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Authors: Liu, Hua-Bo, Liu, Jun, Wang, Zhe, Ma, Li-Ying, Wang, Si-Qi, et al.

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PRIMER NOTE

DEVELOPMENT AND CHARACTERIZATION OF MICROSATELLITE MARKERS IN *PRUNUS SIBIRICA* (ROSACEAE)[1]

HUA-BO LIU[2], JUN LIU[2], ZHE WANG[2], LI-YING MA[2], SI-QI WANG[2], XING-GU LIN[2], RONG-LING WU[2], AND XIAO-MING PANG[2,3]

[2]Center for Computational Biology, National Engineering Laboratory for Tree Breeding, Key Laboratory of Genetics and Breeding in Forest Trees and Ornamental Plants, College of Biological Sciences and Biotechnology, Beijing Forestry University, Beijing 100083, People's Republic of China

- *Premise of the study:* Microsatellite loci were developed for *Prunus sibirica* to investigate genetic diversity, population genetic structure, and marker-assisted selection of late-blooming cultivars in the breeding of *P. sibirica*.
- *Methods and Results:* Using a magnetic bead enrichment strategy, 19 primer pairs were developed and characterized across 40 individuals from three *P. sibirica* wild populations and six individuals of *P. armeniaca*. The number of alleles per locus varied from three to 11 and the observed and expected heterozygosities ranged from 0.063 to 0.917 and 0.295 to 0.876, respectively, in the three *P. sibirica* wild populations. All primer pairs could be successfully amplified in six individuals of *P. armeniaca*.
- *Conclusions:* These microsatellite primer pairs should be useful for population genetics, germplasm identification, and marker-assisted selection in the breeding of *P. sibirica* and related species.

Key words: genetic diversity; microsatellite; *Prunus sibirica*; Rosaceae.

Prunus sibirica L. is an important ecological and economic tree species, widely distributed in the mountain areas of northern and northeastern China, eastern Siberia, and Mongolia (Zhang and Zhang, 2003). Its seeds are used not only in traditional Chinese medicine but also to obtain transformed products, such as almond milk, skin care products, and biodiesel fuel. In 2005, 7.28 million tons of fruit were harvested in China from an area of 1.54 million ha (Zhang and He, 2007). In general, the Siberian apricot is a hardy species. However, it is still vulnerable to late spring frosts that can damage the blossom and the young fruit, seriously impairing fruit production and thus causing major economic losses. For this reason, one of the most crucial goals in apricot breeding is to select late-blooming cultivars that can avoid late spring frosts. Because there is no commercial cultivar, trees have been commonly grown from seeds collected from the wild, and thus the quality cannot be guaranteed, although abundant genetic variation exists in natural populations. However, the level of genetic diversity and population genetic structure of *P. sibirica* still remain unknown. Molecular markers, especially microsatellites, have proven to be powerful for studying the population genetic variation of wild species because of their abundance and high polymorphisms throughout genomes (Tautz, 1989). Here, we report 19 polymorphic microsatellite markers developed for *P. sibirica*.

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[3]Author for correspondence: xmpang@bjfu.edu.cn

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METHODS AND RESULTS

Genomic DNA of *P. sibirica* was extracted from fresh healthy leaves using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). Microsatellites were isolated from an individual tree using a magnetic bead enrichment strategy, as described in Nunome et al. (2006), with minor modifications. Approximately 20 µg of genomic DNA was digested with each enzyme, *Alu*I and *Hae*III (New England Biolabs, Ipswich, Massachusetts, USA), and then ligated to a double-stranded linker (F: 5′-GTTTAGCCTTGTAGCAGAAGC-3′; R: 5′-pGCTTCTGCTACAAGGCTAAACAAAA-3′) using T4 DNA ligase. To select fragments containing microsatellites, ligation products were hybridized with a 5′-biotinylated repeat oligonucleotide probe $(GA)_{12}$ at 60°C overnight. Hybridization products were captured with streptavidin-coated magnetic beads (Promega Corporation, Madison, Wisconsin, USA) and recovered by PCR using the linker forward primer (5′-GTTTAGCCTTGTAGCAGAAGC-3′). The PCR products were purified using Wizard SV Gel and PCR Clean-Up System (Promega Corporation), and then the 3′ end of the PCR products was adenylated. The adenylated PCR products were ligated to pGEM-T Easy Vector (Promega Corporation) and then transformed into competent *Escherichia coli* TOP10 cells (Biomed Tech, Beijing, China). A total of 384 positive clones were selected and tested by PCR using vector primers T3/T7 and primer $(AC)_{12}$. In total, 166 clones with positive inserts were sequenced with an ABI PRISM 3730xl DNA sequencer (Applied Biosystems, Foster City, California, USA).

