Isolation of 16 Microsatellite Markers for Spiraea alpina and S. mongolica (Rosaceae) of the Qinghai–Tibet Plateau

Authors: Khan, Gulzar, Zhang, Faqi, Gao, Qingbo, Jiao, Xiujie, Fu, Pengcheng, et al.

Source: Applications in Plant Sciences, 2(1)

Published By: Botanical Society of America

URL: https://doi.org/10.3732/apps.1300059

Applications in Plant Sciences 2014 2(1): 1300059

PRIMER NOTE

ISOLATION OF 16 MICROSATELLITE MARKERS FOR *SPIRAEA ALPINA* AND *S. MONGOLICA* (ROSACEAE) OF THE QINGHAI–TIBET PLATEAU[1]

GULZAR KHAN[2,3,5], FAQI ZHANG[2,4,5], QINGBO GAO[2], XIUJIE JIAO[2], PENGCHENG FU[2], RUI XING[2], JINHUA ZHANG[2], AND SHILONG CHEN[2,6]

[2]Key Laboratory of Adaptation and Evolution of Plateau Biota, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810001, People's Republic of China; [3]University of the Chinese Academy of Sciences, Beijing 100039, People's Republic of China; and [4]Key Laboratory of Eco-environments of Three Gorges Reservoir Region, Ministry of Education, School of Life Sciences, Southwest University, Chongqing 400715, People's Republic of China

- *Premise of the study:* A set of microsatellite markers were developed to characterize the level of genetic diversity and gene flow in two plant species endemic to the Qinghai–Tibet Plateau, *Spiraea alpina* and *S. mongolica*.
- *Methods and Results:* Using the Fast Isolation by AFLP of Sequences COntaining repeats (FIASCO) method, 16 microsatellite loci showed polymorphisms in both species. In two populations of each species, the number of alleles per locus ranged from three to 18 in *S. alpina* and from four to 30 in *S. mongolica*.
- *Conclusions:* These microsatellite markers provide an efficient tool for population genetic studies and will be used to assess the genetic diversity and spatial genetic structure of *S. alpina* and *S. mongolica.*

Key words: gene flow; genetic diversity; microsatellite markers; population genetics; Qinghai–Tibet Plateau; *Spiraea*.

Spiraea alpina Pall. and *S. mongolica* Maxim. (Rosaceae subfam. Spiraeoideae) are perennial shrubs, found in western China and some areas of Mongolia and Siberia. The two alpine plants usually grow on sunny slopes or ridges. They are widespread across the Qinghai–Tibet Plateau and adjacent highlands, at altitudes of 2000–4500 m (Lu et al., 2003). Due to high levels of morphological variation, the genus *Spiraea* L. has been classified in several ways by different authors into various subgenera, sections, and series (Lu et al., 2003; Potter et al., 2007). A recent phylogeographic analysis of cpDNA variations in *S. alpina* indicated that this alpine shrub survived in multiple refugia during the Last Glacial Maximum and that earlier glaciations may have triggered deep intraspecific divergence (Zhang et al., 2012). However, the phylogeographic analysis based on one uniparentally inherited cpDNA fragment may only partly recover the phylogeographic history of a species. Biparentally inherited simple sequence repeat (SSR) markers with more polymorphism and information are necessary for a better understanding of the genetic structure and phylogeographic history of *S. alpina* and *S. mongolica.* In this study, we isolated 16 polymorphic microsatellite primers to facilitate the investigation in further studies for these two species.

[1]Manuscript received 12 July 2013; revision accepted 18 September 2013.

The authors thank Ms. Wang Wenjuan (Northwest Institute of Plateau Biology, Chinese Academy of Sciences) for her assistance with the study. This research was supported by the National Natural Science Foundation of China (grant no. 31270270).

[5]These authors contributed equally to this work.

