Applications in Plant Sciences

PRIMER NOTE

Isolation and characterization of microsatellite loci for *Prunus mongolica* (Rosaceae)

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PREMISE OF THE STUDY: Microsatellite primers were developed in *Prunus mongolica* (Rosaceae), a relict flora endemic in arid areas of the Asian interior, to investigate the genetic diversity, phylogeography, population structure, and history of the species.

METHODS AND RESULTS: Fifty-one microsatellite loci, including di-, tri-, and tetranucelotide repeats, were identified using transcriptome sequencing and bioinformatic screening. The number of alleles ranged from seven to 11 and the levels of observed and expected heterozygosity ranged from 0.545 to 1.000 and 0.600 to 0.989, respectively. Most of the primers also amplified in a group of congeneric species (*P. triloba*, *P. davidiana*, *P. persica*, *P. cerasifera*, and *P. serrulata*).

CONCLUSIONS: This set of microsatellite loci is useful for studying the genetic diversity of *P. mongolica*. In addition, they can also be used to investigate the population structure, phylogeography, and landscape genetic patterns of congeneric species.

KEY WORDS genetic diversity; microsatellite; polymorphism; *Prunus mongolica*; Rosaceae.

Prunus mongolica Maxim. (Rosaceae) is a relict flora that is endemic to arid areas of the Asian interior. *Prunus mongolica* was listed in the China Plant Red Data Book as endangered; this has resulted from mining, firewood collection, industrialization, and urbanization, which have led to habitat loss and fragmentation (Fu, 1992). Scientific conservation requires a full understanding of genetic diversity, phylogeny, and population structure. Genetic studies are limited in *P. mongolica*; therefore, it is necessary to develop microsatellite loci for use in the species.

Microsatellites, also known as simple sequence repeats (SSRs), have been widely used as DNA markers in studies of population genetics, phylogeography, landscape genetics, and parentage analysis due to their high level of polymorphism, putative neutrality, codominant inheritance, and ease of scoring (Moriguchi et al., 2015). In this study, we isolated and characterized 51 polymorphic microsatellite loci for *P. mongolica* by transcriptome sequencing and bioinformatic screening.

METHODS AND RESULTS

Total RNA was extracted from fresh leaves of one individual of *P. mongolica* using the EasyPure Plant RNA Kit (TransGen Biotech Inc., Beijing, China) following the manufacturer's protocol. A cDNA library was prepared using the TruSeq Stranded Total RNA Sample Prep Kit (Illumina, San Diego, California, USA) and sequenced on a HiSeq 3000 sequencing platform (Illumina).

Extra (adapters and other Illumina-specific sequences) and low-quality sequences were removed from the raw data using the software Trim Galore version 0.4.4 (Li et al., 2015). FastQC version 0.11.5 software (Yang et al., 2013) was used to analyze the quality control of the pre-processed data. A total of 53,866 contigs were obtained by de novo assembly using Trinity version 2.3.2 (Grabherr et al., 2011). SSR detection was performed with MISA version 1.0 (Thiel et al., 2003) with the following criteria: ≥6 repeat units for dinucleotides, ≥5 for tri-, and ≥4 for tetra-, penta-, and hexanucleotides. Primer sets were designed with Primer3 using the default parameters (Koressaar and Remm, 2007; Untergasser et al., 2012). A total of 19,498 SSRs were identified from 15,837 contigs, and 6069 primer sets were designed. Two hundred primer sets that amplified di-, tri-, and tetranucelotide repeats with ≥6, ≥5, and ≥4 repeat units, respectively, were selected to test for polymorphism. Raw transcriptome data were deposited in the National Center for Biotechnology Information Short Read Archive (BioProject no. PRJNA418876, BioSample no. SAMN08038438).

Because of habitat loss, only 32 *P. mongolica* individuals were sampled from three populations (Appendix 1). Total genomic DNA was extracted from 0.15 mg of leaf tissue using a modified cetyltrimethylammonium bromide (CTAB) method (Wang et al., 2010). All PCRs were performed in total volumes of 15 μL: 1 μL

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TABLE 1. Characteristics of 51 microsatellite loci developed in *Prunus mongolica*.