A total of 144 clones contained simple sequence repeat (SSR) loci, of which 124 were suitable for primer design using Primer3 (version 0.40; Rozen and Skaletsky, 2000). The primer length was set to range from 18 to 23 bp, the annealing temperature (T_a) ranged from 55°C to 63°C, amplification product size ranged from 100 to 300 bp, and GC content ranged from 20–80%. The forward primer of each pair was tagged with an M13-forward tag (5′-TGTAAAACGACGGCCAGT-3′). A third primer (M13F), labeled with a fluorescent molecule (FAM, HEX, ROX, TAMRA), was involved in PCR reactions. These primers were initially screened in eight *P. sibirica* individuals randomly selected from eight wild populations in northern Hebei Province (Appendix 1). The PCR reactions were performed in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems) in a 10-µL reaction volume that contained 1–10 ng genomic DNA, 5 µL of 2× *Taq* PCR mix (Biomed Tech), 0.08 µM of the forward primer, and 0.32 µM of each reverse and fluorescent-labeled M13F primer. Conditions of the PCR amplification were as follows: 94°C for 5 min; 30 cycles

TABLE 1. Characteristics of 19 microsatellite loci developed in *Prunus sibirica.*

Locus	Primer sequences (5′–3′)	Repeat motif	Size (bp)	T_a (°C)	GenBank accession no.
PSL1	F: GTGTGTTGGAGCCTTCCAGT R: TCCTTCGTTTCCTTTTGTGC	$(AG)_7$	173	55	JQ411730
PSL2	F: TGGGGTTCTCTCTTTTCAGG R: GCTCACCCATTCAAGTGGAC	$(AT)_{10}$	153	55	JQ411731
PSL3	F: TCTCTTCTTTCGCTCGCTCT R: GGGTGCCCAGATCAGAAATA	$(TCTTT)_3$	200	55	JQ411732
PSL4	F: AATGTCTGCCCCATTGAAAC R: AGTTTTCTGCGGATTCTTGC	$(AG)_8$	188	55	JQ411733
PSL5	F: TGCAATTGGACGACATTGAC R: TTGCCAGACCCCTATTTGTG	$(CT)_{10}$	248	55	JQ411734
PSL6	F: GTTCAAATGGTCCTCGCATT R: TCTTGTGTTATCCGTCCGTTT	$(GA)_{13}$	180	55	JQ411735
PSL7	F: TTTGGAGGTGGAAGAGGATG R: CTTTGGCCCTCACAACAAGT	$(AG)_{12}$	151	55	JQ411736
PSL8	F: AAGCAGGCTCTAACCAAGCA R: TCCTTTAGTGGCACCCAGAC	$(AG)_9$	218	55	JQ411737
PSL9	F: AATAGTGGTGGGCACAGAGG R: TGTGTGTTGTGTTTTGTGTTTTC	$(CT)_{15}$	167	55	JQ411738
PSL10	F: CCCATGGTATAGAGGATCCAGA R: TGGCGAGAAGAAACCCTAGA	$(TC)_{13}$	295	55	JQ411739
PSL11	F: CCATAACCAAAATGGAGAGCTT R: CCTTGCATGACTTTCCCTTC	$(GA)_6$	221	55	JQ411740
PSL12	F: CACCCCCAACATACCACTTC R: GTGCTGCAAAAGCAAAAACA	$(TC)_7$	143	55	JQ411741
PSL13	F: TTCGTCAGTTGGCTTCTCCT R: CAGTCAAGAGCAGCTGCAAG	$(CT)_{12}$	293	55	JQ411742
PSL14	F: AACCCACATTCCCATGCTTA R: GCATCCGAGTTTGAGAGACG	$(GT)_7$	207	55	JQ411743
PSL15	F: TTGAACTGATCCTCCCCAAG R: TCCCTTTCTTTTTCCCTTCA	$(AG)_{19}$	119	55	JQ411744
PSL16	F: TCGATCAATCAAGGGCAACT R: AACGTATGCCATCGTACCG	$(GA)_{10}$	167	55	JQ411745
PSL17	F: ATGGGATCCCTCTGAGTCCT R: GCTGCTCTTGCCAAATTCA	$(AG)_6$	227	55	JQ411746
PSL18	F: GCCTTAGCTTTCTCTTCTTGGA R: CCCTGTATAAACATCCCCCTTA	$(GA)_{21}$	150	55	JQ411747
PSL19	F: TTCTTAGTAGGGGAGGGAGGA R: CCCCACATAGTTGATAGCAGACA	$(AG)_{10}$	105	55	JQ411748

Note: T_a = annealing temperature.

