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Novel microsatellite markers for *Begonia maxwelliana* and transferability to 23 *Begonia* species of Peninsular Malaysia

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ABSTRACT

Begonias are hyper-diverse and important horticultural plants. Six polymorphic microsatellite markers were developed from CT- and GT-enriched libraries of *Begonia maxwelliana*. The number of alleles per locus ranged from 2 to 12 and the observed heterozygosity ranged from 0.036 to 0.813. Null alleles were detected in one locus (*Bma*161) after Bonferroni correction. All the six markers were amplifiable in 23 selected *Begonia* species with the success rates of 17–100%. On average, species of the same section as *B. maxwelliana* (i.e. sect. *Platycentrum*) yielded higher transferability (91%). These markers will be useful for population genetic studies of the genus *Begonia*.

1. Introduction

Begoniaceae belongs to the Order Cucurbitales and has only two genera, i.e. *Begonia* and *Hillebrandia* (Kiew, 2005). *Begonia* is pantropical with over 1500 species worldwide and Southeast Asia is one of the biodiversity hotspots (Hughes and Pullan, 2007). Begonias are important in the horticultural trade as attractive ornamental plants. Since the 18th century, more than 400 out of 1500 natural *Begonia* species have been cultivated, and thousands of cultivars and hybrids have been developed (Tebbitt, 2005). Hybridisation efforts started in the 1800's in Europe and have continued ever since. Because many *Begonia* species hybridise easily, there is a great potential for many new cultivars to be developed and commercialised. Begonias also have high potential in the pharmaceutical and food industry. They are widely used in traditional medicine to treat various ailments (Laferrière, 1992; Tangjang et al., 2011).

Many *Begonia* species are rare and highly endemic. In Peninsular Malaysia, 88% are endemics (48 out of 54 species), of which 57% are threatened (Chua et al., 2009). This worrying trend sees the urgent need for their conservation, and genetic diversity assessment is needed to understand the population dynamics and to estimate the extinction risks (Frankham, 2005). Despite being a large and important genus, there are very few studies on the genetic diversity of *Begonia* (e.g. Hughes et al., 2003; Hughes and Holingsworth, 2008; Twyford et al., 2013).

Microsatellite is currently the most practical, informative and widely-used tool in population genetic study. It is codominant, highly reproducible and has enormous extent of allelic diversity. In begonias, microsatellites have been developed for *Begonia sutherlandii* Hook. f. from Africa (Hughes et al., 2002a), *Begonia socotrana* Hook. f. from Socotra (Hughes

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et al., 2002b), *Begonia fenicis* Merr. from Asian islands (Nakamura et al., 2012), and *Begonia nelumbiifolia* Cham. & Schltdl. and *Begonia heracleifolia* Cham. & Schltdl. from America (Twyford et al., 2013).

In Peninsular Malaysia, *B. maxwelliana* King is endemic and locally abundant in the states of Perak, Pahang and Kelantan. It is a herbaceous plant growing on stream rocks in the forests.

This study aims to develop microsatellite markers for *B. maxwelliana* using an enrichment approach and to test the transferability of the markers in 23 other *Begonia* species of Peninsular Malaysia, as microsatellite markers are known to be transferable to closely related species. Besides genetic diversity assessment, the markers developed can also be used for DNA fingerprinting and studies of gene flow, mating system and hybridisation.

2. Materials and methods

2.1. Plant materials and DNA extraction

Total genomic DNA was extracted from fresh leaves of an individual of *B. maxwelliana* from Bukit Larut Forest Reserve (FR), Perak, Peninsular Malaysia. A voucher (FRI 70306) was deposited at the Kepong Herbarium (KEP), Forest Research Institute Malaysia (FRIM). The CTAB method (Murray and Thompson, 1980) was adopted and modified according to Lee et al. (2002). The extracted DNA was subsequently treated with RNase for 20 min at 65 °C and purified using the columns of DNeasy Plant Mini Kit (QIAGEN, USA).

