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DEVELOPMENT AND CHARACTERIZATION OF MICROSATELLITE MARKERS IN _PRUNUS SIBIRICA_ (ROSACEAE)¹

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• 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.

_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, _AluI_ and _HaeIII_ (New England Biolabs, Ipswich, Massachusetts, USA), and then ligated to a double-stranded linker (F: 5'-GTGGTAGCGCTGGTACGAA-3'; R: 5'-GCGTTGCCTGCAACAGGCTGTTACAAA-3') using T4 DNA ligase. To select fragments containing microsatellites, ligation products were hybridized with a 5'-biotinylated repeat oligonucleotide probe (GA)₅ 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'-GTGGTAGCCTGGTACGAAAGC-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). 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 Skaltsky, 2000). The primer length was set to range from 18 to 23 bp, the annealing temperature (Tₘ) 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′-TGT AA AA CG-3′) and the reverse with an M13-reverse tag (5′-TGT TTA TAC AAC-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...

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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: GTGGTGGAGGCCTTCAGTG | (AG)_7       | 173       | 55       | JQ411730             |
|       | R: GTGCTTTTCCTTTTGCT    | (CT)_5       | 153       | 55       | JQ411731             |
| PSL2  | F: TGGGGTTCTCTCTTTTCT    | (AT)_10      | 188       | 55       | JQ411733             |
|       | R: AGTCTCTGCGGATTCTTGGC | (CT)_10      | 248       | 55       | JQ411734             |
| PSL3  | F: TCTCTCTTTTGCTGGTCTTT | (TCTTT)_3    | 200       | 55       | JQ411732             |
|       | R: GGTGCCCAAGATCACGAAAATA | (GA)_13   | 180       | 55       | JQ411735             |
| PSL4  | F: AAGTCTCGCCCACTTAGAAC | (AG)_8       | 218       | 55       | JQ411737             |
|       | R: TGGCAGACCCCTAATTGTG | (CTT)_13     | 180       | 55       | JQ411738             |
| PSL5  | F: TGCAATTGGACGACATTGAC | (CT)_10      | 248       | 55       | JQ411734             |
|       | R: TTGCCAGACCCCTAATTGTG | (CTT)_13     | 180       | 55       | JQ411735             |
| PSL6  | F: GTTCAAATGGTCCTCGCATT | (GA)_13     | 295       | 55       | JQ411736             |
|       | R: CTTTGGCCCTCACAACAAAGT | (GA)_13   | 151       | 55       | JQ411736             |
| PSL7  | F: TTTGAGGAGGAAGGATGAGT | (AG)_9       | 218       | 55       | JQ411737             |
|       | R: CTTGGCCCTCACAACAAAGT | (GA)_13     | 151       | 55       | JQ411736             |
| PSL8  | F: AAGCAGGCTCTTCAACAGCAG | (AG)_9     | 218       | 55       | JQ411737             |
|       | R: TGGCAGACCCCTAATTGTG | (CTT)_13    | 180       | 55       | JQ411735             |
| PSL9  | F: AATAGTGGTGGGCACAGAGG | (CTT)_12    | 255       | 55       | JQ411740             |
|       | R: TGGCAGACCCCTAATTGTG | (CTT)_13    | 255       | 55       | JQ411741             |

Note: T_a = annealing temperature.

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

| Locus | Pop. 1 (N = 12) | Pop. 2 (N = 12) | Pop. 3 (N = 16) | *P. armeniaca* (N = 6) |
|-------|-----------------|-----------------|-----------------|-------------------------|
|       | 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 | 0.409 | 4 | 0.438 | 0.363 | 3 | 0.667 | 0.611 |
| PSL4  | 9 | 0.750 | 0.830 | 7 | 0.545 | 0.733 | 6 | 0.750 | 0.777 | 5 | 0.667 | 0.611 |
| PSL5  | 10 | 0.583 | 0.813 | 6 | 0.264 | 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 | 6 | 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_o = observed heterozygosity; H_e = expected heterozygosity; N = sample size for each population.
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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 GenAIEx 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          | Jiu, Hebei |
| A05  | Luotuohuang   | 4-11          | Mentougou, Beijing |
| A06  | Akeqiaoerpaing | 12-1          | Hetian, Xinjiang |

LITERATURE CITED

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of leaf tissue. Phytocenology Bulletin 19: 11–15.

NUNOME, T., S. NEGORO, K. MIYATAKE, H. YAMAGUCHI, AND H. FUKUOKA. 2006. A protocol for the construction of microsatellite enriched genomic library. Plant Molecular Biology Reporter 24: 305–312.

PEAKALL, R., AND P. E. SMOURSE. 2006. GenAIEx 6: Genetic analysis in Excel. Population genetics software for teaching and research. Molecular Ecology Notes 6: 288–295.

ROZEN, S., AND H. SKELETKEY. 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.

TAUTZ, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Research 17: 6463–6471.

ZHANG, J. Y., AND Y. HE. 2007. Development of apricot industry belt in northern China. Northern Fruits 1: 33–35 (in Chinese with English abstract).

ZHANG, J. Y., AND Z. ZHANG [eds.]. 2003. Chinese fruit tree: Apricot. China Forestry Press, Beijing, China.

http://www.bioone.org/loi/apps