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TECHNICAL NOTE

Isolation and characterization of fourteen microsatellites from a tree peony (*Paeonia suffruticosa*)

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Abstract Tree peonies are well known garden flowers and very important medicinal plants with some 1,000 cultivars. Most of the cultivars are believed to be of hybrid origin and their wild parents are suffering from severe depopulation. To rejuvenate the traditional cultivars and assess the genetic diversity of the critically endangered wild species for conservation purpose, it is crucial to use suitable molecular markers. In this study, we isolated 14 polymorphic microsatellite loci from an enriched genomic library. These loci were verified by re-cloning and re-sequencing and their characteristics were tested with 20 individuals. On average there are 5.5 alleles per locus, and mean values of observed heterozygosity and expected heterozygosity are 0.41 and 0.67, respectively.

Keywords *Paeonia suffruticosa* · Microsatellites · SSR primers · Polymorphism

Tree peonies are famous ornamental flowers frequently seen in gardens nearly all over the world. People enjoy their large, showy, colorful and fragrant flowers. Now there are more than 1,000 varieties under cultivation in China and they are believed to be domesticated or bred from five wild species, i.e., *Paeonia cathayana* (D. Y. Hong et K. Y. Pan), *P. jishanensis* (T. Hong et W. Z. Zhao), *P. ostii* (T. Hong et J. X. Zhang), *P. qiui* (Y. L. Pei et D. Y. Hong), and *P. rockii* (S. G. Haw et L. A. Lauener) T. Hong and J. J. Li ex, D. Y. Hong (Zhou unpublished). Some cultivars raised in western countries involved distantly related species *P. delavayi* Franch and *P. ludlowi* (Stern et Taylor) D. Y. Hong as well. However, because most cultivars were selected from open pollinated offspring, their exact parents remain unknown, which excludes the possibility of rejuvenation of old cultivars, especially traditional cultivars with histories of several hundred years. Documentation of the parentages of cultivars relies heavily on suitable molecular markers.

Conservation of wild tree peonies also requires precise estimation of genetic diversity using proper molecular markers. Historical domestication for ornamental purpose and immoderate hunting for root barks for medicinal use had led to eradication of wild populations of several species. There are only two wild populations of *P. qiui*. Worse than all, only a few individuals of *P. cathayana* and *P. ostii* were found in the wild. By gathering occasional individuals together to form a population is the last chance to evaluate the genetic diversity of the critically endangered wild tree peonies if suitable molecular markers were available.

Although there are many kinds of molecular markers, very few can fulfill the needs mentioned above. Microsatellite or simple sequence repeat (SSR) has been proved to be a useful marker for the study of population genetics, parentage analysis and genetic resources assessment, and seems to be the most promising choice for its co-dominance and hypervariability. In this paper, we report the isolation of 14 microsatellite loci and their characteristics in tree peonies.

Total genomic DNA was extracted from fresh leaves of a tree peony (*P. suffruticosa* Andrews) collected from the Beijing Botanical Garden of Institute of Botany, the

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Table 1 Characterization of the 14 microsatellite loci for *Paeonia suffruticosa*

