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TECHNICAL NOTE

Isolation and characterization of 15 microsatellite markers from wild tea plant (*Camellia taliensis*) using FIASCO method

Jun-Bo Yang · Jing Yang · Hong-Tao Li · Yu Zhao · Shi-Xiong Yang

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Abstract *Camellia taliensis* is one of the most important wild tea plants in China, especially in Yunnan Province. In this study, we described the development of 15 microsatellite markers from the genome of *C. taliensis* using the protocol of fast isolation by AFLP of sequences containing repeats (FIASCO). Polymorphism of each locus was assessed in 24 samples collected from six wild populations of *C. taliensis*. The average allele number of the microsatellites was four per locus, ranging from 2 to 7. The observed and expected heterozygosities varied from 0.076 to 0.5833 and from 0.1560 to 0.6917, respectively. Cross-species amplification in other three tea plants showed eleven of them holding promise for sister species. These polymorphic SSR markers would be useful tools for population genetics studies and assessing genetic variations to establish conservation strategy, molecular identification and molecular breeding on this tea plant and its allied species and varieties in section Thea genus *Camellia*.

Keywords *Camellia taliensis* · FIASCO · Microsatellite markers · Polymorphism · Tea plant

J.-B. Yang · J. Yang · H.-T. Li
SW China Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences, 650204 Kunming, Yunnan, China

J.-B. Yang
e-mail: jbyang@mail.kib.ac.cn

J. Yang
e-mail: yangjingb@mail.kib.ac.cn

H.-T. Li
e-mail: lihongtao@mail.kib.ac.cn

Y. Zhao
Jiangxi Agricultural University, Jiangxi, China
e-mail: zy_92612.student@sina.com

J.-B. Yang · S.-X. Yang (✉)
Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, 650204 Kunming, Yunnan, China
e-mail: shxyang@mail.kib.ac.cn

Tea is one of the most popular healthy beverages throughout the world (Mondal 2002). It contributes greatly to wealth and job opportunities in many Asian, African and South American countries. *Camellia taliensis* is one of the important wild tea source in China, particularly in Yunnan province among tea plants, including five species and two varieties (Chen et al. 2000), namely *Camellia taliensis* (W. W.Smith) Melchior, *C. tachangensis* F. C. Zhang, *C. crassicolumna* Chang, *C.gymnogyna* Chang, *C. sinensis* (L.) O. Kuntze, *C. sinensis var. assamica* (Masters) Kitamura and *C. sinensis var.pubilimba* Chang, belonging to section Thea (L.) Dyer, genus Camellia L. and family Theaceae, which originated from Yunnan province in southwestern China (Yu 1986; Hasimoto and Takasi 1978). Some studies have been assessed for tea plants using different DNA markers including microsatellite markers, or simple sequence repeat (SSRs) (Hung et al. 2008; Kaundun and Matsumoto 2002; Zhao et al. 2008). Compared with other fingerprinting, such as RAPD, ALFP, ISSR, microsatellites show numerous advantages since they are locus-specific, codominant, highly reproducible and usually highly polymorphic (Powell et al. 1996). However, no nuclear microsatellite primers have been reported for *Camellia taliensis*. In order to devise adequate conservation and management strategies for this high economic important wild tea plant and its sisters, we developed

microsatellite markers (SSR) from *C. taliensis* using the fast isolation by AFLP of sequences containing repeats (FIASCO) protocol of Zane et al. (2002).

Genomic DNA samples were extracted from dry leaf tissue, which was ground in liquid nitrogen, using a CTAB methodology (Doyle and Doyle 1987). Total genomic DNA (about 500 ng) derived were completely digested with *Mse*I and then ligated to an *Mse*I AFLP adaptor. A diluted digestion–ligation mixture (1:10) was amplified with adaptor-specific primers (5′-GATGAGTCCTGAG TAAN-3′). Amplified DNA fragments, with a size range of 200–800 bp, were enriched for repeats by magnetic bead selection with a 5′-biotinylated $(AC)_{15}$ and $(AG)_{15}$ and $(AAG)_{10}$ probe, respectively. Enriched fragments were amplified again with adaptor-specific primers. Polymerase chain reaction (PCR) products were purified using an EZNA Gel extraction kit (Omega Bio-Tek). Purified DNA fragments were ligated into the pGEM-T vector (Promega), and transformed into DH5αcells. Positive clones were tested by PCR using $(AC)_{10}$/$(AG)_{10}$/$(AAG)_7$ and T_7/Sp_6 as primers, respectively. In total, 180 clones with positive inserts were sequenced with an ABI PRISM 3730XL DNA sequencer. A total of 120 (67%) sequences were found to contain microsatellite repeats, and 52 of them were suitable for designing locus-specific primers, using the primer 5.0 program (Clarke and Gorley 2001).

