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TECHNICAL NOTE

Development and characterization of microsatellite loci for *Rosa odorata* var. *gigantea* Rehder & E. H. Wilson (Rosaceae)

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Abstract Eighteen microsatellite markers were developed from *Rosa odorata* var. *gigantea* (Rosaceae), including 11 new microsatellite markers and 7 modified microsatellite loci having been developed from other *Rosa* species. About 27 wild individuals from 3 populations were used to screen polymorphism of these 18 microsatellite makers. The average allele number of these markers was 3.9 per locus, ranging from 2 to 9. The expected and observed heterozygosities varied from 0.2711 to 0.8043 and from 0.0370 to 0.5556, respectively. Cross-species amplification in other eight *Rosa* species showed their potential use for congeneric species. These microsatellite primers will be used for population genetics studies, constructing genetic linkage maps or locating quantitative trait locus (QTL) of *R. odorata* var. *gigantea* and related species.

Keywords *Rosa odorata* var. *gigantea* · Microsatellite · Population genetics

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Rosa odorata var. *gigantea* is one of the seven species contributing to the modern commercial roses (Wylie 1954), for its special fragrance, giant growth, nearly evergreen foliage and large solitary flowers. It is mainly distributed in Yunnan province of China, and is also found in Myanmar, North Thailand, North Vietnam (Ku and Robertson 2003). This species grows on open slopes, roadsides at altitudes of 1,400–2,700 m (Ku and Robertson 2003). Land reclamation and medical excess usage resulted in a rapid decline of population size, and wild populations are extinct at many original distributed areas (by author's field inspection). It has been listed as a species of concern in Yunnan province and a conservative species of China. The population genetics information will be very useful for designing conservation and management strategies for this important wild ancestor of modern roses. However, no microsatellite primers for *R. odorata* var. *gigantea* has been reported. In this study, we developed 18 microsatellite markers from *R. odorata* var. *gigantea*, including 11 new markers and 7 modified loci having been developed from other congeneric species.

Genomic DNA samples were extracted using cetyltrimethylammonium bromide (CTAB) extraction method of Doyle and Doyle (1987). The isolation of microsatellite loci was performed according to the fast isolation by AFLP of sequences containing repeats (FIASCO) (Zane et al. 2002). Briefly, total genomic DNA were digested with a restriction enzyme-MseI (NEB), and then ligated to an MseI AFLP adaptor. The ligation products were pre-amplified with MseI-N primers (5′-GAT GAG TCC TGA GTA AN-3′) and were hybridized with 5′-biotinylated $(AC)_{15}$, $(AG)_{15}$ and $(AAG)_{10}$ probes in a 250 µl solution (SSC 4.2X and SDS 0.07%) at 48°C for 2 h. DNA having hybridized with probes was captured by biotin–streptavidin (Promega). After amplifying with MseI-N primers again,

PCR products were purified using PCR products purifying Kit (Sangon), then ligated into the PGEM®-T vector (Promega) according to the manufacturer's instructions and propagated in *E. coli*, DH5α strain (Tiangen). Only those clones with its size from 200 to 800 bp were selected and tested using $(AG)_{10}$/$(AC)_{10}$/$(AAG)_7$ and T7/SP6 as primers, respectively. Totally, 100 clones were sequenced with an ABI PRISM 3730XL sequencer. The repeats were screened and counted with software Tandem Repeats Finder 4.0(Gary 1999). About 77 sequences (77%) were found to contain microsatellite. Finally, 27 locus-specific primers were designed using Primer Premier 5.0 (Clarke and Gorley 2001). Furthermore, 73 previously published microsatellite primer pairs from other *Rosa* species (Zhang et al. 2006; Kimura et al. 2006; Hibrand-Saint Oyant et al. 2007) were also tested by the following procedure.

Table 1 Microsatellite loci developed from *Rosa odorata* var. *gigantea*: locus name, repeat motif, primers' sequence, PCR annealing temperature (T_a), number of alleles (A), expected heterozygosity (H_E), observed heterozygosity (H_O)

