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Development of microsatellite markers for *Qualea grandiflora* (Vochysiaceae), a typical species of the Brazilian cerrado[1]

Lia Maris Orth Ritter[2,3], Miklos Maximiliano Bajay[2], Mariza Monteiro[2], Renata G. V. C. Souza[2], Maria Andréia Moreno[2], and Paulo Yoshio Kageyama[2]

[2]Universidade de São Paulo, Escola Superior de Agricultura Luiz de Queiroz (ESALQ/USP), Av. Padua Dias 11, Piracicaba, São Paulo 13400-970, Brazil

- *Premise of the study:* Microsatellite primers were developed to investigate genetic diversity and population structure of *Qualea grandiflora*, a typical species of the Brazilian cerrado.
- *Methods and Results:* Eight microsatellite loci were isolated using an enrichment cloning protocol. These loci were tested on a population of 110 individuals of *Q. grandiflora* collected from a cerrado fragment in São Paulo State, Brazil. The loci polymorphism ranges from seven to 19 alleles and the average heterozygosity value is 0.568, while the average polymorphic information content is 0.799.
- *Conclusions:* The developed markers were found to be highly polymorphic, indicating their applicability to studies of population genetic diversity in *Q. grandiflora*.

Key words: cerrado; genetic diversity; microsatellites; *Qualea grandiflora*; simple sequence repeat markers; Vochysiaceae.

The cerrado is the second largest biome of Brazil, with an estimated biodiversity of more than 13 000 plant species (Sano et al., 2008). It has the richest flora among the world's savannas, and its endemism reaches 44% (Myers et al., 2000). Nevertheless, it is under constant human action and this, among other factors, has led to habitat fragmentation and loss of genetic diversity (Sano et al., 2008). Recent studies showed that the Brazilian cerrado has been reduced to 50% of its original area, thus evidencing a great need for comprehensive conservation programs. Nevertheless, the few genetic studies for species of the cerrado that have been carried out have focused on species of economical interest, without taking into account ecological factors (Machado et al., 2004).

To diminish these effects, advances in molecular biology have opened new perspectives for conservation research through the application of genetic tools. Simple sequence repeat (SSR) markers have been used effectively in conservation biology and molecular ecology (Solé-Cava, 2001). We report the development and characterization of eight polymorphic microsatellite loci, which could be used in future studies on genetic diversity, possibly leading to sustainable strategies for the conservation of *Qualea grandiflora* Mart. (Vochysiaceae), a typical tree species of the cerrado. The wood of *Q. grandiflora* is frequently used in industry, such as in furniture construction and paper making. It is also suitable for landscaping, production of dyes (extracted from its seeds and fruits), and asthma treatment (Almeida et al., 1998).

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[3]Author for correspondence: liamaris@gmail.com

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METHODS AND RESULTS

Total genomic DNA was extracted from fresh leaf of a single individual of *Q. grandiflora* (voucher: *Ritter LMO, HUPG, 17891*; herbarium of the Universidade Estadual de Ponta Grossa, Paraná, Brazil), using a cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1990). A microsatellite-enriched genomic library was constructed following the protocol of Billotte et al. (1999). The *Rsa*I enzyme (Invitrogen, Carlsbad, California, USA) was used to digest the genomic DNA of one genotype of *Q. grandiflora*, enriched in microsatellite fragments using $(CT)_8$ and $(GT)_8$ motifs. These enriched fragments were cloned into pGEM-T (Promega, Madison, Wisconsin, USA), and ligation products were used to transform Epicurian Coli XL1-Blue *Escherichia coli* competent cells. The positive clones were selected using the beta-galactosidase gene and grown overnight in liquid medium containing ampicillin (100 µg/mL). A total of 288 clones were sequenced using the adapter Rsa 21 (5′-CTCTTGCTTACGCGTGGACTA-3′) and Rsa 25 (5′-TAGTCCACGCGTAAGCAAGAGCACA-3′) in an ABI 377 automated sequencer (Applied Biosystems, Foster City, California, USA) using the BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems) in a 10 µL volume reaction. Its vectors were removed from each of the sequences by VecScreen (http://www.ncbi.nlm.nih.gov/VecScreen). Eight primer pairs were designed for SSR flanking regions using Primer3 (Rozen and Skaletsky, 2000) according to these criteria: annealing temperature ranged from 54° to 58°C and GC content between 40% and 60%. Each primer pair was designed to amplify a fragment ranging between 100 and 300 bp, and primers were tested using 110 samples from one population of *Q. grandiflora* from Brotas city (22°22′11.31″S, 48°1′23.69″W). PCR amplifications were performed in 25 µL reaction volume consisting of buffer (20 mM Tris-HCl [pH 8.4], 50 mM KCl, and 1.5 mM $MgCl_2$) and containing 20 ng of genomic DNA, 0.8 mM of each primer, 0.2 mM dNTPs, and 1 U *Taq* DNA polymerase (Invitrogen). The amplification program for all primers consisted of an initial denaturing step at 94°C for 1 min, followed by 35 cycles of amplification (94°C [1 min], 1 min at the specific annealing temperature of each primer pair [Table 1], 72°C [1 min]) and a final elongation step at 72°C for 10 min. Amplification products were confirmed by electrophoresis in 7% denaturing polyacrylamide gels and silver stained (Creste et al., 2001). Allele