For characterisation of microsatellite loci, leaf samples of 32 individuals of *B. maxwelliana* from the same population in the Bukit Larut FR were collected and dried in silica gel. For cross-species amplification, leaf samples and representative vouchers of 23 selected *Begonia* species from Peninsular Malaysia were collected and deposited at the KEP (Table 1). The sample size ranged from one to four, depending on availability. Total genomic DNA was extracted from leaf samples of 0.1 g (fresh) or 0.05 g (dry) following the DNeasy Plant Mini Kit (QIAGEN) protocol, with minor modification, i.e., twice the amount of Buffers AP1 and AP2 were used.

2.2. Development of microsatellite markers

CT- and GT-enriched libraries were constructed according to Ng et al. (2009). Briefly, approximately 5 μg of the total genomic DNA was digested with *Nde* II (Promega Corporation, USA) and separated on a 1.6% agarose gel. Fragments of 300–1000 bp were excised, extracted using MinElute Gel Extraction Kit (QIAGEN) and ligated into *Sau*3A1 cassettes (TaKaRa Bio Inc., Japan). Nicks were repaired using DNA polymerase I (TaKaRa Bio Inc.). Linker-ligated DNA fragments were enriched for CT and GT repeats via selective hybridisation using biotinylated $(CT)_{15}$ and $(GT)_{15}$, retrieved with streptavidin coated magnetic beads (Promega Corporation) and washed with buffers. The fragments were amplified with C1 cassette primers (TaKaRa Bio Inc.) to confirm enrichment success prior to digestion with *Nde* II to remove the cassettes. The resulting fragments were then cloned into pUC118 *Bam*HI/BAP (TaKaRa Bio Inc.), transformed into *Escherichia coli* TOP 10 competent cells

Table 1
Twenty three species of Peninsular Malaysian *Begonia* selected for cross-species amplification. Leaf material – Dry (D), Fresh (F).

Section	Species	Leaf material	No. of sample (s)	Source locality	Voucher no.
Diploclinium	*Begonia lowiana* King	D	1	Cameron Highlands, Pahang	FRI 50975
Heeringia	*B. sibthorpiodes* Ridl.	D	1	Langkawi, Kedah	FRI 70636
Parvibegonia	*B. integrifolia* Dalzell	F&D	2	Langkawi, Kedah	FRI 70634
	B. phoeniogramma Ridl.	F	4	Bukit Lagong & Gabai, Selangor	FRI 60550
	B. sinuata Meisn.	D	2	Sungai Nipah, Terengganu	–
Petermannia	*B. holttumii* Irmsch.	F&D	4	Sungai Nipah, Terengganu & Kota Damansara, Selangor	FRI 70609
	B. jiewhoei Kiew	F&D	3	Gua Musang, Kelantan	FRI 70623
	B. wrayi Hemsl.	D	1	Sungai Larut, Perak	–
Platycentrum	*B. abdullahpieei* Kiew	F	4	Bintang Hijau, Perak	FRI 64721
	B. aequilateralis Irmsch.	F	4	Bukit Lagong & Kota Damansara, Selangor	FRI 49297 & 54198
	B. herveyana King	F	4	Bukit Senggeh, Melaka	FRI 49292
	B. decora Stapf	F&D	3	Cameron Highlands, Pahang	FRI 68487
	B. koksunii Kiew	F	1	Gerik, Perak	–
	B. pavonina Ridl.	D	2	Cameron Highlands, Pahang	RK 4729
	B. rhyacophila Kiew	F	2	Bintang Hijau, Perak	–
	B. tampinica Irmsch.	F	4	Bukit Tampin, Negeri Sembilan	RK 5181
	B. venusta King	D	1	Cameron Highlands, Pahang	RK 5187
Reichenheimia	*B. forbesii* King	D	4	Sungai Larut, Perak	FRI 70307
	B. foxworthyi Burkill *ex* Ridl.	F	2	Gua Musang, Kelantan & Bentong, Pahang	FRI 70625
	B. ignorata Irmsch.	F	4	Gunung Senyum, Perak	FRI 60008
	B. rajah Ridl.	F	3	Endau-Rompin, Johor & Terengganu	FRI 47082
	B. reginula Kiew	D	4	Serting, Negeri Sembilan	FRI 70309
Ridleyella	*B. kingiana* Irmsch.	F	3	Gua Musang, Kelantan; Ipoh, Perak & Langkawi, Kedah	FRI 64302 & 70347