

PRIMER NOTE

# Isolation of polymorphic microsatellite markers for *Begonia sutherlandii* Hook. f.

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## Abstract

Seven polymorphic microsatellite loci have been characterized for investigating population structure in the patchily distributed herb *Begonia sutherlandii*. Two loci (BSU3 and BSU4) exhibited population specific null alleles; primer redesign and allele sequencing for one of these loci showed two transition mutations in the original primer site. Two loci exhibited imperfect repeat polymorphisms due to single base pair indels in the flanking region (locus BSU6) and in the microsatellite region itself (BSU7). Transversion mutations were also found in the microsatellite region of locus BSU7. The remaining three loci amplified in all individuals tested and appeared to conform to a simple stepwise mutation pattern.

*Keywords:* *Begonia*, Kwazulu-Natal, microsatellites

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*Begonia sutherlandii* is one of the most widespread *Begonia* species in Africa, with an east Afromontane distribution from Tanzania to the northern part of the Transkei in South Africa. It occurs in a narrow habitat range, on wet and shaded steep slopes, particularly in proximity to rivers or waterfalls in forested areas. Given its habitat preference and the naturally fragmented distribution of montane forest in eastern and southern Africa (Lawes 1990; Eeley *et al.* 1999), *B. sutherlandii* occurs sporadically, often with considerable distances of unsuitable semi-arid habitat separating populations.

The amount of gene flow between geographically disjunct populations is an important factor in determining to what extent and how quickly populations can become adapted to local conditions, as gene flow from 'foreign' populations can retard differentiation (Barton 2001). Species of *Begonia* appear to have poor powers of pollen and seed dispersal, leading to the expectation that populations may become isolated over relatively short distances. This may be one of the factors leading to the evolution of high species diversity in the genus (*Begonia* contains about 1400 species). Microsatellite markers have been developed to examine population genetic structure in *B. sutherlandii*, to establish over what scales population differentiation occurs.

DNA enriched for microsatellite sequences was obtained following a method based on Edwards *et al.* (1995) and Squirrell & Wolff (2001), with the modifications that *Tsp509I* restriction enzyme (AATT; New England Biolabs Inc.) and the *Tsp509I* PCR adapters of White & Powell (1997) were used. The nylon membranes to which the genomic DNA was hybridized were prepared with 6 µg each of (GA)<sub>13</sub>, (CA)<sub>13</sub> and (AAG)<sub>8</sub> per single piece of 8 × 8 mm Hybond® (Amersham Pharmacia Biotech) N + membrane.

The enriched DNA was cloned using a PCR-Script™ Amp Cloning Kit (Stratagene), and the sequence of the insert in recombinant clones was determined using a Thermo-sequenase II dye terminator cycle sequencing kit (Amersham) and an ABI 377 sequencer. One hundred and ten clones were sequenced, and ca. 70% contained microsatellites of eight or more repeat units. (AC)<sub>n</sub> was the most common motif, accounting for approximately 70% of the microsatellites found. Thirty-three sequences were chosen for primer design, which was carried out using PRIMER-3 (<http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi>) with the modified parameters of Beasley *et al.* (1999) as the starting point.

The primers were tested in 10 µL polymerase chain reactions (PCR) containing: 10 ng genomic DNA, 1 × PCR buffer (16 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 67 mM Tris-HCl (pH 8.8), 0.01% Tween-20), 2.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 0.5 µM of each

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**Table 1** Characterization of seven microsatellite primers in *Begonia sutherlandii*

Locus	Repeat in clone	Primer sequence (5'-3')	$T_{an}$	Size range (bp)	No. alleles	$H_E$	$H_O$	(n)	GenBank no.
BSU1	(CT) <sub>10</sub>	AAAAGCCTTACTATATAATGACAA CGACCAAGAAAATAAATGAAAT	55	100–122	10	0.531	0.428	245	AF467454
BSU2	(AG) <sub>14</sub>	CCCTTTCTCTTACCCGTTTCCTT TCATAACCAAACCCAATCTCACC	55	114–140	14	0.539	0.458	256	AF467455
BSU3	(CT) <sub>18</sub>	CATGGCTCTAGTAGTTTCTTCATTT GTAGTGCAACGGCAATGATGAC	55	79–105	11	0.441	0.403	156	AF467456
BSU4	(CT) <sub>14</sub>	TGGAGGAAACATATCACGAAGAAA CCAAGTCTTATGGAAGGATGAACA	55	120–144	14	0.230	0.141	224	AF467457
BSU5	(AG) <sub>12</sub>	GTCPTTCTCAACCCACAGACAA GACCTGTCCATTTGCAAAATCTC	55	148–199	22	0.382	0.356	257	AF467458
BSU6	(TC) <sub>18</sub>	CTCTGGGCTAATAACCATAACC CTAGTAAGATCATTTACAGATACGA	53	162–198	21	0.623	0.588	237	AF467460
BSU7	(CT) <sub>8</sub> CC(TC) <sub>8</sub>	TGTCCTCGCAGAATATGTTCACT TTTAACCAAGCCATGAATGTT	53	134–163	9	0.231	0.186	210	AF467459

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, annealing temperature ( $T_{an}$ )°C for 20 s, 72 °C for 30 s, finishing with a final extension step at 72 °C for 15 min, using a GeneAmp 9600 thermocycler (Perkin Elmer). The products were visualized on a 2% agarose gel. Out of 33 primer pairs, five gave no product, one gave several bands and 27 gave a product consisting of one or two bands of size similar to that in the clone. Of these 27 loci, only 7 produced one or two bands per individual and were polymorphic when the profiles were examined in detail by including 4 µM TAMRA labelled dCTP (PE Applied Biosystems) in the PCR and analysing the products on an ABI 377 sequencer. One of each of these primer pairs was labelled using either FAM, TAMRA or JOE fluorescent dyes (PE Applied Biosystems) for use in screening populations.

Loci BSU4 and BSU3 exhibited population specific null alleles, where none of the individuals in one (BSU4) or two (BSU3) populations gave a PCR product. The primers for BSU4 were redesigned (5'-3': forward AATCTCTTGA-GATGGAGGAAACA, reverse GTTGGTAACTTGGTAT-GGTGGA; original primer sequences are shown in Table 1) to anneal outside the original primers, and these successfully amplified products in the population previously exhibiting nulls. Sequencing the product showed two transition (C-T) mutations 2 and 12 bases from the 3' end of the original reverse primer binding site. The clone from which the primers for BSU3 were designed was too short to permit the design of further primers. The products of two loci (BSU6 and BSU7) showed alleles with single base pair length differences; sequencing alleles from homozygous individuals showed this was due to an extension of one base pair in a T<sub>8</sub> region adjacent to the (TC)<sub>18</sub> microsatellite in locus BSU6, and the loss of one cytosine from the CC

motif in locus BSU7; this locus also showed two transversion mutations (C-A) in the microsatellite region in some individuals.

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