Conserv Genet (2009) 10:1497–1499
DOI 10.1007/s10592-008-9767-y

TECHNICAL NOTE

Isolation and characterization of microsatellite markers for the endangered *Monimopetalum chinense* (Celastraceae)

Hai-Sheng Li · Guo-Wen Xie · Xiao-Yong Chen

Received: 9 November 2008 / Accepted: 14 November 2008 / Published online: 29 November 2008

Abstract We describe the isolation and characterization of 13 polymorphic microsatellite loci in the endangered *Monimopetalum chinense* (Celastraceae) using the Fast Isolation by AFLP of Sequences Containing repeats (FIASCO) protocol in this paper. The 13 loci were tested in 20 individuals from two populations. The number of alleles per locus varied from 2 to 8, with an average of 3.8. The expected and observed heterozygosities ranged from 0.05 to 0.87 and from 0.05 to 0.55, respectively. Two of the 13 loci were biased from Hardy–Weinberg equilibrium. No significant linkage disequilibrium was found among all loci. These polymorphic SSR markers will be useful in studying population genetics of *M. chinens*e.

Keywords *Monimopetalum chinense* · Microsatellites · Heterozygosity · Population genetics

Monimopetalum chinense Rehd. (Celastraceae) is a stoloniferous woody vine endemic to eastern China (Xie and Zhang 1999). In recent decades, it has been subjected to a rapid demographic decline, mainly due to habitat destruction, and is classified in the red book of endangered flora of China (Fu 1992) as an endangered species. Long-term management strategies for conservation of *M. chinense* need knowledge about the genetic variation assigned within and among populations, as well as about gene flow. The few studies available so far have been carried out using dominant ISSRs (Xie et al. 2005). In this study, we have developed and characterized co-dominant microsatellite markers to investigate the spatial genetic structure of this species.

Genomic DNA was extracted from silica gel-dried leaves collected in the wild using the modified CTAB method (Fan et al. 2004). Microsatellite loci from enriched $(AG)_n$ and $(AC)_n$ library were isolated using the fast isolation by AFLP of sequences containing repeats (FIASCO) protocol (Zane et al. 2002) with minor modifications. About 450 ng genomic DNA was digested with *Mse*I restriction enzyme (New England Biolabs) and then ligated to *Mse*I AFLP adaptor (5′-TAC TCA GGA CTC AT-3′/ 5′-GAC GAT GAG TCC TGA G-3′) using T4 DNA ligase (Fermentas). The digestion–ligation mixture was diluted (1:10) and amplified with AFLP adaptor-specific primers (5′-GAT GAG TCC TGA GTA AN-3′, i.e., *Mse*I-N) under the PCR program of initial denaturation of 3 min at 95°C, followed by 20 cycles of 30 s at 94°C, 1 min at 53°C, 1 min at 72°C. For enriching the fragment contained SSR, the PCR products were denatured at 95°C for 5 min, and then hybridized with 5′-biotinylated probe $(AG)_{15}$ and $(AC)_{15}$ in 250 μl hybridization solution (4.2× SSC, 0.07% SDS, 0.6 μM probe) at 48°C for 2 h. Hybridization products were selectively captured with 300 μl Strepatavidin MagneSphere® Paramegnetic Particles (Promega), which were prepared by washing in 150 μl TEN_{100} (10 mM Tris–HCl, 1 mM EDTA, 100 mM NaCl, pH 7.5) for three times. The mixture was incubated at room temperature for 50 min with constant gentle agitation. The beads-probe-DNA

H.-S. Li · X.-Y. Chen (✉)
Department of Environmental Sciences, East China Normal University, 200062 Shanghai, China
e-mail: xychen@des.ecnu.edu.cn

H.-S. Li
Department of Biology, Guangdong Education Institute, 510303 Guangzhou, China

G.-W. Xie
College of Life Sciences, Guangzhou University, 510006 Guangzhou, China