Locus	Repeat motif	Primer sequence (5′-3′)	Allele size (bp)	T_a (°C)	A	H_E	H_O
Rog3	$(CT)_8$	F: ATCATAGCAATGGTGGCA R: AATCGCAGGACTAAACAAAC	158–180	59	3	0.3075	0.1481
Rog4	$(TC)_{11}$	F: AAACACCGTCTGGAAATAG R: TTGTTTGGTATTATTTGCTG	132–138	56	3	0.3305	0.1111[a]
Rog5	$(GA)_{10}$	F: ATTTTAGTTTCCTAGAGCAGA R: ATCGTGGTATTTCATCGG	141–153	58	2	0.3305	0.2593
Rog9	$(AG)_{13}$	F: TCCTGAAAACGAAGCCTCC R: TTCTCCGCCCTATCCAATG	136–152	62	4	0.3298	0.2963
Rog10	$(TC)_{17}$	F: TTGAAGTAGGTGTTGCCG R: TTCCTGAAAACGAAGCCT	166–178	60	3	0.2970	0.3333
Rog14	$(CA)_8$	F: GATTATTATGGCAAGGACTC R: CAAAAACTCCAGTACTATGTCT	121–149	56	4	0.6562	0.1852[a]
Rog18	$(AG)_{17}$	F: ACACGCACACTCTCTTCTACTC R: GTTTCCCAGCCTTCACCT	240–262	57	5	0.3319	0.2963
Rog22	$(GGA)_4$	F: CACTCCGATTTTCTTCAAG R: GTCTATTCGCCAATGTCC	265–283	55	2	0.2830	0.0370[a]
Rog25	$(CT)_{26}$	F: ACTGGCAGTTCCATCAGAAA R: CGTTGACGACCCCTACCT	255–291	65	9	0.8043	0.5556[a]
Rog26	$(AC)_{10}$	F: CCGCACCCTACCTAAAAT R: ATTGAATGGTTGAGATGCC	123–141	58	4	0.6240	0.4815
Rog27	$(TG)_{10}$	F: CTGGTGGAATATTTCATGC R: TGGAATACAGAATCAAGAACA	201–221	55	2	0.3305	0.4074
CL2996*[1]	$(CCG)_6$	F: GCCACCATAGCCAGAGACAT R: AGAAGAAGTTGACGACAGGGAC	171–183	55	4	0.4298	0.3333
CL2980*[1]	$(AG)_9$	F: GTGCTGTCTTCCTCTTTTTC R: ACCAGTGACAGACCATTCTC	152–176	57	4	0.4661	0.1111
CTG623*[1]	$(CT)_{13}X(CT)_6$	F: CCTGACCCAACAAGAGTAAT R: TGAGAAAGAAAGGAAGCAA	127–139	56	4	0.6855	0.4074
H22C01*[1]	$(TC)_{16}$	F: TCATAACCAACCATCTCCA R: AGCCTCTTCGTTCTTCTGT	172–188	55	4	0.4528	0.3333
Rw16E19*[1]	$(AC)_{21}$	F: CAACAAACACGAGGAATGAA R: ATGGCTTGGATACTTGATGAG	166–198	55	5	0.7023	1.0000
Rw34L6*[1]	$(AG)_8$	F: CCTTTAGACTCGGGACCAC R: TCATCCAATATCATTTCAGC	138–158	55	5	0.2711	0.0741
Rw18N19*[2]	$(CTT)_8$	F: CCCGAGAAAGAGACAGTAAAAT R: CACCGACTCCGAGCAAAG	197–209	55	3	0.4088	0.0370

* Modified primers from literature: (1) Hibrand-Saint Oyant et al. 2007; (2) Zhang et al. 2006

[a] Indicated that the observed heterozygosity is significantly different from the expected heterozygosity under Hardy–Weinberg equilibrium ($P < 0.01$)

Table 2 Cross-species amplification of 11 new microsatellite loci developed from *Rosa odorata* var. *gigantea* in other related *Rosa* species

Species (locus)	Rog3	Rog4	Rog5	Rog9	Rog10	Rog14	Rog18	Rog22	Rog25	Rog26	Rog27
R. wichuraiana	+	+	+	+	+	+	+	+	−	+	+
R. rugosa	+	+	+	+	+	+	+	−	−	−	+
R. longicuspis	+	+	+	−	+	+	+	+	+	−	+
R. banksiae	+	+	+	−	+	+	+	−	−	+	+
R. laevigata	+	+	−	+	+	+	+	+	−	+	+
R. multiflora	+	+	+	+	+	+	+	−	+	−	+
R. chinensis var. *spontanea*	−	+	+	+	+	+	+	+	−	+	+
R. gallica	+	+	+	+	+	+	+	+	−	+	+

The symbol "+" indicated successful PCR amplification and "−"suggested failed PCR amplification

Genetic polymorphisms of these 100 loci were tested in 27 wild individuals collected from three distantly distributed populations. Polymerase chain reactions (PCR) were performed in final volumes of 15 µl contained 7.5 µl 2× Taq PCR MasterMix (Tiangen; 0.1 U Taq Polymerase/µl, 0.5 mM dNTP each, 20 mM Tris–HCl (Ph8.3), 100 mM KCl, 3 mM $MgCl_2$), 20–50 ng genomic DNA. The PCR protocols included: initial denaturation of 3 min at 94°C followed by 35 cycles with 30 s at 94°C, 30 s at 55–65°C, 1 min at 72°C and a final extension step of 7 min. Amplicons were resolved on 6% denaturing polyacrylamide gels using silver staining sized by a 20 bp DNA Ladder standard (Fermentas).

