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PERMANENT GENETIC RESOURCES

Ten microsatellite loci from *Solms-laubachia eurycarpa* (Brassicaceae)

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Abstract

***Solms-laubachia eurycarpa* is a medicinal herb endemic to the Hengduan Mountains region of south-central China. We screened a partial genomic library enriched for microsatellites and characterized 10 polymorphic loci for *S. eurycarpa*. The number of alleles per locus ranged from five to 15, with an average of 9.6. The observed and expected heterozygosities ranged from 0.2 to 0.725 and from 0.585 to 0.871, respectively. Amplification in closely related taxa was successful for most loci. The results indicate significant potential for the utility of these markers in studying the population genetics of *S. eurycarpa* and related species.**

Keywords: *Solms-laubachia eurycarpa*, cross-species amplification, microsatellites, SSR

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Solms-laubachia eurycarpa (Brassicaceae) is a perennial herbaceous species endemic to scree slope and rock crevice habitats, 3700–4800 m above sea level, in the Hengduan Mountains region of south-central China (Lan & Cheo 1981; Al-Shehbaz & Yang 2001). Compared to other species of *Solms-laubachia*, which tend to be narrow endemics, *S. eurycarpa* is geographically widespread, its range including five provinces (northwestern Yunnan, western Sichuan, southeastern Xizang, southeastern Qinghai and southwestern Gansu; Zhou *et al*. 2001). It is used in Tibetan traditional medicine (Anonymous 1991, 1993), and harvesting has reduced natural populations to the extent that the species is now considered endangered (Fu *et al*. 1998). Conservation of this species for traditional uses will benefit from the knowledge of its genetic diversity within and between populations across its range. To this end, we report in this study the isolation and characterization of 10 polymorphic microsatellites from a genomic library of *S. eurycarpa*.

Genomic DNA was extracted from silica gel-dried leaves using the cetyltrimethyl ammonium bromide (CTAB) extraction method by Doyle & Doyle (1987). Microsatellites were then developed using the enrichment protocol of Glenn & Schable (2005). Genomic DNA from one individual was digested with *Rsa*I and *Xmn*I (New England BioLabs). Resulting fragments were then ligated to SNX linkers using T4 DNA Ligase (New England BioLabs) and enriched for microsatellites with six biotinylated trinucleotide probes [$(AAG)_8$, $(AAC)_6$, $(AAT)_{12}$, $(ACT)_{12}$, $(ATC)_8$, $(AGC)_6$] bound to streptavidin-coated magnetic beads (Dynabeads M-280, Invitrogen). DNA fragments containing microsatellites were captured magnetically and amplified via polymerase chain reaction (PCR) with linker-specific primers. PCR fragments were cloned using the TOPO-TA cloning kit following the manufacturer's protocol (Invitrogen). Positive bacterial colonies (i.e. white colonies) were used as a template for direct PCR in 25 μL reaction containing 1× PCR buffer (10 mM Tris-HCl, 50 mM KCl, PH 8.3), 1.5 mM $MgCl_2$, 10× BSA, 0.12 mM of each dNTP, 0.25 μM of the universal M13 primers and 1 U *Taq* DNA polymerase. Thermal cycling was as follows: an initial denaturing step at 95 °C for 7 min, followed by 35 cycles of 95 °C for 20 s, 50 °C for 20 s, and 72 °C for 90 s. PCR products were cleaned using NucleoFast 96 PCR Plates following the manufacturer's protocol (Macherey–Nagel). DNA sequencing was performed using the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing reactions were precipitated with ethanol and 3 M sodium acetate and run on an ABI 3730

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Table 1 Characteristics of 10 microsatellites isolated from *Solms-laubachia eurycarpa*. T_a, annealing temperature; H_O, observed heterozygosity; H_E, expected heterozygosity; *P*, *P* values for Hardy–Weinberg equilibrium; *indicates deviation from Hardy–Weinberg equilibrium ($P < 0.05$)

Locus	Repeat motif	Primer sequence (5′–3′)	T_a (°C)	No. of alleles	Allele size range (bp)	H_O	H_E	*P*	GenBank Accession no.
Seu4*	$(CTT)_9$	CCGCAGGGTATTCTTTTC TCAATGAAAGCCAATAACCG	58	13	154–179	0.650	0.816	0.0289	EU340331
Seu7	$(AAG)_8$	TCAAAGCTCACAATGAACCA GAACTGCTTTCAAATCCTCAAT	50	15	207–241	0.500	0.585	0.1161	EU340332
Seu8	$(GAT)_{10}$	TTCGCTGTCCTTTTTGAAACT GAAGATCGCTTCTTCGATGC	50	9	177–204	0.725	0.823	0.0593	EU340333
Seu13*	$(AAG)_8$	ACGAAGGAAAACCCTTACCG ACAGCTCAGAGGCGATGAAT	50	12	171–194	0.700	0.871	0.0123	EU340334
Seu15	$(CTT)_7$	GCCCTTTATCTATTTCTCCGT GGATTTTAAGAGCATAGCAAG	50	5	138–153	0.475	0.471	1.000	EU340335
Seu16	$(AAG)_8$	AATATGTGGGAGTAAATC GTCCTAGATGCAACAGCCAG	50	15	170–203	0.525	0.766	0.1108	EU340336
Seu17*	$(GAT)_8$	GGATAGAATTAAGCATAC AAGGGGAGATTGAGGTGGAG	50	8	130–155	0.450	0.740	0.0002	EU340337
Seu22*	$(AAG)_3AAA(AAG)_8$	CCCACGGGATTACCAGTC AAACAAATTGGACAAGAACG	50	6	180–192	0.200	0.620	0.0156	EU340338
Seu23*	$(AGC)_7$	ATGGTTCGATGGAATGGA TGGCCGTTACTCGACCTATC	50	6	157–168	0.395	0.779	0.0000	EU340339
Seu24	$(CTT)_7$	GTCACTCGTTGGTGTAGG TAAAGCCTTCTTGGACTCGG	50	7	224–247	0.600	0.743	0.1931	EU340340

