Genetics and Molecular Research

Development and characterization of microsatellite loci in *Brasenia schreberi* (Cabombaceae) based on the next-generation sequencing

H. Liu[1]*, Z.C. Long[2]*, L.N. Li[2], Q.F. Wang[2], J.M. Chen[2] and K. Liao[2]

[1]College of Life Science, South-Central University for Nationalities, Wuhan, Hubei, China
[2]Key Laboratory of Plant Germplasm Enhancement and Specialty Agriculture, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan, Hubei, China

*These authors contributed equally to this study.
Corresponding author: K. Liao
E-mail: liaokuo@wbgcas.cn

Genet. Mol. Res. 15 (2): gmr.15027886
Received Ouctober 22, 2015
Accepted December 22, 2015
Published April 4, 2016
DOI http://dx.doi.org/10.4238/gmr.15027886

ABSTRACT. To understand genetic variability of the endangered aquatic herb *Brasenia schreberi* (Cabombaceae), we describe 31 microsatellite markers obtained using next-generation sequencing. A total of 24 individuals from the population of Jackson Lake, USA, were genotyped for each marker. Twenty-eight markers were polymorphic. The number of alleles per locus ranged from 1 to 9; the observed and expected heterozygosities ranged from 0 to 1 and from 0 to 0.751, respectively. These markers should be useful tools for genetic variation and conservation studies of *B. schreberi*.

Key words: Aquatic herb; *Brasenia schreberi*; Endangered species; Microsatellite marker; Next-generation sequencing

INTRODUCTION

Brasenia schreberi J.F. Gmelin (Cabombaceae), a perennial floating-leaved aquatic herb (water shield), is distributed widely in temperate and tropical regions of Asia, Australia, Africa, India, and North and South America (Kim et al., 2008, 2012). However, populations of *B. schreberi* are found only rarely in each region. In several countries of East Asia, *B. schreberi* has been listed as a critically endangered species due to human activity and habitat loss (Lee et al., 2005; Zhang and Gao, 2008).

In recent years, genetic variation among *B. schreberi* populations has been demonstrated with a variety of dominant genetic markers, e.g., RAPD (random amplified polymorphic DNA) and AFLP (amplified fragment length polymorphism) (Kim et al., 2008), ISSR (inter-simple sequence repeat) (Zhang and Gao, 2008), and nuclear ribosomal ITS and chloroplast DNA *trn*L-F sequences (Kim et al., 2012). However, the results of these studies are still not sufficient for defining adaptive population differentiation. Next-generation high-throughput DNA sequencing techniques have greatly promoted the development of multiple co-dominant markers, such as simple sequence repeat (SSR) markers (Chen et al., 2015; Yun et al., 2015; Zhang et al., 2015), which have been shown to be useful tools for assessing the genetic diversity and adaptive population differentiation of endangered species. Here, we report the development of microsatellite markers from *B. schreberi* using next-generation sequencing, which will facilitate ongoing studies into the evolutionary history and conservation of this endangered species.

MATERIAL AND METHODS

Fresh leaves of an individual of *B. schreberi* plant were collected from Wuhan Botanical Garden, China, and genomic DNA was extracted using a Plant Genomic DNA Isolation kit (Tiangen, Beijing, China) following the manufacturer instructions. A sequencing library was constructed at Novogene Bioinformatics Technology Co., Ltd. (Beijing, China) according to the manufacturer instructions (Illumina). This library was sequenced using an Illumina HiSeq 2000 Platform at Novogene Bioinformatics Technology Co., Ltd., and generated 5.59 G clean DNA reads. A total of 106,801 SSR loci were identified using the MIcroSAtellite identification tool (MISA) (Thiel et al., 2003) from the resources, and 54,640 primers were successfully designed using Primer3 (http://biotools.umassmed.edu/bioapps/primer3_www.cgi).

Fifty of the designed primers were randomly selected and initially screened using total DNA isolated from dried leaves of six *B. schreberi* individuals collected from the population at Mangshan in Hunan Province. Polymerase chain reaction (PCR) amplifications were performed in a volume of 20 µL containing 0.25 mM each dNTP, 2 µL 10X Taq buffer (10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, and 50 mM KCl), 1 mM each primer, 0.2 U Taq Polymerase (TransGen Biotech Co., Beijing, China), and 25 ng DNA template. Genomic DNA was amplified using an ABI 2720 Thermal Cycler (Applied Biosystems, USA). The PCR program was as follows: an initial denaturation of 5 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s annealing at 54°-56°C (depending on the primers), and 30 s extension at 72°C, with a final extension cycle of 10 min at 72°C. Agarose gel electrophoresis showed that 31 of 50 markers were clearly amplified.

