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Development and characterization of 10 microsatellite loci in *Paeonia lactiflora* (Paeoniaceae)[1]

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- *Premise of the study:* Microsatellite primers were developed in *Paeonia lactiflora* for further population genetic studies.
- *Methods and Results:* Ten dinucleotide microsatellite markers were developed using an enriched genomic library. They were identified in 58 individuals, and nine markers were also amplified in its cultivars. The number of alleles per locus ranged from three to 11, with a mean of six. The observed and expected heterozygosities ranged from 0.1662 to 0.9140 and from 0.0841 to 0.8157, respectively.
- *Conclusions:* These microsatellite markers will facilitate further studies on population genetics variation and genetic structure of *P. lactiflora.*

Key words: microsatellites; *Paeonia lactiflora*; population genetics; SSR.

Paeonia lactiflora Pall., a famous ornamental and officinal plant, is widely cultivated worldwide (Guo and Wang, 2008). Due to habitat damage and excessive collecting of the seeds, the natural populations of *P. lactiflora* have dramatically declined in recent decades, causing a significant loss of genetic resources for the improvement of the cultivars. Therefore, the assessment of the wild population's genetic diversity through the use of molecular markers is urgently needed, particularly for the conservation of genetic resources, identification of cultivars, and selection of parents for hybridization breeding (Varshney et al., 2005). For that purpose, the key step relies on selecting successful sets of molecular markers, particularly codominant ones like microsatellites. In *Paeonia* L., however, available microsatellite markers are relatively limited (Wang et al., 2008). Up to the present, 10 polymorphic microsatellite primers for the Chinese peony *P. lactiflora* have been reported (Li et al., 2010). In this study, we created a set of polymorphic microsatellites for *P. lactiflora* that were confirmed to be reproducible in assessing genetic diversity within and among the populations.

METHODS AND RESULTS

Fresh leaves of wild material of *P. lactiflora* were collected in late spring in Chifeng, Hebei Province, China. Only a single individual was used for the initial DNA extraction. Genomic DNA was extracted from silica gel–dried leaves using the CTAB method (Doyle and Doyle, 1987), and the construction of a microsatellite-enriched library followed Glenn and Schable (2005) with slight modifications. Genomic DNA was digested with *Mse*I (New England Biolabs, Shanghai, China) and then ligated to the double-strand *Mse*I adapter (5′-TACTCAGGACTCAT-3′/5′-GACGATGAGTCCTGAG-3′). The ligated DNA was randomly linked to one of four single-strand biotinylated microsatellite probes (5′-$(CA)_{15}$-Biotin, 5′-$(GA)_{15}$-Biotin, 5′-$(AT)_{15}$-Biotin, 5′-$(ACT)_{15}$-Biotin). Hybridized DNA was captured by streptavidin-coated paramagnetic beads (Dynabeads M-280 Streptavidin, Dynal Biotech, Oslo, Norway) and gathered with a magnetic particle–collecting unit (MPC-S, Dynal Biotech). The enriched DNA was amplified using *Mse*I adapter-forward as a primer, and the product was purified, ligated into pGEM-T Easy Vector (Promega Corp., Madison, Wisconsin, USA), and cloned in One Shot Top10 Chemically Competent *E. coli* cells (TransGen Biotech, Beijing, China). Three hundred thirty-five positive clones were selected and sequenced, of which 235 (~70%) contained SSRs. Of these, 107 sequences had suitable lengths for primer design using Primer Premier 5.0 (PREMIER Biosoft International, Palo Alto, California, USA). Primers with melting temperatures between 52°C and 62°C were designed for 107 microsatellite flanking sequences to obtain amplified fragments of 100–400 bp. Developed primer pairs were assessed using 67 samples (from 58 wild plants and nine cultivars) by PCR amplification. PCR amplifications were performed in 15 μL, total volumes containing ~70 ng genomic DNA, 10 μM of each primer, and 1× PCR Mix (Tiangen Biotech, Beijing, China). The PCR program consisted of 5 min of initial denaturation at 95°C followed by 30 cycles of denaturation at 95°C for 45 s, a primer-specific annealing temperature (Table 1) for 30 s, extension at 72°C for 50 s, and a final extension at 72°C for 8 min. Finally, 10 pairs of primers (Table 1) were chosen because they showed clear bands of a single locus. The forward primer was labeled with one of the fluorescent dyes (FAM, JOE, or ROX) for polymorphism detection on an ABI 3730xl DNA analyzer (Applied Biosystems, Foster City, California, USA).

Fifty-eight *P. lactiflora* individuals from two wild populations were sampled from two separate populations: 35 individuals from population DHT (40.57°N, 115.77°E) in Chicheng County, Hebei Province, and 23 individuals from population YLS (37°50′N, 111°28′E) in Jiaocheng County, Shanxi Province, to assess polymorphisms of the microsatellite loci. A voucher specimen of *P. lactiflora* (PE, #00023099) is deposited at the Institute of Botany, Chinese Academy of Science. The number of alleles per locus (*A*), the observed and expected heterozygosity (H_o and H_e), and the deviations from Hardy–Weinberg equilibrium (HWE) were analyzed using POPGENE software (Yeh et al.,

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TABLE 1. Characteristics of 10 polymorphic microsatellite markers in *Paeonia lactiflora*. Information on each primer pair includes forward and reverse sequences, repeat motif, size range of the original fragment (bp), annealing temperature (T_a), and GenBank accession number.