Locus	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)	T_a (°C)	Function	GenBank accession no.
TR3320	F: CGCCTCCTCTCTCTCTCTA R: CCCAGAAAAGATTAAGGCTAC	$(TC)_{19}$	127–175	55	EIN3-binding F-box protein 1	MG682092
TR5622	F: AAACGAGAGCAGGATCAATA R: CGTAGTTTGGGTTGGAGTC	$(CAA)_8$	127–179	55	B3 domain-containing transcription factor	MG682093
TR9296	F: GCAGAGAACTTATCGCTTATG R: CATCTGGAAAGTGAATCTGAG	$(TTTA)_6$	133–201	55	Glyoxylate/hydroxypyruvate reductase HPR3	MG682094
TR10339	F: ACTCTTCTCTCCATTGAGTCC R: GTCCTCATCTGGGAAATTTAG	$(CT)_{20}$	105–141	55	Protein MIZU-KUSSEI 1	MG682095
TR10936	F: AGAAGATAGAGGGATGGGAGT R: CTCTCTGCAACAACCAAAAC	$(AG)_{21}$	153–181	56	GATA transcription factor 11	MG682096
TR12256	F: GAGAGAGAAAAATTGCAGTCC R: CCATGGAGATTAAGAGGAAGA	$(TCT)_7$	131–177	55	Unknown	MG682097
TR12298	F: CAATAGCCAAAACCCTCTC R: GCCTTCCATTTCTCTATGTCT	$(TC)_6$	123–169	54	DNA-directed RNA polymerases II, IV, and V	MG682098
TR12728	F: AAATCTAAGCCCACGTACATC R: CACCCTGGTAACTCATAATCA	$(GA)_{18}$	153–193	56	Protein-tyrosine-phosphatase MKP1	MG682099
TR14394	F: TGAGCTACAGACAGGTGAACT R: ACACAACACACCACTTTTCTC	$(GAA)_7$	143–169	55	Transcription factor bHLH25	MG682100
TR16484	F: GTGTTGGCTAGAGTGACTTTG R: CGCCACACCTAAGTTTTT	$(TC)_8$	157–195	56	UDP-glycosyltransferase 74F2	MG682101
TR17663	F: TCATCTCCTCTACCTTTTTCC R: GGTCATGATCTCTGCTTGATA	$(CT)_{19}$	115–169	55	Unknown	MG682102
TR17745	F: CCTTCCATTTCTCTGTTCTCT R: CACTCAACCAAATCATAGCTC	$(TC)_{21}$	111–149	55	Aquaporin PIP1-2-like	MG682103
TR18640	F: AACTAGAGAGAGAGACGCACA R: GTTCTTTCGCATTCTCAGAC	$(GA)_{16}$	91–139	55	Succinate-CoA ligase [ADP-forming] subunit beta, mitochondrial	MG682104
TR18685	F: CAGAAGTCAGAGCAGAGTCAG R: CTACCGGGAGATATTTCAGAT	$(ACAG)_6$	139–169	55	VQ motif-containing protein 9	MG682105
TR18807	F: GTCCTTAAGCAGCCCAGTA R: AGAGCTTCGATGTCAGAGAC	$(CT)_{20}$	121–173	55	Probable acyl-activating enzyme 17, peroxisomal	MG682106
TR19174	F: CTTGAGAGGAGGAAAAAGAAG R: GCTGTCTGGGGTTATAAATTC	$(AGA)_8$	145–217	55	Transcription factor PRE6-like	MG682107
TR19201	F: TATTGCACTCTCCTATGAACC R: GTTGAGACTCTTGCTGTTGTT	$(CAG)_7$	139–167	55	Auxin response factor 7 (LOC110749255), transcript variant X2	MG682108
TR20374	F: CTTCCATCCACACTTACAT R: GAGGGACTGAATAAACCTGAG	$(GA)_8$	149–207	55	Uncharacterized protein At1g04910	MG682109
TR21328	F: AAGGTGAAGGGTGAAGATACT R: AAACACAATCACAGAGAGAGC	$(GA)_{12}$	141–195	55	Magnesium-chelatase subunit ChlH, chloroplastic	MG682110
TR21918	F: CCTCCTTCTGTCTCTCTCTTC R: CAGTTAAACCACCAGCACTAC	$(CT)_{12}$	125–187	55	E3 ubiquitin-protein ligase RNF144A	MG682111
TR20030	F: ATAACCCCAAGCTCTCTCTC R: AGGAGGTAGGAGATGGAGTG	$(CTC)_8$	131–161	55	*Prunus persica* receptor-like protein 51	MG682112
TR22071	F: CAGAAGAAAAAGAAGGAGAGC R: TGTCAACCATACTCACCTCAT	$(GAA)_7$	131–161	55	*Prunus avium* cysteine proteinase inhibitor 12-like, transcript variant X6	MG682113
TR23600	F: CAGCTCTCTCTTTCTCTCTCC R: ACTTCCACTACCAGAATCCAT	$(TC)_8$	141–183	55	Probable serine/threonine-protein kinase DDB_G0282963	MG682114
TR24122	F: GGTCTGTTAATGGAGGTTCTT R: CACCAATATTGAGAGAGAGAC	$(CT)_{13}$	133–169	56	UDP-*N*-acetylglucosamine–dolichyl-phosphate	MG682115
TR25043	F: AACCAACCCAACCCACAC R: GGGGGAGAACCTATCTTATATT	$(ACAT)_6$	111–165	57	*N*-acetylglucosaminephosphotransferase	MG682116
TR25166	F: CAGGCTCTGTAGTTCGATTC R: TGTATGTTAGTAAGGCGAAGG	$(TCT)_8$	147–179	55	Gibberellin 20 oxidase 1	MG682117
TR25414	F: CAGCAGAGAGACCAGAATAGA R: GAGCATTATAGCTGTGTGTGG	$(TCCA)_6$	103–125	55	Uncharacterized protein At2g17340-like	MG682118