Table 1 Primers sequences and characterization for 13 polymorphic microsatellite loci for *Monimopetalum chinense*

Locus	Primer sequence (5′-3′)	Motif	Ta (°C)	Size range (bp)	*A*	H_O	H_E	GenBank accession #
Mch03	F: ATTTGAATGCTGGAATGTAT R: GAAAATAATGTGCAATCCTC	$(TG)_{24}$	46	126–158	5	0.35	0.70	FJ390487
Mch16	F: ACCAAATGCACTGTTATC R: TGATTTATACAGAGCAAG	$(CA)_7$-$(AC)_3$ imperfect	56	143–145	2	0.15	0.14	FJ390488
Mch21	F: GGTTGGAAATTTTCATACTG R: AACTTCAAGCGGAATCTGTC	$(AT)_5(GT)_9$	64	162–204	4	0.55	0.68	FJ390491
Mch24	F: CCTCAGAGCATTGTAAGCAT R: CGTGTCTTCCTTGGACTTCA	$(AG)_{10}$	65	163–169	2	0.25	0.22	FJ390492
Mch27	F: CACGGCTGCTCTTCTGGTTA R: TTGCACCGACTAGCTCAATC	$(TC)_{13}$	58	159–183	3	0.10*	0.67	FJ390493
Mch28	F: ACAAGCGACACCAAACTCAC R: AAACAGAGGATGCTGAAACG	$(TC)_3C(TC)_{17}$	54	119–169	8	0.55	0.87	FJ390494
Mch29	F: CTGGTTTCGGTTCTTGGAT R: TAACACCACAAATGGACGA	$(TG)_{15}$	48	210–242	5	0.50	0.78	FJ390495
Mch31	F: CGAAGACCATGTTATGCTGA R: CGAGGCAAGGCTTATCTTATTT	$(AG)_9$	56	137–143	2	0.05	0.05	FJ390496
Mch33	F: TCAGACAAGGGGTACAGGAA R: GAAAGGGGAAAATGGGGAAT	$(CT)_{14}$	65	208–220	3	0.45	0.37	FJ390497
Mch51	F: CTTTTCCCGTCCCTATCTCT R: GCTCACATACCTCAATTTCG	$(CT)_{19}$	63	157–169	4	0.45	0.72	FJ390498
Mch53	F: GTTGGTGACAGGCTCATTTA R: CCAACTAACATGCAATCTCA	$(CT)_{11}$	61	175–185	2	0.10	0.18	FJ390499
Mch55	F: TTTGTCACCCCTATCCTTCT R: CGATCCTGCCTTTACTTCTC	$(AG)_{27}$-$(AG)_5$	61	226–246	4	0.20*	0.75	FJ390500
Mch58	F: ATAGGCTGGTCAAAGAAGAA R: TACAAGACTGGGTACGGTTA	$(CT)_{12}$	61	212–248	5	0.35	0.71	FJ390501

Ta, PCR annealing temperature; *A*, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity

* Indicates the observed heterozygosity is significantly biased from Hardy–Weinberg expectation ($P < 0.01$)

complex was separated by a magnetic field. Three low stringency washes in 500 µl of Ten_{1000} (10 mM Tris–HCl, 1 mM EDTA, 1 M NaCl, pH 7.5) for 8 min were performed followed by three washes in 500 µl high stringency buffer (0.2× SSC and 0.1% SDS) for 8 min at room temperature. Captured DNA fragments were released by incubating for 5 min at 95°C in 50 µl of TE (Tris–HCl 10 mM, EDTA 1.0 mM, pH 8.0). Recovered DNA fragments were amplified with *Mse*I-N primers for 30 cycles as described above. The PCR products were purified with Gel Extraction Kit (Bioteke, Beijing). The purified fragments were ligated into pMD18-T plasmid vector (TaKaRa) and transformed into *Escherichia coli* strain (JM109, TaKaRa) following the manufacturer's instructions. Transformants were plated on Luria-Bertani agar plates containing ampicillin, X-gal and IPTG and insert-containing clones were picked out and tested by PCR using $(AG)_{10}$ or $(AC)_{10}$ and $M13^+/M13^-$ as primers, respectively. Identified clones were sequenced on an ABI PRISM3730 automated sequencer. The sequences containing motifs repeating more than five times were considered as microsatellites.

