

PRIMER NOTE

Polymorphic microsatellite markers for the Socotran endemic herb *Begonia socotrana*

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Abstract

Six polymorphic microsatellite markers have been developed to examine population structure and outcrossing rates in the narrow-range endemic *Begonia socotrana*. Only two of the markers amplify products in its recently discovered sister species *B. samhaensis*. All of the loci amplify in winter-flowering *Begonia* hybrids derived from *B. socotrana*, revealing little polymorphism and demonstrating the narrow genetic base of the material used in their production.

Keywords: *Begonia*, microsatellites, Socotra

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Begonia socotrana (sect. *Peltaugustia*) is a bulbiferous herb endemic to the island of Socotra in the northern Indian Ocean, where it grows in sheltered, north-facing crevices in the Haggier mountains. It is listed by the IUCN as 'vulnerable' (Walter & Gilliet 1998). To investigate patterns of population genetic structure and outcrossing rates in this species, we have developed six polymorphic microsatellite markers.

DNA enriched for microsatellites hybridizing to an (AC)₁₃ oligomer was isolated from *B. socotrana* following the method of White & Powell (1997), with the modification that no size selection was performed on the initial genomic DNA digest or on the post-enrichment polymerase chain reaction (PCR) fragments.

The enriched DNA was ligated into a ZAP Express *EcoRI* vector, followed by packaging using a ZAP Express Predigested Gigapack III Gold cloning kit (Stratagene). Plaques were lifted from a plating of the library using Pasteur pipettes, placed in 500 µL of SM buffer (30 mM NaCl, 1 mM MgCl₂, 50 mM Tris pH 7.5, 0.01% gelatin) with 20 µL of chloroform and left to diffuse overnight at 4 °C. The resulting recombinant phage suspensions were screened for microsatellites using a three-primer PCR as follows: 1 µL of the phage suspension was combined in a total volume of 10 µL with 1 µM M13 F primer, 1 µM M13 R primer, 1 µM of (AC)₁₃ oligomer, 1× PCR buffer [16 mM (NH₄)₂SO₄, 67 mM

Tris-HCl (pH 8.8), 0.01% Tween-20], 2.5 mM MgCl₂, 0.2 mM dNTPs and 0.5 units of *BioTaq* (Bioline). An initial denaturing step at 94 °C for 5 min was followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, carried out in a GeneAmp 9600 thermocycler (Perkin Elmer). The PCR products were run on a 2% agarose gel; positive clones were identified by the presence of more than one band. Plasmids were extracted from positive plaques following the manufacturers protocol, and the sequence of the insert was determined using a Thermosequenase II dye terminator cycle sequencing kit (Amersham) and an ABI 377 DNA sequencer.

Approximately 25% of the colonies screened contained microsatellites, and 24 (out of 75) were chosen for primer design, which was carried out using Primer-3 (Rozen & Skaletsky 1998) with the modified parameters of Beasley *et al.* (1999) as the starting point. Three clones were discarded owing to the possibility of them being chimeric, as they showed varying combinations of identical flanking regions around different microsatellites; these products may be the result of premature strand termination and subsequent priming off other microsatellites during the PCR bulking of the enriched DNA.

The primers were tested in 10 µL PCR reactions containing 10 ng genomic DNA, 1× PCR buffer [16 mM (NH₄)₂SO₄, 67 mM Tris-HCl (pH 8.8), 0.01% Tween-20], 2.5 mM MgCl₂, 0.2 mM dNTPs, 0.5 µM of each primer and 0.5 units of *BioTaq* (Bioline). The reactions were denatured at 95 °C for 7 min, followed by 30 cycles of 95 °C for 30 s, 55 °C for 15 s,

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Table 1 Characterization of six microsatellite primers in *Begonia socotrana*

