689 (DaE12); 3, 0.414 (DaE20); 3, 0.633 (DaE46); 3, 0.148 (DaE67).

CONCLUSIONS

The eight microsatellite loci of *D. alata* developed in the current study are potentially suitable for studies of population genetic structure. Four loci also amplified in another Fabaceae species from the Brazilian savanna, *P. emarginatus*, with similar levels of polymorphism.

LITERATURE CITED

COLLEVATTI, R. G., J. S. LIMA, T. N. SOARES, AND M. P. C. TELLES. 2010. Spatial genetic structure and life history traits in Cerrado tree species: Inferences for conservation. *Natureza & Conservacao* 8: 54–59.

CRESTE, S., A. TULMANN NETO, AND A. FIGUEIRA. 2001. Detection of single sequence repeat polymorphisms in denaturing polyacrylamide sequencing gels by silver staining. *Plant Molecular Biology Reporter* 19: 299–306.

DAYANANDAN, S., K. S. BAWA, AND K. KESSELI. 1997. Conservation of microsatellites among tropical trees (Leguminosae). *American Journal of Botany* 84: 1658–1663.

DICK, C. W., AND M. B. HAMILTON. 1999. Microsatellites from the Amazonian tree *Dinizia excelsa* (Fabaceae). *Molecular Ecology* 8: 1765–1766.

DOYLE, J. J., AND J. L. DOYLE. 1987. Isolation of plant DNA from fresh tissue. *Focus (San Francisco, Calif.)* 12: 13–15.

GOUDET, J. 2002. FSTAT 2.9.3.2.: A program to estimate and test gene diversities and fixation indices. Website http://www2.unil.ch/popgen/softwares/fstat.htm [accessed 18 January 2012].

MARTINS, W. S., D. C. S. LUCAS, K. F. S. NEVES, AND D. J. BERTIOLI. 2009. WebSat: A web software for microsatellite marker development. *Bioinformation* 3: 282–283.

ROZEN, S., AND H. J. SKALETSKY. 2000. Primer3 on the WWW for general users and for biologist programmers. *In* S. Krawetz and S. Misener [eds], Bioinformatics methods and protocols: Methods in molecular biology, 365–386. Humana Press, Totowa, New Jersey, USA.

SANO, S. M., J. F. RIBEIRO, AND M. A. BRITO. 2004. Baru: Biologia e uso. Embrapa, Planaltina, Distrito Federal, Brazil.