TABLE 2. Variability of 19 SSR loci in three populations of *Prunus sibirica* and six individuals of *P. armeniaca.*

	Pop. 1 (N = 12)			Pop. 2 (N = 12)			Pop. 3 (N = 16)			*P. armeniaca* (N = 6)		
Locus	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e
PSL1	4	0.750	0.601	5	0.583	0.646	5	0.563	0.609	3	0.667	0.611
PSL2	4	0.667	0.698	3	0.500	0.517	4	0.500	0.518	3	0.667	0.653
PSL3	5	0.583	0.517	4	0.583	0.469	4	0.438	0.363	3	0.667	0.611
PSL4	9	0.750	0.830	7	0.545	0.793	6	0.750	0.777	5	0.667	0.611
PSL5	10	0.583	0.813	6	0.364	0.764	9	0.688	0.773	6	0.667	0.792
PSL6	8	0.500	0.747	8	0.500	0.823	9	0.800	0.824	5	0.000	0.778
PSL7	10	0.750	0.844	10	0.833	0.865	9	0.563	0.777	3	0.667	0.611
PSL8	4	0.727	0.682	4	0.750	0.552	3	0.563	0.541	2	0.333	0.444
PSL9	9	0.636	0.798	7	0.750	0.806	8	0.750	0.805	4	0.833	0.694
PSL10	7	0.833	0.757	4	0.583	0.510	4	0.563	0.662	4	0.667	0.736
PSL11	8	0.833	0.826	11	0.833	0.833	6	0.625	0.768	3	0.667	0.500
PSL12	11	0.667	0.802	6	0.833	0.764	7	0.750	0.777	6	0.667	0.806
PSL13	5	0.417	0.472	6	0.583	0.726	7	0.688	0.730	5	0.833	0.764
PSL14	4	0.333	0.295	5	0.833	0.674	6	0.563	0.570	4	0.833	0.583
PSL15	10	0.909	0.876	10	0.727	0.847	9	0.813	0.832	6	0.833	0.778
PSL16	9	0.250	0.795	8	0.364	0.826	4	0.063	0.408	3	0.000	0.611
PSL17	6	0.417	0.809	4	0.727	0.694	4	0.688	0.588	3	1.000	0.611
PSL18	10	0.917	0.847	7	0.500	0.771	9	0.625	0.855	8	0.833	0.861
PSL19	5	0.833	0.601	3	0.417	0.542	3	0.533	0.504	3	0.500	0.403

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

at 94°C for 30 s, 55°C for 40 s, and 72°C for 45 s; followed by eight cycles at 94°C for 30 s, 53°C for 40 s, and 72°C for 45 s; and a final extension at 72°C for 10 min. PCR products were genotyped using an ABI 3730xl DNA Analyzer with GeneScan-500LIZ size standard (Applied Biosystems) and GeneMarker software (SoftGenetics, State College, Pennsylvania, USA). A total of 52 primers successfully amplified products with expected size and simple banding patterns. These primers were screened further for polymorphism and transferability using 40 individuals of *P. sibirica* from three wild populations (Appendix 1) and six individuals of *P. armeniaca* L. (Appendix 2). Finally, 19 of 52 primers successfully amplified in all individuals of *P. armeniaca* and revealed high levels of polymorphism (Table 1). Using the software GenAlEx version 6.4 (Peakall and Smouse, 2006), we found the number of alleles per locus varied from three to 11 in three *P. sibirica* wild populations and from two to eight in *P. armeniaca* individuals. The observed and expected heterozygosities ranged from 0.063 to 0.917 and 0.295 to 0.876, respectively, in three *P. sibirica* wild populations, and from 0 to 1 and 0.403 to 0.861 in *P. armeniaca* (Table 2).

CONCLUSIONS

We reported the development of 19 genomic SSR markers from enriched genomic SSR libraries in *P. sibirica*, providing valuable tools for genetic studies in *P. sibirica* and related species, such as population genetics, germplasm identification, and marker-assisted selection.

Appendix 1. Geographic localities of samples of *Prunus sibirica* used in this study.

Code	Locality	Sample sizes	Geographic coordinates
S01	Yanqing, Beijing, China	1	40°21′N, 116°00′E
S02	Chicheng, Hebei, China	1	41°08′N, 115°54′E
S03	Chongli, Hebei, China	1	41°09′N, 115°06′E
S04	Fengning, Hebei, China	1	41°23′N, 117°06′E
S05	Luanping, Hebei, China	1	40°52′N, 117°37′E
S06	Pingquan, Hebei, China	1	41°19′N, 118°47′E
S07	Pingquan, Hebei, China	1	41°16′N, 118°58′E
S08	Weichang, Hebei, China	1	42°01′N, 118°01′E
Pop. 1	Pingquan, Hebei, China	12	41°16′N, 118°58′E
Pop. 2	Chifeng, Neimenggu, China	12	41°53′N, 120°16′E
Pop. 3	Weichang, Hebei, China	16	42°01′N, 118°01′E

Appendix 2. Samples of *Prunus armeniaca* used in this study. The samples are deposited at the Institute of Forestry and Pomology, Beijing Academy of Agriculture and Forestry Science.

Code	Cultivar name	Original code	Origin
A01	Longwangmao	6-2	Mentougou, Beijing
A02	Yiwofeng	18-8	Zhulu, Hebei
A03	Shushanggan	5-1	Yili, Xinjiang
A04	Chuanzhihong	8-10	Julu, Hebei
A05	Luotuohuang	4-11	Mentougou,Beijing
A06	Akeqiaoerpang	12-1	Hetian, Xinjiang

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