Based on the initial screening results, all of the 31 primer pairs were selected and used to test for polymorphism in 24 individuals from a population located in Jackson Lake, Florida, USA (85°11'W, 30°45'N). Forward primers were labeled with 6-FAM. PCR was performed using the same reaction and cycling conditions as were used in the initial screening. PCR products were separated using an ABI 3730 XL automated sequencer (TsingKe Biotech, Beijing, China) and

visualized using the GeneScan system (Applied Biosystems, Foster City, CA, USA). The number of observed alleles per locus (N_A), observed (H_O) and expected (H_E) heterozygosities, and deviations from Hardy-Weinberg equilibrium (HWE) for each locus in the test population of *B. schreberi* were calculated using the program of GenAlex 6.5 (Peakall and Smouse, 2012).

RESULTS AND DISCUSSION

Twenty-eight of the 31 tested markers were polymorphic. The N_A ranged from 1 to 9. The H_O and H_E ranged from 0 to 1 and from 0 to 0.751, respectively (Table 1). All of the loci, except for locus BS29, showed significant deviations from HWE, which could be due to the extensive clonal reproduction of this species. These markers will facilitate further investigation into the adaptive genetic variation and conservation of *B. schreberi*.

Table 1. Primer sequences and characteristics of the 31 microsatellite loci in *Brasenia schreberi*.

Locus	Primer sequence (5'-3')	Repeat motif	Allele size (bp)	Ta (°C)	N_A	H_O	H_E	GenBank accession No.
BS1	GAACGAATGAATAACCAGAGATG ATTTCCTAGCCTGCCTCCAT	$(TG)_7(AG)_8$	127-138	58	4	0.125	0.721	KT899583
BS2	TTGTAGAAGTGCCACGATGC CGCTTCCATTCTCTACCCAG	$(CT)_9(CA)_7$	147-159	59	6	0.250	0.594	KT899584
BS3	TCAAAACGTGAGCATGAACAA TAACCATTCAAGAAACCCCG	$(CT)_8(AT)_6$	115-130	60	4	0.125	0.572	KT899585
BS4	TGTAGCCGACCCCATCTAGT GGTTTCCACGTAACACGTCA	$(GTT)_5$	128-212	59	4	0.125	0.558	KT899586
BS5	GGAACATGAGCAAGTGCAGA TTGGCAAGTTATACCCTCCG	$(AG)_{10}$	273-291	60	3	0.125	0.525	KT899587
BS6	ATCCCCGTGTCACACTAAGC GGACCTGGGTCATATTAGGGA	$(TA)_7(GA)_7$	209-218	60	4	0.375	0.537	KT899588
BS7	GGAGTGCAATAGGCAAAAGC TGTTTCATTTTACAAAACGTGGA	$(ATG)_5$	118-184	60	4	1.000	0.593	KT899589
BS8	CCAAAGATTCACACATTGGAAA CTATGAAGTCACCAGGTGCG	$(AG)_{10}$	138-160	60	6	0.417	0.751	KT899590
BS9	CTTGGAAAGAGGGCTTTGTG TGAGGTGAATGATTTTTGCATC	$(AAG)_5$	265-297	60	3	0.000	0.392	KT899591
BS10	TAACAGGAGCCGACGCTAGT GTTGTACCGAAGCCTGAAGC	$(CT)_6$	270-281	60	2	0.000	0.080	KT899592
BS11	CCACTAAGACTTCCGGTGCT GCGGCATGCATTACTCCTAT	$(GAGAG)_5$	274	59	1	0.000	0.000	KT899593
BS12	GGGGCTTCATTTATTGGGAT CTGCTTTTCCATGGCATTTC	$(TC)_7$	252-280	60	4	0.000	0.451	KT899594
BS13	AATGGTGAAGGAAAGCATGG GTGTCACGCACGCAGATAGT	$(CA)_6$	267-281	60	4	0.125	0.194	KT899595
BS14	GTGTCACGCACGCAGATAGT GCAAGCTCATTTTGCTTTCTT	$(TG)_6$	178	60	1	0.000	0.000	KT899596
BS15	TCGAGGTTTTCCTCTGCCTA CATTTGCATGATTGATCGCT	$(TC)_6$	231-268	60	4	1.000	0.631	KT899597
BS16	CCGAAGAAGAAGTTCCTCCC CATCAGCTGCCTCATCTTCA	$(GAA)_7$	105-114	60	4	0.792	0.614	KT899598
BS17	TCAGAGCGACAGTGAGGAGA CCAAAATCAGCATGTGCATC	$(TGA)_5$	184	60	1	0.000	0.000	KT899599
BS18	CAAATCCCACATGTCTCACG GCTTTTGGAATCTGCCAAACTA	$(T)_{11}$	168-177	60	4	0.958	0.591	KT899600
BS19	CTGCTGAAGGATTCTCGGAC TGTACAGCACTTGCAGGTAAGAA	$(CTA)_5$	263-289	60	2	0.000	0.413	KT899601
BS20	GCAAGGATGATGTTTGAGCA GAAATATATTCATGATGTGGAGTATGA	$(TA)_7(TG)_8$	100-161	60	5	1.000	0.639	KT899602
BS21	AAGGCGGAAAAGAGGAAGAG GAGGATGGCTGATTGTGGTT	$(TG)_8$	141-148	60	2	0.000	0.497	KT899603
BS22	AAGTATGCCCAACCAGCAAA AAGCTAACCGTTTCATCTTTATCA	$(TG)_7(AG)_9$	218-231	60	6	0.417	0.491	KT899604
BS23	AATAGACACGACCGGTTTGG AAACCAAACCAAATGACCCA	$(AAC)_6$	105-141	60	4	0.917	0.650	KT899605
BS24	TCGATCTTTGAAATTCGATAGTTG ATGCACCAAACCAAAACACA	$(CT)_6$	100-127	59	4	0.042	0.322	KT899606
BS25	AAACTCCACCAGATCAACCG AAAAATGCCCCCTTCTCTGT	$(AG)_7$	186-288	60	3	0.000	0.288	KT899607
BS26	TCAAACGATAAAAACATTTGAAGAA CGATCGGTCTGATCTTGGAT	$(TC)_9$	307-313	59	5	0.250	0.360	KT899608
BS27	AAACCTCTGGTACTTGGAGACTTG TGCAGTTTGTCTTTTCCAAGG	$(AT)_8$	107-187	60.	8	0.000	0.743	KT899609
BS28	GGCCTAGATTCCTTGCTTCC CGGCAATCATTTCTGTTTTT	$(AC)_6$	241-280	60	5	0.708	0.655	KT899610
BS29	AATCAGATCGGACCACGAAC GCATGTTCTAGAGGAAAGAGCC	$(GT)_6$	230-270	60	6	0.417	0.698	KT899611
BS30	AGGATCGAGTTGTTTTCCCC CGTGGTGTAAGGCCAAGAGA	$(AG)_8$	216-232	60	4	0.667	0.575	KT899612
BS31	ACCTAGTTTCTCACCGCCAG GGAAGAGAAGGGAGATGAAGG	$(CT)_{10}$	132-175	59	9	1.000	0.731	KT899613