(continues)

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TABLE 1 (Continued)

Locus	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)	T_a (°C)	Function	GenBank accession no.
TR26908	F: TATCTTCATCGCTCTCTTCTG R: CTGGGGTCTTAATTAGACGTT	$(CAT)_{10}$	111–149	55	Galactomannan galactosyltransferase 1	MG682119
TR27527	F: CACCAAACCTTTCTCTCTCTT R: ACAAAGTCGATCAGCATCTC	$(CT)_{27}$	117–165	54	Transcription factor HY5-like, transcript variant X1	MG682120
TR29327	F: CTGCCGTCTATCTCTCTCTCT R: CAAGTTTGGTGCTCGTATCTA	$(CT)_{6}$	113–145	56	Probable fructokinase-4	MG682121
TR30962	F: CTATGGGTCTGGTTATGGAA R: CTCCCAGCCAATATCTCAG	$(GGA)_{11}$	101–161	55	Cytosolic 5′-nucleotidase 3, transcript variant X5	MG682122
TR31156	F: CTCTGCCTCTTTCTCTTCTCT R: ATCCCCCTACTTTCTTGC	$(AG)_{14}$	117–155	55	Putative glycine-rich cell wall	MG682123
TR31399	F: GAAGAACAAGAAGGGCAAG R: CAACACTAACACCACCAGATT	$(AG)_{20}$	117–167	55	Structural protein 1	MG682124
TR31622	F: AAGCCAGAACCCTTATCTCTA R: GCCTGAATTGAGTGAACTGTA	$(AAC)_{5}$	125–179	55	Unknown	MG682125
TR32795	F: CAAGTTGATTTCTTCCTCTCC R: TATAACGCCTCTCTCTGTTGA	$(AGA)_{13}$	119–155	55	Peamaclein-like	MG682126
TR33107	F: AAAACAGAGAGGACGATGG R: GCTCTAGGTGTTGGGTTAAAT	$(TTGT)_{6}$	139–171	55	BEL1-like homeodomain protein 9, transcript variant X1	MG682127
TR35128	F: GCCTGAATTGAGTGAACTGTA R: AAGCCAGAACCCTTATCTCTA	$(TTG)_{8}$	129–181	56	Glutamine–fructose-6-phosphateaminotransferase [isomerizing] 2	MG682128
TR35536	F: CTCTTGTGTTCATCGGCTAC R: ATCTCACCCGTCTCTTTATTC	$(TGA)_{6}$	153–175	55	Calcium-dependent protein kinase 13	MG682129
TR37605	F: CTTTCTAACCCCTCTGTGTCT R: TATGAACAGCTTACCTTCGTC	$(TC)_{21}$	117–153	55	BEL1-like homeodomain protein 9, transcript variant X2	MG682130
TR37669	F: CCTCTTTGATCTCCTTCCATA R: GAAGACGACCTTTTTCATACC	$(TCT)_{8}$	141–181	55	Glutamate synthase [NADH], amyloplastic, transcript variant X3	MG682131
TR37811	F: GATCAAGGAGGAAGACAAAGT R: GCAGAGAGAGAGAAAGAGGAA	$(AG)_{26}$	105–145	55	Heterogeneous nuclear ribonucleoprotein 1	MG682132
TR38169	F: GATCATGAAGTTGCAGAAGAG R: ATGTGCCCTTCCTGTGAG	$(TGG)_{10}$	143–185	56	Probable WRKY transcription factor 12	MG682133