TABLE 1. Characteristics of eight microsatellite loci of *Qualea grandiflora*.

Locus	GenBank accession no.	Primer sequences (5′–3′)	Repeat motif	T_a (°C)	Allele size range (bp)
Qgr1	JN230425	F: CACTGGCGATTCATTTCTCA R: GCCCAACCGAGTAAACAAG	$(TC)_{14}$	58	156–194
Qgr2	JN230426	F: CGACGATGAGTTTCATTAGC R: TGATTGAGAATGGGGGACT	$(CT)_8$	58	208–222
Qgr3	JN230427	F: ATACAATGTCGGGGAGGAC R: CCACGTAAAACTCAATTCATCG	$(CT)_{12}$	56	206–242
Qgr4	JN230428	F: TCAGAGCACTCAAGCAACG R: GCAAACTAATGGAGGAGGA	$(GA)_{11}$	58	224–260
Qgr5	JN230429	F: CGCAAAATCCCCATCTTATC R: TGCTCCTTGAGTGCTGTGA	$(TC)_{20}$	58	210–238
Qgr6	JN230430	F: GGCTTTATGTCTTTTGGTTTG R: CTTCTTCTTCTTCCTCGCAGA	$(CT)_{17}$	58	204–238
Qgr7	JN230431	F: GAATGTGTTCCAAACCAGTG R: AAGTGTGCGTGTGCGTGT	$(TC)_{11}(CA)_8$	54	186–208
Qgr8	JN230432	F: ATGCGTAAGCGTAAAATCTC R: ACATTGCCACGGGAGTAAG	$(CT)_{11}$	58	212–252

Note: T_a = optimal annealing temperature.

TABLE 2. Results of initial primer screening in *Qualea grandiflora*.

Loci	*N*	H_o	H_e	PIC	HWE[a]
Qgr1	9	0.734	0.68	0.629	0.66
Qgr2	7	0.27	0.77	0.725	0
Qgr3	15	0.552	0.869	0.848	0
Qgr4	19	0.439	0.878	0.859	0
Qgr5	15	0.794	0.893	0.875	0.21
Qgr6	11	0.688	0.881	0.856	0.04
Qgr7	12	0.53	0.821	0.787	0
Qgr8	12	0.538	0.843	0.817	0
Average	12.5	0.568	0.829	0.799	—

Note: — = not applicable; H_e = expected heterozygosity; H_o = observed heterozygosity; HWE = Hardy–Weinberg equilibrium; *N* = number of alleles; PIC = polymorphic information content.

[a] Results less than 0.5 indicate deviation from HWE.

scoring was carried out using the 10 bp DNA Ladder (Invitrogen) as size standard. Measures of heterozygosity were made using genetic data analysis (Lewis and Zaykin, 2000). Measures of polymorphic information content and number of alleles (Table 2) were made using CERVUS 3.0 software (Kalinowski et al., 2007). The linkage disequilibrium was tested using an adjusted *P* value for 5% nominal level using genetic data analysis (Lewis and Zaykin, 2000).

Based on this analysis, no disequilibrium was detected among all loci. After Bonferroni correction, all loci depart significantly from Hardy–Weinberg equilibrium (5%), except for $Q_{gr}1$ and $Q_{gr}5$. The number of alleles found in the loci ranged from seven to 19, and the estimation of expected heterozygosity was usually less than observed heterozygosity, indicating positive values of the fixation index. The average polymorphic information count (PIC) value is 0.799, which means that the loci are providing a high degree of genetic information.

CONCLUSIONS

The developed microsatellite loci showed high levels of polymorphism in *Q. grandiflora*. These markers constitute an important resource that can be further used in the assessment of genetic diversity of this species in the Brazilian cerrado, therefore allowing a better understanding of its reproductive characteristics.

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