Development and Characterization of Microsatellite Markers for Melastoma dodecandrum (Melastomataceae)

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PRIMER NOTE

DEVELOPMENT AND CHARACTERIZATION OF MICROSATELLITE MARKERS FOR *MELASTOMA DODECANDRUM* (MELASTOMATACEAE)[1]

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- *Premise of the study:* Microsatellite markers were developed for *Melastoma dodecandrum* to investigate the genetic diversity of this species and to detect hybridization and introgression in *Melastoma*.
- *Methods and Results:* Fourteen microsatellite loci were characterized by screening primers developed using two simple sequence repeat (SSR)–enriched libraries. Based on the genotyping of two natural populations, 13 loci were polymorphic and the number of alleles per locus ranged from two to 15. The observed and expected heterozygosities for the 13 loci ranged from 0.235 to 0.941 and 0.219 to 0.922, respectively. Cross-species amplification was successful for all 14 loci in each of two congeneric species, *M. candidum* and *M. sanguineum*.
- *Conclusions:* These polymorphic SSR markers could be used as multilocus molecular makers to study the population genetics of *M. dodecandrum*, as well as hybridization and introgression among *Melastoma* species.

Key words: cross-species amplification; hybridization; *Melastoma dodecandrum*; Melastomataceae; microsatellite.

The genus *Melastoma* L. (Melastomataceae) is distributed in tropical and subtropical Asia and northern Australia (Meyer, 2001). Previous studies have identified around 50–100 species within this genus (Chen, 1984; Wagner et al., 1999); however, only 22 species were recognized in the latest taxonomic revision by Meyer (2001). Natural hybridization between two species of this genus has been reported, with more cases of interspecific hybridization being expected, because many species in this genus have overlapping geographic distributions and flowering periods (Dai et al., 2012). According to the Flora of China, there are nine *Melastoma* species in China, which are distributed south of the Yangtze River (Chen, 1984). *Melastoma dodecandrum* Lour. is a small shrub that is distributed primarily in southern China, extending to northern Vietnam (Chen, 1984). Unlike other *Melastoma* species, which are restricted to the area south of the Nanling Mountains, *M. dodecandrum* extends north to Zhejiang Province, exhibiting higher levels of cold tolerance (Chen, 1984). Population genetic studies hold promise toward inferring the dispersal routes of this species and studying molecular adaptation to colder climates. Microsatellite markers are codominant and often highly polymorphic, and hence are increasingly used in population genetic studies. However, no microsatellite markers have been available in *Melastoma* to date to investigate the genetic diversity of this species, or hybridization and introgression in this genus. In this study, we developed and characterized 14 microsatellite markers for *M. dodecandrum* and tested their transferability to two other congeneric species, *M. candidum* D. Don and *M. sanguineum* Sims.

METHODS AND RESULTS

Two populations of *M. dodecandrum* were sampled from Dafu Mountain (22°56′18″N, 113°19′08″E) and Maofeng Mountain (23°17′07″N, 113°20′18″E) in Guangzhou, Guangdong, China. In addition, three individuals each of *M. candidum* and *M. sanguineum* were collected from Dafu Mountain. Voucher specimens (Dafu Mountain population: RZ20111015; Maofeng Mountain population: RZ20111006; *M. sanguineum*: RZ20111016; *M. candidum*: RZ20111017) were deposited in the Herbarium of Sun Yat-sen University (SYS). Genomic DNA was extracted from silica-dried leaves with the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). The genomic DNA of one individual of *M. dodecandrum* from Dafu Mountain was used for the development of microsatellites. Approximately 300 ng of genomic DNA was digested with *Mse*I restriction enzyme (New England Biolabs, Ipswich, Massachusetts, USA), and then ligated to an *Mse*I adapter (5′-TACTCAGGACTCAT-3′/5′-GACGATGAGTCCTGAG-3′) using T4 DNA ligase (New England Biolabs). The ligation product was diluted and amplified with the *Mse*I-N primer (5′-GATGAGTCCTGAGTAAN-3′) under a PCR program with initial denaturation of 3 min at 95°C; followed by 30 cycles of 30 s at 94°C, 60 s at 53°C, and 60 s at 72°C; and a final extension of 10 min at 72°C. The PCR products were denatured at 95°C for 5 min and hybridized with each of the two 5′-biotinylated probes, $(AG)_{15}$ and $(AC)_{15}$, in 300 µL of hybridization solution (20× saline sodium citrate [SSC], 10% sodium dodecyl sulfate

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TABLE 1. Characteristics of 14 microsatellite loci of *Melastoma dodecandrum*.