Locus name	Motif sequence	Primer sequence (5′–3′)	Size (bp)	*Ta* (°C)	*Na*	H_E	H_O	GenBank accession No.
P01	$(GA)_8$	F:AAGGTTGTGATCCACTCT	221	53	8	0.8393	0.3684	FJ024283
		R:ACAACTCAAATATGCAGAG						
P02*	$(GA)_{19}$	F:CAAAGCCTCAAGAAACTCCCTA	132	58	2	0.2615	0.1000	FJ024284
		R:GATGTACAACACCAAGATGCAA						
P03	$(GA)_{10}$	F:ATGTCACCGAAAGTTGTGC	291	54	7	0.8051	0.2632	FJ024285
		R:AAAGCCTGGTGCAGTTATT						
P04*	$(AC)_5$	F:TAGTCACGCACCTGCTCAC	240	58	4	0.3599	0.1053	FJ024286
		R:CTCTATTCCTGTCGCTAGTCG						
P05	$(AG)_9$	F:TCGCCCAACCTGTCGTGGAGAT	286	50	4	0.5718	0.1000	FJ024287
		R:TTGAATAGAGCGGAATGGAAAA						
P06*	$(TC)_5CCC(TC)_5(CA)_8$	F:GTTATAGAACCACTGACAT	321	58	6	0.7496	0.3684	FJ024288
		R:TGAGAGACAAATAATCGTG						
P07	$(TC)_{26}$	F:GGTATTGTTCTCTGTGTGGT	273	56	6	0.8063	0.6667	FJ024289
		R:GAAACCAAACTCAAACTCG						
P08*	$(TC)_{24}$	F:GTGGTCATACCTAGATAAAT	228	58	4	0.4708	0.1579	FJ024290
		R:ATCAGAACTACCTAAGAAGACTAAG						
P09	$(CT)_{17}$	F:GCCACAAGAAAACAAAAACC	267	54	6	0.7705	0.1500	FJ024291
		R:CCTTCACCACTACTTCCCCAT						
P10	$(CT)_{20}$	F:CACAAAACTCCTTCATCTTC	339	58	6	0.8179	0.5500	FJ024292
		R:ATCGTCAATTAGAATCAGAC						
P11	$(CT)_{15}TTTT(CT)_5$	F:CCCTATTGACGAATGGAT	399	56	2	0.4923	0.7000	FJ024293
		R:GGAGTATTGGGTGGTGTG						
P12*	$(TC)_9TTTCTCTCTA(TC)_5$	F:TTGGTTGGTGAAGGTGTT	324	50	6	0.7487	0.9000	FJ024294
		R:CTTCGATAACCGCAGGAGGAT						
P13	$(TC)_6$	F:TATAAATGGGAAGCAGACTCAA	311	50	7	0.8090	0.4500	FJ024295
		R:TATACTCAGCCTCGAAAAGAAG						
P14	$(TC)_{20}TTTCTAGT(TATC)_5$	F:CAAACTACCTGAATGTTCGGCTC	323	50	9	0.8654	0.8000	FJ024296
		R:CATCAAATTACCAAAGAAATCCT						
Mean over all loci			–	–	5.5	0.6692	0.4057	–

* Indicates significant departure from Hardy–Weinberg equilibrium after checked by MICRO-CHECKER version 2.2.3

Size, expected fragment size from each primer pairs; *Ta*, annealing temperature; *Na*, number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity; GenBank accession no, the GenBank accession numbers of the sequences based on which primers were designed

Chinese Academy of Sciences. The experimental procedures of genomic library construction, enrichment of microsatellite library, cloning and sequencing of the target fragments, sequence editing, primer designing and polymorphism testing of the loci were substantially the same as Tian et al. (2008). Twenty tree peony samples were used to characterize the microsatellite loci. One further step was taken to verify the authenticity of the PCR products from the 14 loci. PCR products separated from the purification gels were cloned and sequenced again.

The genotype data matrix was checked by MICRO-CHECKER version 2.2.3 (van Oosterhout et al. 2004) for the presence of null alleles and analysed for each locus using POPGENE version 1.31 (Yeh and Yang 1999) to estimate the observed and the expected heterozygosity (H_O and H_E), the deviations from Hardy–Weinberg equilibrium (HWE) and the pairwise linkage disequilibrium (LD).

Two hundred and forty positive clones with different sizes of PCR products ranged from 200 bp to 900 bp were sequenced. Fifty-eight (24.02%) clones were found harboring microsatellites, among which 44 (18.17%) contain unique fragments. These fragments contain 44 dinucleotide repeat motifs, six trinucleotide repeat motifs and one tetranucleotide repeat motif, among which 24 are perfect, seven imperfect and 13 compounds according to Weber (1990). Of the 44 dinucleotide repeat motifs, 35 are $(GA/CT)_n$, 9 are $(CA/GT)_n$. Neither $(AT/TA)_n$ nor $(GC/CG)_n$ repeat motif

was detected in this study, even though $(AT/TA)_n$ is said to be the most abundant in plants, probably their palindromic structure really works (Powell et al. 1996).

Forty-four pairs of primers were designed to amplify the fragments with microsatellite loci. Among them 14 (31.82%) were polymorphic (Table 1), 4 (9.09%) were monomorphic, and 27 not yielded the expected PCR products. All the 14 polymorphic loci were verified containing the expected sequences and repeat motifs.

Seventy-seven alleles were detected in a population of 20 samples. As shown in Table 1, the number of alleles per locus varied from 2 to 8 with an average of 5.5 and the H_O and H_E ranged form 0.10 to 0.90 and 0.26 to 0.87, respectively. Most loci were tested deviating significantly from HWE ($P < 0.05$) (Table 1), indicating that the assumptions of HWE are violated. Significant LD ($P < 0.05$) was observed between six pairs of loci (P01–P04, P01–P14, P03–P04, P04–P14, P05–P13, and P06–P09). Since *P. suffruticosa* is a cultivated species and this species is mostly of hybrid origin, LD between some loci is not surprising. The microsatellite loci isolated and tested to be polymorphic in this study are expected to be useful in documenting the origin of cultivars and assessing genetic diversity of the tree peonies for conservation purpose.

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