TR38361	F: GGATGTGCTTTACTGGAGAA R: CTCCTTCTAATCCCTTCAGC	$(TGA)_{8}$	127–155	56	Probable phospholipid-transporting ATPase	MG682134
TR41171	F: TAGAAACTATGTGCGTGTGTG R: CTTCAGGGAGGCTATAAATTC	$(GA)_{15}$	123–175	55	4, transcript variant X2	MG682135
TR42254	F: ATTGCTTGCTCTGTCTCTGTA R: CTTATTACCCACTACCCCAAC	$(AAG)_{5}$	141–181	55	Homeobox-leucine zipper protein HOX11-like, transcript variant X2	MG682136
TR42785	F: TCTCTGAAATTCTCTCTGCTC R: TCTTTCTTCCTCCTCTCTCAC	$(GTGC)_{6}$	117–147	55	Formin-like protein 4	MG682137
TR46482	F: CCACCTTCTTATAGCGACTG R: AAGTACGATACAAGCACTCCA	$(TATG)_{6}$	133–193	55	Probable E3 ubiquitin-protein ligase RZFP34, transcript variant X4	MG682138
TR47534	F: CGATCTCCTTCCAGACCT R: CGACATCCTCAAGAACATCTA	$(GTG)_{6}$	119–157	55	Auxin efflux carrier component 1	MG682139
TR47552	F: ACAATCTCTTCGTCTCTGGTT R: GCTCCAGTGTACGTTGTAAG	$(AG)_{19}$	121–177	54	UDP-glycosyltransferase 76E2-like, transcript variant X2	MG682140
TR50321	F: AAGACCACTCACAACTCCAC R: CTTTGAAACACTGTTCTGGTC	$(CTT)_{8}$	263–293	55	Cyclic nucleotide-gated ion channel 1-like, transcript variant X1	MG682141
TR52495	F: TGATGAGCTTGCCATTCT R: CTTCTTGTCCGCTAATTCAC	$(GAA)_{8}$	147–173	55	G-box-binding factor 4, mRNA	MG682142

Note: T_a = annealing temperature.

of DNA, 5.5 μL of sterilized deionized water, 7.5 μL of 2× EasyTaq PCR Supermix (TransGen Biotech Inc.), and 0.5 μL each of forward and reverse primers (each forward primer was fluorescently labeled with FAM, HEX, or TAMRA). PCR amplification was carried out using the following thermal conditions: initial denaturation for 5 min at 94°C; then 35 cycles of denaturation for 30 s at 94°C, annealing for 30 s at the annealing temperature (Table 1), and 20 s at 72°C; followed by a final extension for 10 min at 72°C. PCR products were genotyped on an ABI PRISM 3730 genetic analyzer (Applied Biosystems, Waltham, Massachusetts, USA) with a GeneScan 500

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TABLE 2. Genetic diversity of 51 microsatellite loci in three populations of *Prunus mongolica*.[a]