[6] Author for correspondence: slchen@nwipb.cas.cn

doi:10.3732/apps.1300059

METHODS AND RESULTS

Total genomic DNA was extracted from silica gel–dried leaves of *S. alpina* following the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). Microsatellite loci from an enriched $(AG)_n$ library were isolated using the Fast Isolation by AFLP of Sequences COntaining Repeats (FIASCO) method with minor modifications (Zane et al., 2002). Approximately 300 ng of genomic DNA were completely digested with *Mse*I (New England Biolabs, Beverly, Massachusetts, USA), and then ligated to an *Mse*I AFLP adapter (5′-TACTCAGGACTCAT-3′/5′-GACGATGAGTCCTGAG-3′) using T4 DNA ligase (New England Biolabs). The diluted digestion-ligation mixture (1 : 10) was amplified with adapter-specific primers (5′-GATGAGTCCTGAGTAAN-3′). For enrichment, the PCR products were denatured at 95°C for 5 min, then hybridized with two 5′-biotinylated probes, $(AC)_{15}$ and $(AG)_{15}$, respectively, in a 250-µL hybridization solution (4× saline sodium citrate [SSC], 0.1% sodium dodecyl sulfate [SDS], 0.5 µmol/L probe) at 48°C for 2 h. Streptavidin-coated magnetic beads (New England Biolabs) were used to separate and capture the DNA fragments hybridized to the probe at room temperature for 20 min, followed by two washing steps: three times in TEN_{100} (10 mM Tris-HCl, 1 mM EDTA, 100 mM NaCl [pH 7.5]) for 8 min and three times in TEN_{1000} (10 mM Tris-HCl, 1 mM EDTA, 1 M NaCl [pH 7.5]) for 8 min. The separated single-stranded DNA fragments were amplified with adapter-specific primers as described above. The PCR products, after purification using a CASpure PCR Purification Kit (Sangon, Shanghai, China), were ligated into the pGEM-T Easy Vector (Promega Corporation, Madison, Wisconsin, USA) according to the manufacturer's instructions, then transformed into *Escherichia coli* TOP10 competent cells (Trans Gen Biotech, Beijing, China). Transformants were plated, and insert-containing clones were selected by blue-white screening with ampicillin, X-Gal, and isopropyl-β-D-1-thiogalactopyranoside (IPTG). Positive clones were tested by PCR using $(AC)_{10}$/$(AG)_{10}$ and $M13^+$/ $M13^-$ as primers.

Sequencing reactions and analysis of 120 positive clones were carried out with an ABI 3730xl DNA sequencer (Applied Biosystems, Foster City, California, USA), again following the manufacturer's instructions. These sequences were analyzed for repeat motif regions of microsatellites using the software SSRHunter (Li and Wang, 2005). Of these, 78 clones had microsatellite motifs, and primers were designed with Primer3 software (Rozen and Skaletsky, 2000). Polymorphisms of all loci with designed primer pairs were assessed with 92 individuals in two populations of each species from Nangqian in Qinghai Province (population code: NQ) and Hongyuan in Sichuan Province (population code: HY), People's Republic of China (Appendix 1). The PCR reactions were performed in a 15-μL reaction volume containing 0.8 μL of template DNA (10–100 ng), 1.5 μL of 10× buffer, 0.15 μL of dNTPs (10 mM each), 0.5 μL of each primer (10 mM), 5 U of *Taq* (TaKaRa Biotechnology Co., Dalian, China), and 11.4 μL of ddH_2O. The PCR cycling profile included an initial step of 5 min at 95°C; followed by 30 cycles of 50 s at 94°C, 50 s at annealing temperature for each primer (Table 1), and extension for 30 s at 72°C; followed by a final extension step at 72°C for 7 min. PCR products were then electrophoresed by QIAxcel Advanced System (QIAGEN, Hilden, Germany). Out of the 60 primer pairs, 21 pairs generated amplification products of the expected sizes in which 16 primer pairs displayed polymorphisms among the populations of the two species (Table 1).

MICRO-CHECKER version 2.2.3 (van Oosterhout et al., 2004) was used to assess null alleles and scoring errors. The number of alleles per locus (*A*), observed (H_o) and expected heterozygosities (H_e), deviations from Hardy–Weinberg equilibrium (HWE), and linkage disequilibrium (LD) between all pairs of polymorphic loci were calculated with GENEPOP version 4.0.10 (Rousset, 2008). Across the two populations of *S. alpina*, *A* ranged from three to 18, H_o ranged from 0.043 to 0.870, and H_e ranged from 0.126 to 0.950. In *S. mongolica*, *A* ranged from four to 30, H_o ranged from 0.040 to 1.000, and H_e ranged from 0.544 to 0.968. Some loci showed significant deviation from HWE (Table 2).