Locus	Primer sequences (5′–3′)	Repeat motif	Size (bp)	T_a (°C)	GenBank accession no.
C33	F: GGGCTGAGACTTGAGAAAGAGA	$(AC)_{10}$	208	55	JX126109
	R: GTTTTCCCGTGGAACAAAAA				
C38	F: TTCACCCTCCTCTAATGACCTC	$(AC)_5$	201	55	JX126110
	R: CTATGTCGATGGGTGGATGATT				
C54	F: TGTGTTATTGGCAAGACAAAGG	$(GT)_7$	214	55	JX126111
	R: AGAGATCTCGATTATGCCCAAG				
C67	F: CGCCTCGAGTCTTTCTTTCTT	$(AC)_8$	201	55	JX126112
	R: CATACAAATCCCAGCAAATGTT				
C71	F: GTTACAGCCCACCAGAAAGAAA	$(AG)_{10}(TAC)_{10}$	250	55	JX126113
	R: CTTAGCCCTTGTCCGTAAGAAT				
C73	F: CGGCCGACATTTGAAGTTAGTA	$(AC)_6$	199	55	JX126114
	R: GGGTCGTTTTATCCATCTTCTTC				
C91	F: GTTTTCCCGTGGAACAAAAA	$(AC)_{10}$	204	55	JX126115
	R: ACCGATGCATATTTTGGAATTT				
C96	F: TTCTGGTCCCCAGTACTTCAAT	$(CA)_8$	217	55	JX126116
	R: TCGTTGGGGAATCTTTACAGTC				
C102	F: GAGAAGAAAATTTTTACATGTCCCTA	$(AC)_{10}$	190	55	JX126117
	R: ATGGCAAATACCAGCAGATGAT				
G80	F: GAATGACAACACCGGCAAG	$(CT)_{11}$	204	55	JX126118
	R: CCTCCACGTTCTCTTGAATGTC				
G90	F: TAAGCAACAGGCAAGAATGAGA	$(AG)_9$	201	55	JX126119
	R: GTGACCCTTGCCTAGTTTTGAC				
G92	F: GGGCTGAGACTTGAGAAAGAGA	$(GA)_{10}$	224	55	JX126120
	R: TTAGGAGAGGATCGTCTGATGC				
G107	F: CCAATCAGTCAGCCACAAAGT	$(CT)_6$	184	55	JX126121
	R: GGAGACCTAACTTCATGTCAATG				
G119	F: GAAAATAAAAACAGCCGGATTG	$(CT)_6$	200	55	JX126122
	R: GACTTCATTGGATCAAGACACG				

Note: T_a = annealing temperature.

[SDS], 100 µM probe) at 48°C for 2 h to construct two independent microsatellite libraries. Streptavidin magnesphere paramagnetic particles (Promega Corporation, Madison, Wisconsin, USA) were used to separate the DNA fragments hybridized to the probes. The fragments were washed three times in TEN_{100} for 8 min and three times in TEN_{1000} for 8 min. The recovered DNA fragments were amplified with adapter-specific primers by following the same procedure as mentioned above. The PCR products were purified with the EZgene Gel/PCR Extraction Kit (Biomiga, San Diego, California, USA), ligated into pMD18-T vector (TaKaRa Biotechnology Co., Dalian, Liaoning, China), and then transformed into *E. coli* strain DH5α (TaKaRa Biotechnology Co.). Transformants were selected on Luria–Bertani (LB) agar medium containing 100 mg/mL ampicillinum natricum. Two hundred forty positive clones were selected and tested by PCR using $(AG)_{10}$, $(AC)_{10}$, and M13 universal primers, of which 197 clones contained potential microsatellite motifs. These positive clones were sequenced on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, California, USA). Sequences containing more than five tandem repeats were considered as microsatellites.