Locus	Repeat in clone	Primer sequence (5'–3')	Size range (bp)	No. of alleles	H_O	H_E	(<i>n</i>)	GenBank no.
B17b	(CT) ₂₆ (CT) ₁₀ (CT) ₁₃	TCCCGATATTCACAACATATCAC ATGATGGACCCCGTATCACAT*	300–386	31	0.809	0.895	133	AF403057
B63	(AT) ₃ (AC) ₈	CTTAAGCTTCATACTCCAATCAC* GTTTTGAACCTTGAGAATACTAGTGAG	176–190	4	0.099	0.137	94	AF403054
B128	(CTCACA) ₆	TTCCTTTGACAGTTTGTGTGT* AATTTCCGGTAATCAGCAGACAGG	148–172	6	0.502	0.524	143	AF403052
B130	(AC) ₂₄	GCACCTCCTTTTGATGATACACC* CCTAGTCTCTTCACTTATCACAAGGT	105–125	11	0.680	0.704	140	AF403053
B215	(AC) ₂₅ (AT) ₆	CGCGTTAAAAATATGTGAAGCAC TACTATGTGGCAAGCCTCAAACA*	73–81	6	0.520	0.547	143	AF403055
B226	(AC) ₉	GGACGGTGTTAGGCCCTTCTAT* CAATAGTTGTGGATGCAAGGTGA	163–181	7	0.688	0.685	140	AF403056

*Fluorescently labelled primer.

72 °C for 30 s, using a GeneAmp 9600 thermocycler (Perkin Elmer).

Of 24 primer pairs tested, six gave no product, the remaining 18 amplified products which were close to the size of the cloned sequence when run on a 2% agarose gel. Initial population screens of these 18 primer pairs were undertaken by including 4 µM fluorescent dye (TAMRA) labelled dCTP (PE Applied Biosystems) in the PCR and analysing the products on an ABI 377 DNA sequencer. Twelve primer pairs gave banding patterns which could not be interpreted as single loci, and one was monomorphic. The remaining six loci produced one or two bands per individual and were polymorphic; one primer from each pair was fluorescently labelled for use in further population genetic surveys (Table 1).

Although an (AC)₁₃ oligomer was used for the precloning enrichment, other repeat motifs were found in the cloned products, such as (CT)_{*n*} repeats (e.g. locus B17b) and a (CTCACA)₆ repeat located in an open reading frame (locus B128). Primers for one of the loci (B125) amplified products ≈120 bases shorter than expected, and sequencing of the products obtained from population samples showed this was due to a drastic shortening of the microsatellite region.

Of the six loci, only two amplified products (both monomorphic) from the sister species of *Begonia socotrana* which occurs on the neighbouring island of Samha (*Begonia samhaensis*; Hughes & Miller, 2002). All loci amplified products in the winter-flowering *Begonia* Hiemalis cultivars,

which are derived from a cross between *B. socotrana* and *Begonia* × *Tuberhybrida*. Only two of the loci were polymorphic in the cultivars (B17b and B226), both of which showed two alleles, present only in the homozygous state. No products were amplified from the other *Begonia* species tested (*B. sutherlandii*, *B. geranioides*, *B. dregei* and *B. fallax*).

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References

- Beasley EM, Myers RM, Cox DR, Lazzaroni LC (1999) Statistical refinement of primer design parameters. In: *PCR Applications* (ed. Innis MA), pp. 55–71. Academic Press, San Diego.
- Hughes M & Miller AG (2002) A new endemic species of *Begonia* (Begoniaceae) from the Socotra archipelago. *Edinburgh Journal of Botany*, in press.
- Rozen S & Skaletsky HJ (1998) *Primer 3*. Code available at: <http://www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi>.
- Walter KS & Gilliet HJ, eds (1998) *1997 IUCN Red List of Threatened Plants*, p. 73. IUCN, Morges, Switzerland & Cambridge.
- White G & Powell M (1997) Isolation and characterization of microsatellite loci in *Swietenia humilis* (Meliaceae): an endangered tropical hardwood species. *Molecular Ecology*, 6, 851–860.