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Short Communication

Isolation of microsatellite loci for *Rhodiola alsia* (Crassulaceae), an important ethno-medicinal herb endemic to the Qinghai-Tibetan plateau

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ABSTRACT. *Rhodiola alsia*, which has been used widely in traditional Chinese medicine for a considerable time, grows on moist habitats at high altitude near the snow line. Microsatellite loci were developed for *R. alsia* to investigate its population genetics. In total, 17 polymorphic microsatellites were developed based on ESTs from the Illumina HiSeq™ 2000 platform. The microsatellite loci were checked for variability using 80 individuals of *R. alsia* sampled from four locations on the Qinghai-Tibet Plateau. The total number of alleles per locus ranged from 10 to 20, and the observed heterozygosity ranged from 0.000 to 1.000. The null allele frequency ranged from 0.000 to 0.324. These microsatellites are expected to be helpful in future studies of population genetics in *R. alsia* and related species.

Key words: *Rhodiola alsia*; Simple sequence repeat; Population genetics; Qinghai-Tibet Plateau

INTRODUCTION

The Qinghai-Tibet Plateau (QTP) is an important biodiversity hub for studies of evolution and speciation. Despite its importance, however, relatively little is known about its flora. To date, most molecular studies of QTP flora have used plastid DNAs and only a few have employed nuclear DNA markers (Liu et al., 2012). The insights that such studies provide into the genetic diversity and evolutionary history of species are of value in exploring the relationships between evolutionary and demographic histories (Avise et al., 1987). *Rhodiola* L. (Crassulaceae) consists of about 90 species, of which 20 are used in traditional Chinese medicine. Their therapeutic activities include preventing high altitude sickness and eliminating fatigue (Arora et al., 2005). *Rhodiola alsia* is a popular ethno-medicinal herb exclusively endemic to QTP. This species is found on moist habitats near the snow line at high altitude (Wu et al., 2001). In a previous study, 17 microsatellite markers were developed for 4 species of *Rhodiola* (You et al., 2013). However, the markers did not successfully amplify target sequences in *R. alsia*. In this study, we designed novel SSR (simple sequence repeat) markers for *R. alsia* based on microsatellite loci of biparentally inherited nuclear DNA. In total, 17 polymorphic SSR markers were isolated and characterized from expressed sequence tag (EST)-based sequences. These SSR markers are different from those developed previously with regard to sequence, repeat motifs and size (You et al., 2013). The microsatellite markers developed here will provide an efficient tool for population genetic studies and for assessing genetic diversity and spatial genetic structures at a larger scale in *R. alsia* and its close congeners.

MATERIAL AND METHODS

Eighty *R. alsia* individuals from four populations [Qingshuihe (18 individuals), Xiewu (21 individuals), Shiqu (18 individuals) and Dingqing (23 individuals)] were used in this study (Table 1). Fresh leaves were collected and dried using silica gel. Total genomic DNA was extracted using the modified cetyltrimethylammonium bromide method (Gao et al., 2012). Microsatellite markers were detected in *R. alsia* based on EST obtained using an Illumina HiSeq™ 2000 platform (Illumina, San Diego, CA, USA). The SSRs were isolated by Microsatellite (MISA; http://pgrc.ipk-gatersleben.de/misa/) using Unigenes as reference. Only SSR lengths longer than 150 bp upstream and downstream were used. SSR marker parameters were set to identify perfect mono-, di-, tri-, tetra-, penta-, and hexanucleotide motifs with minimum repeats of 12, 6, 5, 5, 4, and 4, respectively. One hundred SSR loci were selected randomly from the identified loci. All the primers were designed by BGI-Shenzhen (Shenzhen, Guangdong, China) using the primer 3-2.3.4 software (http://primer3.sourceforge.net/). These primers were used to identify highly polymorphic loci in the 80 individuals from the four populations.

Table 1. Locality information for populations of *Rhodiola alsia* used in the study.

Population code	Location	Sample size	Voucher No.	Geographic coordinates	Altitude (m)
QSH	Qingshuihe, Qinghai Province, China	18	Chen2002021	34°05'N, 97°37'E	4670
XW	Xiewu, Qinghai Province, China	21	Chen2002023	33°12'N, 97°26'E	4350
SQ	Shiqu, Sichuan Province, China	18	Chen2002151	33°08'N, 97°33'E	4390
DQ	Dingqing, Tibet Autonomous Region, China	23	Chen2002056	31°41'N, 94°55'E	4900

The voucher specimens are deposited in the Herbarium of the Northwest Institute of Plateau Biology (HNWP), Xining, Qinghai Province, China.