CONCLUSIONS

The SSR markers developed here are efficient to estimate genetic diversity in *S. alpina* and *S. mongolica*. Their use at larger spatial scales will provide detailed information about the distribution of genetic diversity in both species. Fine-scale genetic structure studies will enable us to estimate levels of historical gene flow in these species. Such information is useful for building and testing hypotheses on the history of the Qinghai–Tibet Plateau in response to climatic and geologic changes. The markers are also expected to be helpful in future studies of genetic variation and population ecology in these and other species in the subfamily Spiraeoideae.

LITERATURE CITED

Doyle, J. J., and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf material. *Phytochemical Bulletin* 19: 11–15.

Li, Q., and J. M. Wang. 2005. SSRHunter: Development of local searching software for SSR sites. *Hereditas* 27: 808–810.

Lu, L. D., C. Z. Gu, C. L. Li, C. Alexander, B. Bartholomew, R. B. Anthony, E. B. Davide, et al. 2003. Rosaceae. *In* Z. Y. Wu and P. H. Raven [eds.], Flora of China, vol. 9, 46–434. Science Press, Beijing, China, and Missouri Botanical Garden Press, St. Louis, Missouri, USA.

Potter, D., T. Eriksson, R. C. Evans, S. Oh, J. E. E. Smedmark, D. R. Morgan, M. Kerr, et al. 2007. Phylogeny and classification of Rosaceae. *Plant Systematics and Evolution* 266: 5–43.

Table 1. Characteristics of 16 microsatellite loci developed in *Spiraea alpina* and *S. mongolica*.

Locus	Primer sequences (5′–3′)	Repeat motif	Fragment size (bp)	T_a (°C)		GenBank accession no.
				S. alpina	*S. mongolica*	
SA1	F: ATGGCACGAACTATTGAATG R: GAATGACACGCAATCTATCC	$(GA)_{29}$	220–292	52	53	KC894821
SA2	F: TCCACCACAAGCCCAGTC R: GAGTAACCCAATCACCACAG	$(AG)_{17}$	128–168	53	53	KC894833
SA3	F: GTCAGCGTAATGCGGATG R: CACAAGGCACCACATAGG	$(AG)_{20}$	234–280	53	53	KC894822
SA4	F: GGAGATCGGCTGAAGAAG R: CCACACCCAATCACAACT	$(AG)_{16}$	116–164	53	53	KC894823
SA5	F: TTCACATCCAAGCAGTTCA R: GGTAAGCATCCAAGTCCAT	$(GA)_{15}$	248–256	52	53	KC894824
SA6	F: GGACTTCTGTTCATACCATAC R: GTCAGCGTAATGCGAATG	$(TC)_{40}$	280–320	51	51	KC894825
SA7	F: TCAATCGCACGACAATCC R: TCAAACCTCAAACCCTAAT	$(CT)_8$	120–160	53	53	KC894834
SA8	F: GCGTCCAAGACTAATCCA R: ACCGCCTCAGAACTCACC	$(TC)_{10}$	119–209	53	53	KC894835
SA9	F: ACGGACCTGCGGAGAATG R: CGCTCACAAACCCAACTAACA	$(GA)_5$	137–173	53	53	KC894836
SA10	F: GTGAAACGAGCTGAAGGT R: GCCATCTTGGAGATATACGA	$(AG)_{27}$	331–381	50	52	KC894826
SA11	F: CGAGGTGCTTCAAATTACAA R: ATGGAGGAGGATGCTTAGT	$(AG)_{30}$	214–238	53	51	KC894827
SA12	F: CAATCGAAGAGTGAAGAAGAG R: CCTGGTTACTATAGCAATGGA	$(AG)_{15}$	240–302	52	53	KC894828
SA13	F: CTGTTCAACCTACAATCCAA R: CCAGATCCTAACTACTATCGT	$(AG)_{30}$	172–228	51	54	KC894830
SA14	F: AGAATGCTCGCTACCTGC R: GATGTTCGGGCTTGCTAC	$(TG)_8$	180–220	53	53	KC894832
SA15	F: GCTTGGACGGATGGAGAT R: TCGCCAGTCTACTTGCTT	$(AG)_{18}$	160–250	52	54	KC894829
SA16	F: CAGAGGGAAGGAGAAGTCA R: TCCGTCCAAAGTCATCGT	$(GA)_{20}$	186–250	50	50	KC894831

Note: T_a = annealing temperature.