	Wulanchabu population (n = 10)			Chifeng population (n = 10)			Eerduosi population (n = 12)		
Locus	A_e	H_o	H_e	A_e	H_o	H_e	A_e	H_o	H_e
TR3320	8.500	0.889	0.922	8.500	0.889	0.869	9.000	1.000	0.925
TR5622	11.000	1.000	0.928	10.500	1.000	0.963	9.500	0.833	0.880
TR9296	7.000	1.000	0.967	8.000	1.000	0.970	7.000	1.000	0.925
TR10339	11.000	1.000	0.900	11.000	1.000	0.832	10.000	1.000	0.935
TR10936	10.500	1.000	0.922	10.000	1.000	0.889	9.500	0.909	0.926
TR12256	10.000	0.889	0.837	9.500	0.800	0.600	9.500	0.800	0.847
TR12298	11.000	1.000	0.941	10.500	1.000	0.858	9.500	1.000	0.906
TR12728	10.000	1.000	0.948	10.000	1.000	0.954	9.000	1.000	0.874
TR14394	11.000	0.778	0.895	10.500	0.900	0.758	9.500	0.750	0.699
TR16484	10.500	1.000	0.908	10.000	1.000	0.921	9.500	1.000	0.909
TR17663	10.500	1.000	0.980	10.500	1.000	0.961	9.000	1.000	0.960
TR17745	11.000	1.000	0.974	10.500	1.000	0.953	9.500	1.000	0.928
TR18640	10.500	1.000	0.989	10.500	1.000	0.953	10.000	1.000	0.939
TR18685	11.000	0.875	0.908	10.000	0.800	0.858	9.000	0.833	0.790
TR18807	9.500	1.000	0.942	10.500	1.000	0.967	9.000	1.000	0.939
TR19174	11.000	0.889	0.810	10.500	1.000	0.853	9.500	1.000	0.938
TR19201	11.000	1.000	0.858	11.000	0.900	0.884	10.000	0.917	0.888
TR20030	11.000	1.000	0.941	10.500	1.000	0.953	9.500	0.917	0.928
TR20374	10.500	1.000	0.974	10.000	1.000	0.963	9.500	1.000	0.952
TR21328	11.000	1.000	0.963	11.000	1.000	0.958	10.000	1.000	0.960
TR21918	11.000	1.000	0.954	10.500	1.000	0.921	9.500	1.000	0.960
TR22071	11.000	0.889	0.817	10.500	0.900	0.942	9.500	0.750	0.928
TR23600	10.500	1.000	0.908	10.500	1.000	0.935	9.000	1.000	0.949
TR24122	9.000	1.000	0.922	8.500	0.900	0.937	9.500	1.000	0.925
TR25043	10.500	0.778	0.882	10.000	0.600	0.705	9.500	0.727	0.870
TR25166	11.000	1.000	0.915	10.500	1.000	0.963	9.500	1.000	0.920
TR25414	10.500	1.000	0.733	9.500	1.000	0.832	9.000	0.889	0.771
TR26908	10.000	0.900	0.884	10.500	0.889	0.791	9.500	0.909	0.905
TR27527	11.000	0.900	0.863	11.000	0.900	0.837	10.000	0.833	0.920
TR29327	10.000	1.000	0.928	10.000	1.000	0.876	9.000	1.000	0.952
TR30962	9.500	1.000	0.867	9.000	1.000	0.935	8.500	1.000	0.932
TR31156	10.000	0.889	0.974	10.000	1.000	0.915	9.000	1.000	0.931
TR31399	10.500	1.000	0.961	10.000	1.000	0.979	9.500	1.000	0.935
TR31622	11.000	0.800	0.884	11.000	1.000	0.916	10.000	0.833	0.851
TR32795	11.000	0.900	0.921	11.000	1.000	0.926	10.000	0.917	0.891
TR33107	11.000	0.900	0.911	11.000	0.900	0.874	10.000	0.833	0.909
TR35128	11.000	0.778	0.817	10.500	1.000	0.900	9.500	0.750	0.855
TR35536	10.500	0.778	0.882	10.000	0.900	0.837	9.500	0.909	0.900
TR37605	11.000	0.900	0.926	11.000	1.000	0.895	10.000	1.000	0.935
TR37669	10.500	0.889	0.869	10.500	0.889	0.889	9.000	1.000	0.899
TR37811	10.500	1.000	0.935	10.500	1.000	0.941	9.000	1.000	0.957
TR38169	10.500	1.000	0.941	10.500	1.000	0.961	9.000	0.917	0.935
TR38361	10.000	1.000	0.858	10.500	1.000	0.778	9.500	1.000	0.840
TR41171	10.500	1.000	0.967	10.000	1.000	0.980	8.500	1.000	0.931
TR42254	11.000	0.900	0.921	11.000	0.800	0.921	10.000	0.583	0.851
TR42785	10.000	0.750	0.833	10.000	0.875	0.867	8.000	0.833	0.866
TR46482	9.500	1.000	0.941	10.500	1.000	0.923	8.000	0.833	0.848
TR47534	9.000	1.000	0.817	8.500	0.778	0.680	8.500	0.556	0.804
TR47552	10.000	1.000	0.967	10.000	1.000	0.941	9.000	1.000	0.948
TR50321	9.000	0.800	0.874	10.500	0.857	0.868	8.500	0.545	0.827
TR52495	9.500	0.556	0.843	9.500	1.000	0.902	9.000	0.800	0.805