Of the 96 clones sequenced, all were found to contain simple sequence repeats. However, 38 of the 96 sequences were discarded because either the repeat was too short or the flanking regions of the repetitive sequences were not suitable for designing primers. Fifty-eight primer pairs were designed according to the nucleotide sequences upstream and downstream of the repetitive DNA using the Primer3 (Rozen and Skaletsky 2000). Thirty-four primer pairs were tested using 20 individuals of *M. chinense* from 2 populations. PCRs were conducted using PTC-200 thermal cycler (MJ Research) in 20 µl volume containing the following components: 20 ng of genomic DNA, 0.2 mM of each dNTP, 0.2 µM of each primer, 1× PCR buffer (Mg^{2+} free), 1.5 mM Mg^{2+}, and 1 unit of Taq DNA polymerase (Sagon). The PCR protocols included: initial denaturation at 94°C for 5 min, followed by 35 cycles of 50 s at 94°C, 50 s at primer-specific annealing temperature (Table 1),

1 min at 72°C, and a final extension at 72°C for 10 min. Electrophoresis was performed on 8% denaturing polyacrylamide gels and visualized by silver staining. The band size was scaled using a 50 bp DNA ladder (Fermentas) as the reference.

Twenty-one of the 34 primer pairs tested successfully amplified the target fragments in *M. chinense*, and 13 loci showed polymorphism (Table 1). There were 2–8 alleles per locus, and the average number of alleles per locus was 3.8. The program GENEPOP (Raymond and Rousset 1995) online version (http://genepop.curtin.edu.au/) was used to calculate the expected and observed heterozygosities, conformance to Hardy–Weinberg equilibrium (HWE) expectations, and linkage disequilibrium for the thirteen microsatellite loci (Table 1). The expected (H_E) and observed heterozygosities (H_O) ranged from 0.05 to 0.87 and from 0.05 to 0.55, respectively. Two of the 13 polymorphic loci (Mch27, Mch55) showed significant deviation from Hardy–Weinberg equilibrium (HWE) ($P < 0.01$), which was due to excess of homozygotes. No significant linkage disequilibrium was detected in any locus pair. These 13 polymorphic microsatellite loci presented here could be useful for studying the population genetics of *M. chinense*. This information will play a critical role in planning the genetic management of this endangered species.

Acknowledgments We are grateful to Shen-Min Guan, Yan Chen, Min Liu, Miao-Miao Shi, Mei-Hua Liu, Rong Wang and Lin-Feng Li for their technical assistants. This work was supported by a grant from the National Natural Science Foundation of China (30470146) and Program for 211 Project and New Century Excellent Talents in University (NCET-05-0431).

References

Fan XX, Shen L, Zhang X, Chen XY, Fu CX (2004) Assessing genetic diversity of *Ginkgo biloba* L. (Ginkgoaceae) populations from China by RAPD markers. Biochem Genet 42:269–278. doi:10.1023/B:BIGI.0000034431.15308.57

Fu LG (1992) Chinese plant red book. Science Press, Beijing

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

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana Press, Totowa, NJ, pp 365–386

Xie G-W, Wang D-L, Yuan Y-M, Ge X-J (2005) Population genetic structure of *Monimopetalum chinense* (Celastraceae), an endangered endemic species of eastern China. Ann Bot (Lond) 95:773–777. doi:10.1093/aob/mci087

Xie GW, Zhang ZY (1999) The geographical distribution and population spatial pattern of *Monimopetalum chinense*. Ecol Sci 18:7–11

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