Each polymerase chain reaction (PCR) was carried out using a 20-µL reaction mixture. The PCR master mix contained 2 µL 20-60 ng template DNA, 2 µL 10X PCR buffer, 0.6 µL 2.5 mM dNTPs, 0.5 µL of each primer (100 nM), 0.2 µL Taq polymerase (Takara, Dalian, China) and 14.2 µL ddH_2O. The following amplification conditions were used: denaturation for 5 min at 94°C; followed by 35 cycles of 45 s at 94°C, 30 s at the annealing temperature for each primer and 30 s at 72°C; and, a final extension step of 7 min at 72°C. The PCR products were amplified on agarose and polyacrylamide gels. Loci displaying high levels of polymorphism were then applied to a QIAxcel advanced system (QIAGEN, Hilden, Germany). The total number of alleles, observed heterozygosity, expected heterozygosity, null allele frequency, deviation from Hardy-Weinberg equilibrium, and linkage disequilibrium were calculated using the GENEPOP online package (http://genepop.curtin.edu.au/).

RESULTS

A total of 100 SSR loci were randomly selected for PCR amplification. Of these, 55 were successfully amplified, and 17 SSR loci proved to be highly polymorphic (Table 2). The total number of alleles per locus ranged from 10-20, with an average number of 14.8. The observed and expected heterozygosities ranged from 0.000-1.000 and 0.356-0.929, with an

Table 2. Characteristics of seventeen microsatellite markers developed in *Rhodiola alsia*.

Locus	Primer sequences (5'-3')	Repeat motif	Fragment size (bp)	Ta (°C)	Total No. of alleles	GenBank accession No.
RA50	F: TTCCTCGGTAGCTATGATGATGT R: TAAATGAGTATTGCTTTCGCCTT	$(AT)_8$	153	54	13	KJ804158
RA49	F: TTGTGGAAATCTGTGTTGTTCAG R: TTTCTTTCTGGGTTGTTTTTATCTG	$(AG)_8$	136	54	17	KM657855
RA48	F: ACGCGTTGATGAGAGAGTGTTAT R: ACCATCCAGCTAGCTAAGACCA	$(AG)_7$	138	56	10	KJ804160
RA44	F: TTCTTCAATCTGGAACCTCCTC R: GAGTCCTTTGACGACGAAAACTA	$(CT)_7$	136	56	20	KM657854
RA41	F: GCATGATGACGGAGTAGGAGAT R: CCTAACGTTCAATTTTCACCTCA	$(AG)_7$	142	56	15	KJ804161
RA38	F: CTGTGGAGGATTTGGAAAATATG R: CATACAAGCTTCATCCTTCCATT	$(AG)_7$	156	56	16	KM657853
RA37	F: GAGATTCGGACTCGAAATTGG R: CGCTCAACTTTATTCCTCCTCTT	$(GA)_8$	84	54	14	KM657852
RA36	F: TCTGAACAGAAGATTTGTTTGGC R: CGAAGAAAACAGAACTTTTGACG	$(GA)_8$	123	54	17	KM657851
RA33	F: CCATACATATCGGACAGCTGAAT R: TTGCAACTCGAAGAAAGACAAGT	$(AGG)_7$	110	56	13	KM657850
RA32	F: CAATATTAACCTCACCCCCAATC R: TCTCGTCATGGGTCCAGC	$(CT)_9$	159	54	19	KM657849
RA23	F: TTCTTGTACAATTCAGGCCATCT R: ATGATTATGTTCACCCTATGCCA	$(TG)_8$	143	56	13	KJ804162
RA20	F: GCAGTGGATTGAAAAGATGAGAT R: AAAAACGTGTCCAAGTCTCTCAC	$(TG)_8$	158	56	14	KJ804163
RA19	F: TGTAGGAAAGTTGTTCCTTGTGG R: CCTTTGATCTTTCAGCTCCTCTT	$(AC)_8$	132	56	14	KJ804164
RA18	F: TACTGAATTTGGTGGAGGTTTTG R: CTGGAGACTTCCCAGTACCTTCT	$(AG)_8$	152	56	14	KM657848
RA17	F: GTCTCTCCTCACTGAAACGATGT R: CAACCCCAATTTCAATTTTCTTA	$(CT)_8$	128	56	19	KM657847
RA15	F: CTCCCTCTGCTCAAATTTTTGTT R: TGCTAACCTCTCTACTCCAAGCA	$(AG)_8$	143	56	14	KJ804165
RA14	F: GAGAACAAATGTCAAACCCAAAA R: GGGTTAGATTTGTGTGTCGCTTA	$(CT)_9$	113	54	10	KJ804159