Locus	Primer sequence (5′–3′)	Repeat motif	Size range (bp)	T_a (°C)	GenBank Accession No.
Pmg164	F: CCCTACTTTCTCCCTCCTGCTC	$(AG)_9$	325–369	58	HQ891843
	R: TTGTATTATCTTTGTGCCCCAT				
Pmg183	F: GAGATAAGATGCGACAAAGTAG	$(GA)_{12}$	170–210	56	HQ891844
	R: AGTTATGGTTTACTGGATTCTG				
Pmg153	F: CATTTACTCCTTCGCATTCAAC	$(TC)_{14}$	345–390	58	HQ891845
	R: TAACTCCAGATGGGTATTTTGT				
Pmg117	F: AACACCAAACTACTCTAAAGGG	$(TC)_7TT(TC)_{13}$	320–362	53	HQ891846
	R: TGTATCTGGACACAAGTATGAG				
Pmg50	F: ATCGTTTACCGAATCACCATAC	$(TG)_{11}A(GA)_{16}$	230–270	56	HQ891847
	R: CTTAGAAAATCATCCTTCACCC				
Pmg155	F: TTTGAAGCAAACTTTCATCTGA	$(GA)_9$	278–320	56	HQ891848
	R: GAATCACTCACACGATGTCCTA				
Pmg196	F: CGTGACAATCGTCTCCAATAGC	$(GA)_{13}$	140–180	60	HQ891849
	R: CCAAACACAAACAAAGGAAGC				
Pmg209	F: TCTCCAACCCTTGAATAGCTCA	$(GA)_{19}$	174–224	60	HQ891850
	R: CCTTCCCTATCTCCTCCTCCAC				
Pmg180	F: TTCTCCAACCCTTGAATAGCTC	$(GA)_{19}$	180–214	58	HQ891851
	R: TCTCCTCCTCCACCATTACCAC				
Pmg165	F: AAGAAACCTACCTCAATCAGTC	$(GA)_{18}$	186–226	52	HQ891852
	R: TTCTTTCATCTCCCTTCTACAC				

1997). The pairwise linkage disequilibrium (LD) was evaluated using Arlequin ver. 3.1 (Excoffier et al., 2005).

The number of alleles per locus ranged from three to 11, with an average of six. All loci were polymorphic in the DHT and YLS populations, indicating a high level of polymorphism at the species level (Table 2). The observed and expected heterozygosity per locus ranged from 0.1662 to 0.9140 (an average of 0.4221) and from 0.0841 to 0.8157 (an average of 0.5676), respectively (Table 2). Four loci (Pmg196, Pmg209, Pmg180, Pmg165) in DHT and seven loci (Pmg164, Pmg183, Pmg153, Pmg117, Pmg155, Pmg209, Pmg180) in YLS showed significant deviation from HWE ($P < 0.01$) as a result of heterozygote deficiency. No significant linkage disequilibrium was detected among pairs of loci in each population, suggesting that these microsatellites are independent markers and suitable for genetic studies on *P. lactiflora* populations. Furthermore, nine of the 10 microsatellite markers, except Pmg180, also amplified in nine *P. lactiflora* cultivars ('Danfeng', 'Zijinghong', 'Zhengrunhong', 'Zhongshenghong', 'Honglian', 'Tianxiangjin', 'Xiuqiuhua', 'Ziling', and 'Yanli') with three to six alleles per locus.

TABLE 2. Results of initial primer screening in *Paeonia lactiflora*. For each primer pair, the number of alleles (A), average observed heterozygosity (H_o), and average expected heterozygosity (H_e) are reported. Sample size within each population (N) is indicated in parentheses.

	DHT (N = 35)			YLS (N = 23)		
Locus	A	H_o	H_e	A	H_o	H_e
Pmg164	4	0.6534	0.3416	5	0.7932	0.2023
Pmg183	9	0.3636	0.6273	5	0.2908	0.6938
Pmg153	10	0.2091	0.7796	6	0.3053	0.6796
Pmg117	6	0.2865	0.7033	5	0.6203	0.3715
Pmg50	4	0.4621	0.5302	6	0.3314	0.6541
Pmg155	3	0.5540	0.4396	3	0.9140	0.0841
Pmg196	6	0.5027	0.4902	2	0.7362	0.2580
Pmg209	6	0.3909	0.6004	7	0.1662	0.8157
Pmg180	10	0.1752	0.8131	8	0.1855	0.7968
Pmg165	11	0.1818	0.8065	4	0.3208	0.6645

CONCLUSIONS

Ten newly developed microsatellite markers from *P. lactiflora*, in addition to nine that were amplified in its cultivars, provide a good basis for the investigation of the population genetics and conservation biology of this species, the identification of the genetic diversity of important resources for improvement, and the evaluation of the cultivars.

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