Note: A_e = number of effective alleles; H_e = expected heterozygosity; H_o = observed heterozygosity.
[a]Locality and voucher information are provided in Appendix 1.

size standard (Applied Biosystems). Allele peaks were scored using GeneMarker version 1.3 (SoftGenetics, State College, Pennsylvania, USA). MICRO-CHECKER version 2.2.3 (van Oosterhout et al., 2004) was used to detect genotyping errors due to null alleles, stuttering, or allele dropout. The number of effective alleles and levels of observed and expected heterozygosity were calculated using GENETIX version 4.0 (Belkhir et al., 2001). Deviation from Hardy–Weinberg equilibrium and linkage disequilibrium was tested with GENEPOP version 3.4 (Rousset, 2008).

A total of 51 primer sets produced consistent amplifications in all samples and were polymorphic (Table 1). These 51 loci had high numbers of effective alleles and high levels of observed and

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TABLE 3. Cross-amplification results for the 51 microsatellite loci developed in *Prunus mongolica* in five *Prunus* species.[a,b]

Locus	*P. triloba*	*P. davidiana*	*P. persica*	*P. cerasifera*	*P. serrulata*
TR10339	—	163–195	115–147	133–171	117–151
TR10936	—	137–149	119–146	125–167	149–161
TR12298	—	103–115	—	132–144	105–121
TR12728	—	110–122	105–117	105–127	120–132
TR16484	—	153–165	143–155	133–145	113–125
TR17663	—	131–140	130–142	—	120–129
TR17745	120–126	110–122	123–135	133–145	125–137
TR18640	135–147	153–159	133–145	131–143	—
TR18807	140–149	143–155	133–145	113–119	131–143
TR20374	115–127	151–163	157–169	153–165	151–159
TR21328	111–131	115–136	113–125	117–129	—
TR21918	101–121	110–130	115–135	118–138	111–123
TR23600	—	—	80–100	91–101	—
TR24122	—	—	130–150	139–159	133–153
TR3320	—	—	—	121–133	—
TR27527	140–149	145–160	132–144	141–153	135–147
TR29327	133–145	135–147	130–142	132–138	—
TR31156	—	—	—	—	141–161
TR31399	131–151	111–131	143–163	141–161	—
TR37605	125–145	126–146	121–141	122–142	123–143
TR37811	131–161	121–151	111–141	146–176	132–162
TR41171	131–191	112–142	123–153	119–149	145–175
TR47552	121–141	122–142	111–131	123–143	101–131
TR26908	113–129	133–149	113–125	115–127	131–143
TR30962	111–127	131–143	127–139	123–139	135–147
TR32795	125–137	123–126	147–159	131–161	126–138
TR19174	103–123	121–133	116–128	113–129	132–144
TR19201	142–154	131–161	125–137	126–138	141–171
TR20030	115–135	117–139	123–135	134–146	137–149
TR25166	113–125	132–144	115–135	162–182	133–145
TR31622	151–163	131–143	133–163	142–154	125–137
TR35128	117–129	146–158	135–155	132–144	172–184
TR35536	137–157	117–129	153–165	135–155	127–167
TR37669	123–138	121–133	125–137	122–134	115–127
TR38169	—	—	125–137	—	—
TR38361	133–149	131–147	115–127	124–136	125–137
TR42254	122–134	117–129	115–127	123–135	116–128
TR47534	123–135	115–127	132–144	124–136	125–137
TR52495	117–129	112–124	113–125	117–129	123–135
TR5622	121–151	124–136	142–154	141–153	123–135
TR12256	—	103–115	105–117	113–133	132–152
TR14394	143–155	124–136	121–130	123–135	141–153
TR22071	122–155	114–126	142–154	121–133	113–125
TR50321	—	—	—	123–143	114–124
TR18685	113–125	121–133	135–147	123–135	141–153
TR25043	—	117–137	132–144	124–136	—
TR25414	133–145	123–139	121–133	142–158	152–164
TR33107	121–133	143–155	117–139	—	113–128
TR42785	—	—	—	—	—
TR46482	253–265	221–236	232–244	—	215–227
TR9296	—	—	141–156	122–134	—