Ta = annealing temperature.

average of 0.804 and 0.799, respectively. The null allele frequency ranged from 0.000-0.324, with an average value of 0.040 (Table 3).

Table 3. Results of initial primer screening in four populations of *Rhodiola alsia*.

Locus	Population QSH (N = 18)				Population XW (N = 21)				Population SQ (N = 18)				Population DQ (N = 23)			
	A	H_O	H_E	N_A	*A*	H_O	H_E	N_A	*A*	H_O	H_E	N_A	*A*	H_O	H_E	N_A
RA50	6	0.556	0.719	0.049	6	0.857	0.620*	0.000	4	0.889	0.554*	0.000	9	0.696	0.757*	0.049
RA49	2	0.000	0.356*	0.279	6	0.143	0.633*	0.300	11	0.667	0.759*	0.000	12	0.957	0.884*	0.000
RA48	9	0.611	0.756*	0.120	7	1.000	0.770*	0.000	7	0.944	0.767*	0.000	7	1.000	0.773*	0.000
RA44	10	0.889	0.889	0.000	13	0.952	0.913*	0.000	12	0.833	0.911	0.014	17	1.000	0.929	0.000
RA41	9	0.667	0.863*	0.094	10	0.952	0.807*	0.048	10	0.833	0.884	0.010	14	0.870	0.923	0.042
RA38	5	0.222	0.565*	0.221	10	0.810	0.862*	0.000	10	0.944	0.859	0.000	13	0.957	0.807	0.000
RA37	5	0.944	0.679	0.000	10	0.952	0.854	0.000	8	1.000	0.827*	0.000	11	0.783	0.836	0.000
RA36	9	0.833	0.811*	0.000	8	0.857	0.787	0.012	8	0.882	0.779	0.000	15	0.957	0.873	0.000
RA33	6	0.471	0.633	0.098	8	0.500	0.788*	0.171	6	0.778	0.837	0.040	9	0.773	0.779*	0.031
RA32	11	0.722	0.756*	0.000	10	1.000	0.753	0.000	13	0.944	0.887	0.000	10	1.000	0.748	0.000
RA23	6	0.167	0.746*	0.324	11	0.905	0.858	0.017	9	0.611	0.878*	0.144	12	0.957	0.893	0.000
RA20	8	0.722	0.789	0.004	8	0.810	0.791	0.000	10	0.722	0.902*	0.080	10	0.870	0.802	0.009
RA19	8	0.667	0.790*	0.063	9	0.762	0.787	0.000	9	0.778	0.833	0.019	13	1.000	0.914	0.000
RA18	5	0.500	0.705*	0.131	8	1.000	0.772*	0.000	6	0.944	0.783*	0.000	13	0.870	0.917*	0.060
RA17	9	0.556	0.867*	0.151	14	1.000	0.893	0.000	11	1.000	0.888	0.000	14	1.000	0.876	0.000
RA15	9	0.944	0.844	0.000	13	1.000	0.921	0.000	11	1.000	0.898	0.000	9	1.000	0.853	0.000
RA14	8	0.944	0.805*	0.000	5	1.000	0.674*	0.000	7	0.889	0.817	0.053	7	0.391	0.679*	0.115

A = total number of alleles per locus; H_O = observed heterozygosity; H_E = expected heterozygosity; N_A = null allele frequency; N = sample size for each population. *Significant departure from HWE at P < 0.01. See Table 1 for population abbreviations.

DISCUSSION

This investigation successfully used EST from the Illumina HiSeq™ 2000 platform to develop 17 novel and highly polymorphic SSR markers in *R. alsia*. These markers are expected to be of considerable value in our future studies on genetic variation and conservation in *R. alsia* and related species.

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