In total, 64 clones were found to contain microsatellites, of which 43 with five or more repeats were selected for primer design using Primer3 (Rozen and Skaletsky, 2000). To test these microsatellites, PCR amplifications were conducted using three individuals for each of the three species in a final volume of 20 µL, containing 25 ng of genomic DNA, 10× PCR buffer (with Mg^{2+}), 2.5 mM of each dNTP, 10 µM of each primer set, and 1 U r*Taq* DNA polymerase (TaKaRa Biotechnology Co.). The PCR reactions were carried out under standard conditions for all primers with the following cycling conditions: 3 min of denaturation at 95°C; followed by 30 cycles of 30 s at 94°C, 30 s at 55°C, and 40 s at 72°C; with a final extension of 10 min at 72°C. Amplification products were first electrophoresed through 1.5% agarose gels to assess whether amplification was successful and the expected sizes were obtained. Our results showed that PCR products of expected sizes were successfully amplified for 14 primer pairs in *M. dodecandrum* (Table 1), and that these primer pairs exhibited successful amplification in the two congeneric species, *M. candidum* and *M. sanguineum*. We then labeled the forward primers of the 14 primer pairs with the fluorescent dye FAM (Invitrogen, Carlsbad, California, USA) and conducted the PCR amplifications for both populations of *M. dodecandrum* using the conditions mentioned above. Using ROX 500 as an internal size standard, the fragment sizes of these PCR products were determined on an ABI PRISM 3100 DNA Analyzer with Genotyper 4.0 (Applied Biosystems). Population genetics parameters for *M. dodecandrum* were calculated using POPGENE version 1.31 (Yeh et al., 1999).

All but one of the microsatellite loci exhibited polymorphisms, with the number of alleles per locus ranging from two to 15 (Table 2). The observed and expected heterozygosity from the polymorphic loci ranged from 0.235 to 0.941 and 0.219 to 0.922, respectively. Four loci (C67 and C73 in the Dafu Mountain population, and C33 and C91 in the Maofeng Mountain population) deviated significantly from Hardy–Weinberg equilibrium ($P < 0.05$). There was no significant linkage disequilibrium between locus pairs; therefore, all of the loci should be considered as being independent across the genome.

TABLE 2. Genetic diversity in two populations of *Melastoma dodecandrum*.

	Dafu Mountain (n = 17)			Maofeng Mountain (n = 18)		
Locus	A	H_o	H_e	A	H_o	H_e
C33	7	0.824	0.839	6	0.389	0.737
C38	2	0.353	0.499	3	0.389	0.465
C54	6	0.765	0.752	6	0.778	0.754
C67	5	0.412	0.726	4	0.500	0.671
C71	11	0.941	0.881	12	0.889	0.854
C73	3	0.529	0.604	4	0.778	0.657
C91	7	0.824	0.840	7	0.389	0.737
C96	3	0.235	0.219	3	0.333	0.294
C102	3	0.706	0.570	3	0.556	0.513
G80	15	0.941	0.922	9	0.722	0.779
G90	7	0.647	0.629	3	0.611	0.541
G92	11	0.647	0.620	6	0.778	0.605
G107	1	0.000	0.000	1	0.000	0.000
G119	2	0.471	0.428	2	0.389	0.322

Note: A = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; n = sample size for each population.

CONCLUSIONS

This is the first study to report microsatellite markers for species of *Melastoma*. All of the 14 markers for *M. dodecandrum* show good transferability in two congeneric species. The primers developed here are suitable for investigating the genetic diversity and population structure of *M. dodecandrum* and other congeneric species, and for detecting hybridization and introgression within this genus.

LITERATURE CITED

Chen, J. 1984. Melastomataceae. *In* C. Chen, H. Chang, R. Miau, and T. Hsu [eds.], Flora Reipublicae Popularis Sinicae, vol. 53(1), 152–162. Science Press, Beijing, China.

Dai, S., W. Wu, R. Zhang, T. Liu, Y. Chen, S. Shi, and R. Zhou. 2012. Molecular evidence for hybrid origin of *Melastoma intermedium*. *Biochemical Systematics and Ecology* 41: 136–141.

Doyle, J. J., and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

Meyer, K. 2001. Revision of the Southeast Asian genus *Melastoma* (Melastomataceae). *Blumea* 46: 351–398.

Rozen, S., and H. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers. *In* S. Misener and S. A. Krawetz [eds.], Methods in molecular biology, vol. 132: Bioinformatics methods and protocols, 365–386. Humana Press, Totowa, New Jersey, USA.

Wagner, W. L., D. R. Herbst, and S. H. Sohmer. 1999. Bishop Museum Special Publication 83. Manual of the flowering plants of Hawaii, vol. 2. University of Hawaii and Bishop Museum Press, Honolulu, Hawaii, USA.

Yeh, F. C., R. C. Yang, and T. Boyle. 1999. POPGENE version 1.31: Microsoft Windows–based freeware for population genetic analysis, quick user guide. Center for International Forestry Research, University of Alberta, Edmonton, Alberta, Canada. Website http://ualberta.ca/~fyeh/popgene.html [accessed 16 May 2012].