Note: — = unsuccessful amplification.
[a]Locality and voucher information are provided in Appendix 1.
[b]$N = 5$ for all populations.

expected heterozygosity, ranging from seven to 11, 0.545 to 1.000, and 0.600 to 0.989, respectively (Table 2). Cross-amplification of these 51 primer sets was tested for in *P. triloba* Lindl., *P. davidiana* (Carrière) Franch., *P. persica* (L.) Batsch, *P. cerasifera* Ehrh., and *P. serrulata* Lindl.; most primers amplified in these five species except *P. triloba* (*P. triloba*: 35/51; *P. davidiana*: 43/51; *P. persica*: 46/51; *P. cerasifera*: 45/51; *P. serrulata*: 41/51; Table 3).

CONCLUSIONS

In our study, a total of 19,498 SSRs were identified from 15,837 contigs, and 6069 microsatellite primers were designed. Among the identified SSR loci, 51 microsatellite primer pairs were polymorphic. The 51 novel microsatellite markers can be used to study the genetic diversity, landscape genetics, and phylogeography of the

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species. The microsatellite primers can also be used to genotype congeneric species (*P. triloba*, *P. davidiana*, *P. persica*, *P. cerasifera*, and *P. serrulata*).

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APPENDIX 1. Voucher information for *Prunus* species used in this study.

Species	*n*	Collection locality	Geographic coordinates	Voucher ID[a]	Collector
Prunus mongolica Maxim.	10	Wulanchabu, Inner Mongolia, China	41°56′N, 110°28′E	201607-Pmon-popI-AHB	Zhanyuan Lu
	10	Chifeng, Inner Mongolia, China	43°23′N, 118°40′E	201607-Pmon-popII-AHB	Zhanyuan Lu
	12	Eerduosi, Inner Mongolia, China	39°35′N, 106°46′E	201607- Pmon-popIII-AHB	Zhanyuan Lu
P. triloba Lindl.	5	Hefei, Anhui, China	31°52′N, 117°11′E	201705-Ptri-AHB	Xin Hong
P. davidiana (Carrière) Franch.	5	Hefei, Anhui, China	31°52′N, 117°11′E	201705-Pdav-AHB	Xin Hong
P. persica (L.) Batsch	5	Wuhan, Hubei, China	30°32′N, 114°24′E	201705-Pper-AHB	Xin Hong
P. cerasifera Ehrh.	5	Hefei, Anhui, China	31°52′N, 117°11′E	201705-Pcer-AHB	Xin Hong
P. serrulata Lindl.	5	Hefei, Anhui, China	31°52′N, 117°11′E	201705-Pser-AHB	Xin Hong

Note: n = sample size.
[a]Vouchers are deposited at the herbarium of Anhui University (ANU), Hefei, Anhui Province, China.

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