DNA Analyser (Applied Biosystems). Sequences were analysed for the repeat region using the software EPHEMERIS 1.0 (available at http://www.uga.edu/srel/DNA_Lab/programs.htm). In total, 481 clones were sequenced, yielding 45 potential loci. Primers flanking core microsatellite repeats were developed using PRIMER 3 online software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_http://www.cgi) for 24 loci. All forward primers had M13 tails (5′-TGTAA AACGACGGCCAGT-3′) attached to the 5′ end to allow for labelling with fluorescent M13 primers.

Initial PCR trials were performed on 40 individual *S. eurycarpa* collected from northwestern Yunnan and southeastern Xizang, respectively. Each 10 μL PCR contained approximately 10 ng of genomic DNA, 1× PCR buffer (10 mM Tris-HCl, 50 mM KCl, PH 8.3), 0.16 μM of reverse primer and fluorescent M13 primer, 0.04 μM of forward primer, 0.12 mM of each dNTP, 1.5 mM of $MgCl_2$ and 0.4 U *Taq* polymerase. The thermocycling profile was composed of an initial denaturation step at 94 °C 4 min, 30 cycles of 94 °C for 30 s, 50–58 °C (Table 1) for 30 s, 72 °C for 45 s, eight cycles of 94 °C for 30 s, 53 °C for 30 s, 72 °C for 45 s, with a final extension step of 72 °C for 10 min. PCR products were resolved by capillary electrophoresis on an ABI 3730 DNA Analyser and sized with GENEMAPPER version 3.7 software (Applied Biosystems). In total, 10 primer pairs consistently amplified microsatellite loci and were polymorphic. The program CERVUS 2.0 (Marshall *et al*. 1998) was used to calculate number of alleles and the observed and expected heterozygosities. Linkage disequilibrium and departures from Hardy–Weinberg equilibrium (HWE) were tested using GENEPOP on the web version 3.4 (Raymond & Rousset 1995).

The number of alleles per locus ranged from five to 15, with an average of 9.6. The observed and expected heterozygosities ranged from 0.2 to 0.725 and from 0.585 to 0.871, respectively (Table 1). Five loci, Seu4, Seu13, Seu17, Seu22 and Seu23, deviated significantly from HWE ($P < 0.05$). MICROCHECKER (van Oosterhout *et al*. 2004) analyses indicated the possible presence of null alleles for northwestern Yunnan population at Seu17 locus with estimated frequency of 0.297, and for southeastern Xizang population at Seu4 and Seu23, with estimated frequencies of 0.106 and 0.334, respectively. These potential null alleles might be population-specific. Linkage disequilibrium analyses showed that Seu8 and Seu23 were linked ($P < 0.01$).

The loci were examined in 12 congeneric species and in the closely related *Desideria baiogoinensis* (Yue *et al*. 2006), using PCR conditions outlined above. PCR products were resolved on a 1.5% agarose gel. With the exception of Seu23, successful amplification was common (Table 2). This suite of markers thus appears to have considerable potential as a tool for population genetic studies across *Solms-laubachia* and in closely related genera.

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Table 2 Cross-species amplification tests using 10 microsatellite primers isolated from *Solms-laubachia eurycarpa* in related taxa (one sample per species)

	Locus									
Species	Seu4	Seu7	Seu8	Seu13	Seu15	Seu16	Seu17	Seu22	Seu23	Seu24
Solms-laubachia angustifolia	+	+	+	+	+	+	+	+	–	+
S. calcicola	–	+	+	+	+	+	+	+	–	+
S. grandiflora	+	+	+	+	+	+	+	+	–	+
S. lanata	–	+	–	+	+	–	–	–	–	–
S. linearifolia	+	+	+	+	–	+	+	–	–	–
S. minor	–	+	+	+	–	–	–	–	–	+
S. platycarpa	–	+	+	+	–	+	+	+	–	+
S. pulcherrima	+	+	+	+	+	+	+	+	–	+
S. retropilosa	+	+	+	+	+	–	+	+	–	+
S. sunhangiana	+	+	+	+	+	+	+	+	–	+
S. xerophyta	+	+	+	+	+	+	+	+	–	+
S. zhongdianensis	+	+	+	+	+	–	+	+	–	+
Desideria baiogoinensis	+	+	+	+	+	+	+	+	–	–

+ amplified successfully, – fail to amplify.

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