TABLE 2. Results of initial primer screening in four populations of *Spiraea alpina* and *S. mongolica*.[a]

	S. alpina							*S. mongolica*						
	Population NQ ($N = 22$)			Population HY ($N = 23$)				Population NQ ($N = 20$)			Population HY ($N = 27$)			
Locus	A	H_o	H_e	A	H_o	H_e	Null alleles	A	H_o	H_e	A	H_o	H_e	Null alleles
SA1	15	0.600	0.810	10	0.570	0.800	No	6	0.250	0.740	7	0.518	0.630	Yes
SA2	3	0.000	0.250	4	0.087	0.126*	Yes	18	1.000	0.833*	25	0.700	0.940	No
SA3	14	0.860	0.830*	8	0.870	0.740	No	13	0.650	0.900	10	0.040	0.814	No
SA4	4	0.000	0.318	3	0.000	0.240	No	14	0.800	0.900*	19	0.788	0.888*	No
SA5	18	0.681	0.950	17	0.570	0.900	No	4	0.000	0.544	10	0.410	0.666	No
SA6	13	0.863	0.830*	12	0.600	0.863	No	10	0.650	0.824*	10	0.260	0.814*	No
SA7	10	0.681	0.818	7	0.820	0.776	No	11	0.800	0.850*	13	0.741	0.810	No
SA8	9	0.045	0.809	8	0.130	0.420	No	15	0.850	0.863	22	0.518	0.883	No
SA9	9	0.409	0.796	10	0.570	0.857	No	22	0.900	0.912*	22	0.700	0.841	No
SA10	13	0.455	0.763	8	0.470	0.590*	No	25	1.000	0.968*	30	1.000	0.963*	No
SA11	8	0.600	0.730	11	0.470	0.880	No	9	0.950	0.765*	19	1.000	0.888*	No
SA12	18	0.410	0.825	13	0.520	0.862	No	15	0.800	0.810*	14	0.666	0.800*	No
SA13	18	0.860	0.936	16	0.870	0.904	No	13	1.000	0.866*	18	1.000	0.880*	No
SA14	4	0.000	0.568*	4	0.000	0.652	No	17	0.866	0.913*	25	1.000	0.954	No
SA15	9	0.230	0.630	8	0.280	0.674	No	18	0.800	0.950*	21	0.630	0.910	No
SA16	3	0.000	0.248	3	0.043	0.270*	No	18	0.700	0.900	20	0.333	0.764	No
Mean	10.50	0.418	0.694	8.87	0.430	0.659		14.25	0.751	0.846	17.80	0.644	0.840	

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

[a] Locality information: NQ = Nangqian, Qinghai Province; HY = Hongyuan, Sichuan Province. See Appendix 1 for geographic coordinates and voucher information.

* Significant departure from Hardy–Weinberg equilibrium at $P < 0.01$.

ROUSSET, F. 2008. GENEPOP'007: A complete reimplementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* 8: 103–106.

ROZEN, S., AND H. J. SKALETSKY. 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.

VAN OOSTERHOUT, C., W. F. HUTCHINSON, D. P. M. WILLS, AND P. SHIPLEY. 2004. MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4: 535–553.

ZANE, L., L. BARGELLONI, AND T. PATARNELLO. 2002. Strategies for microsatellite isolation: A review. *Molecular Ecology* 11: 1–16.

ZHANG, F. Q., Q. B. GAO, D. J. ZHANG, Y. Z. DUAN, Y. H. LI, P. C. FU, R. XING, ET AL. 2012. Phylogeography of *Spiraea alpina* (Rosaceae) in the Qinghai–Tibetan Plateau inferred from chloroplast DNA sequence variations. *Journal of Systematics and Evolution* 50: 276–283.

Appendix 1. Locality information for populations of *Spiraea alpina* and *S. mongolica* used in the study. The voucher specimens are deposited in the Herbarium of the Northwest Institute of Plateau Biology (HNWP), Xining, Qinghai Province, People's Republic of China.

Species	Population code	Population locality	Voucher no.	Geographic coordinates	Altitude (m)
S. alpina	NQ	Nangqian, Qinghai Province, China	Chensl6037	31°58′N, 96°30′E	4320
	HY	Hongyuan, Sichuan Province, China	Chensl6099	32°46′N, 102°21′E	3654
S. mongolica	NQ	Nangqian, Qinghai Province, China	Chensl6291	31°58′N, 96°30′E	4320
	HY	Hongyuan, Sichuan Province, China	Chensl6109	31°58′N, 96°30′E	3654