Ta = annealing temperature; N_A = number of alleles observed; H_E = expected heterozygosity; H_O = observed heterozygosity.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank Chun-Feng Yang for his kind assistance in the field work, and Zhi-Zhong Li and Zhi-Yuan Du for their help in the laboratory. Research supported by grants from the National Natural Science Foundation of China (#31570220 and #31200170) and the Open Funding Project of the Key Laboratory of Aquatic Botany and Watershed Ecology, Chinese Academy of Sciences.

REFERENCES

Chen WS, Zhao G, Jian SG and Wang ZF (2015). Development of microsatellite markers for *Suriana maritima* (Surianaceae) using next-generation sequencing technology. *Genet. Mol. Res.* 14: 14115-14118.http://dx.doi.org/10.4238/2015.October.29.31

Kim CY, Na HR and Choi HK (2008). Conservation genetics of endangered *Brasenia schreberi* based on RAPD and AFLP makers. *J. Plant Biol.* 51: 260-268. http://dx.doi.org/10.1007/BF03036125

Kim CY, Jung JD, Na HR, Kim SW, et al. (2012). Population genetic structure of the endangered *Brasenia schreberi* in South Korea based on nuclear ribosomal spacer and chloroplast DNA Sequences. *J. Plant Biol.* 55: 81-91. http://dx.doi.org/10.1007/s12374-011-9193-4

Lee HW, Choung HL, Roh TH, Kwon YH, et al. (2005). Categorization and Conservation of the Threatened Plant Species in Environmental Impact Assessment. Korea Environment Institute, Seoul.

Peakall R and Smouse PE (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research - an update. *Bioinformatics* 28: 2537-2539. http://dx.doi.org/10.1093/bioinformatics/bts460

Thiel T, Michalek W, Varshney RK and Graner A (2003). Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). *Theor. Appl. Genet.* 106: 411-422.

Yun YE, Yu JN, Nam GH, Ryu SA, et al. (2015). Next-generation sequencing identification and characterization of microsatellite markers in *Aconitum austrokoreense* Koidz., an endemic and endangered medicinal plant of Korea. *Genet. Mol. Res.* 14: 4812-4817. http://dx.doi.org/10.4238/2015.May.11.13

Zhang FQ, Lei SY, Gao QB, Khan G, et al. (2015). Isolation of microsatellite loci for Rhodiola alsia (Crassulaceae), an important ethno-medicinal herb endemic to the Qinghai-Tibetan plateau. *Genet. Mol. Res.* 14: 5266-5269. http://dx.doi.org/10.4238/2015.May.18.18

Zhang GF and Gao BQ (2008). Analysis on genetic diversity and genetic structure of *Brasenia schreberi* in Jiangsu and Zhejiang Provinces revealed by ISSR Markers. *J. Lake Sci.* 20: 662-668. http://dx.doi.org/10.18307/2008.0516