Of the 27 new primers designed for *R. odorata* var. *gigantea*, 20 successfully amplified the target regions, 11 of them displayed polymorphism (Table 1). For those previously published microsatellite markers, 47 of 73 succeeded in amplifying and 38 showed polymorphism. In order to get more specific primers for *R. odorata* var. *gigantea*, we cloned PCR products of 17 loci with high polymorphism, and then performed PCR and sequencing using the methods mentioned above. Finally, 7 modified microsatellite primer pairs were designed (Table 1). Thus, we got 18 specific primers in all.

Population genetic analyses were performed using the program GENEPOP version 4.0(Raymond and Rousset 1995). The number of alleles per locus (A) ranged from 2 to 9 (averaged at 3.9). The expected (H_E) and observed (H_O) heterozygosities varied from 0.2711 to 0.8043 (averaged at 0.4468) and from 0.0370 to 0.5556 (averaged at 0.3004), respectively. Among the 11 new microsatellite markers, four loci (Rog4, Rog14, Rog22, and Rog25) were deviated significantly from Hardy–Weinberg equilibrium ($P < 0.01$), probably due to excessive homozygosity and the population structure within samples. Tests for linkage disequilibrium were run in FSTAT version 2.9.3.2 (Goudet 1995). Significance levels were adjusted using sequential Bonferroni corrections (Rice 1989). No loci showed significant linkage disequilibrium after Bonferroni correction. The 11 new primer pairs were tested in other 8 important horticultural species or closely related taxa as *R. wichuraiana*, *R. rugosa*, *R. longicuspis*, *R. banksiae*, *R. laevigata*, *R. multiflora*, *R. chinensis* var. *spontanea* and *R. gallica* (Table 2). Most of them can be amplified successfully, except locus Rog25 (Table 2).

The 11 polymorphic loci developed in this study are the first set of microsatellite markers for *Rosa odorata* var. *gigantea*. Associating with 7 modified loci mentioned above, these microsatellite markers will be used to investigate population genetics and conservation biology of *R. odorata* var. *gigantea* and other *Rosa* species. They are also useful for constructing genetic linkage maps and locating quantitative trait locus (QTL) for *Rosa* sepecies.

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References

Clarke KR, Gorley RN (2001) PRIMER v5: user manual/tutorial. PRIMER-E Ltd., Plymouth, p 91

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemistry Bull 19:11–15

Gary B (1999) Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res 27:573–580. doi:10.1093/nar/27.2.573

Goudet J (1995) FSTAT (Version 1.2): a computer program to calculate F-statistics. J Hered 86:485–486

Hibrand-Saint Oyant L, Crespel L, Rajapakse S (2007) Genetic linkage maps of rose constructed with new microsatellite markers and locating QTL controlling flowering traits. Tree Genet Genomes 4:11–23. doi:10.1007/s11295-007-0084-2

Kimura T, Nishitani C, Iketani H et al (2006) Development of microsatellite markers in rose. Mol Ecol Notes 6:810–812. doi:10.1111/j.1471-8286.2006.01352.x

Ku TC, Robertson KR (2003) Rosa (Rosaceae). In: Wu ZY, Raven PH (eds) Flora of China, vol. 9. Science Press, Beijing; Missouri Botanical Garden Press, St. Louis, pp 371

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Rice WR (1989) Analyzing tables of statistical tests. Evolution Int J Org Evolution 43:223–225. doi:10.2307/2409177

Wylie AP (1954) The history of garden roses, part I. J Roy Hort Sci 79:555–571

Zane L, Bargelloni L, Patarnello T (2002) Strategies for microsatellite isolation: a review. Mol Ecol 11:1–16. doi:10.1046/j.0962-1083.2001.01418.x

Zhang LH, Byrne DH, Ballard RE et al (2006) Microsatellite marker development in rose and its application in tetraploid mapping. J Am Soc Hortic Sci 131:380–387. doi:10.1016/j.scienta.2005.09.004