Polymorphisms of all 52 microsatellite loci were assessed in 24 samples of *Camellia taliensis* from six natural populations in Yunnan province. The PCR reactions were performed in 15 μl of reaction containing

Table 1 Specific primer sequences and characterization for 15 microsatellite loci isolated from *Camellia taliensis*

Locus	Repeat motif	Primer sequences (5′–3′)	*T*a (°c)	Allele size (bp)	*A*	H_O	H_E	Genbank accession no.
A5	$(AG)_{12}$	F: ATGCTATTCCTCCGTCTC R: TTCAGGGATTGGTTTCTC	54	113–119	3	0.2083	0.1959	FJ542023
A37	$(AAG)_9$	F: TCTGCCCTTCCCTAAATC R: ATGTTTGGTCTCGGTTGTT	54	170–182	4	0.3750	0.6445	FJ542020
A63	$(TTC)_5$	F: CGATACCCTTCGCTCACC R: AGCCCATTCCCTTTGTCC	58	171–193	5	0.2083	0.6268	FJ542019
A74	$(AAG)_5$	F: TTTCAATATATACAATTGTTCTCTC R: TTACTCCTACTGGGCTTT	53	178–186	4	0.2083	0.3194	FJ542018
C5	$(AC)_{12}$	F: ATCAAACCCTAACCCTTCC R: GTGTTGGTGGCTTACCTT	55	231–239	3	0.4370	0.4160	FJ542017
C8	$(CT)_8$	F: ATGGGAGTAGAGGTAGGC R: AACAAACTGAACCCGTAG	54	210–240	5	0.5417	0.4033	FJ542016
G3	$(AG)_{19}$	F: TCCAAGCCTCAAGACCAA R: CAAGGACGAATAAGCAAAA	53	235–243	3	0.076	0.5680	FJ542014
G19	$(AG)_{13}$	F: ATAATCCCACAAGAAAAGTATACAG R: TTCGATAAGGATGGCAGT	53	240–310	7	0.4583	0.6056	FJ542013
G20	$(CT)_8$	F: AGCTCAAATATGCAATCA R: CAAACCTTTTCTTCTCCT	50	126–134	3	0.3333	0.2837	FJ542012
G23	$(CT)_5$	F: AAACCCTTCACTTCCTTC R: AGCCATTCCCATACACTT	53	155–171	4	0.1667	0.1560	FJ542011
G47	$(AG)_5$	F: TTCCTCCACCTCATTCAT R: AGGGGAGACAGACAAAGC	55	168–174	2	0.4583	0.4034	FJ542009
G74	$(CT)_{12}$	F: CTCTGATTCTAGACCATTCGAAGAC R: TATCCCTACCTCAACTATCA	55	250–290	5	0.2917	0.5435	FJ542007
G79	$(CT)_{11}$	F: CCCAATGAATGTCCAGAA R: ATCGACGAGTCCAGATAA	53	230–270	5	0.3750	0.6815	FJ542006
Q5	$(CT)_{15}$	F: TCTCCGATCCCAACCTAA R: CGAATAAACTGCGATAAACA	54	242–274	6	0.3333	0.5470	FJ542005
Q6	$(AG)_{23}$	F: CATTACCTCCAATCTCCG R: CACGCTCATCTCCTCTTT	55	229–263	6	0.5833	0.6917	FJ542022

*T*a, PCR annealing temperature; *A*, number of alleles revealed; H_O, observed heterozygosity; H_E, expected heterozygosity

30–50 ng genomic DNA, 0.6 μM of each primer, 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 $MgCl_2$). PCR amplifications were conducted under the following conditions: 97°C for 3 min followed by 30–36 cycles at 94°C for 30 s, at the annealing temperature for each specific primer (optimized for each locus, Table 1) for 30 s, 72°C for 1 min, and a final extension step at 72°C for 7 min. PCR products were separated on 8% polyacrylamide denaturing gel using a 20 or 10 bp ladder molecular size standard (Fermrntas; O'rangeRuler™) by silver staining.

In all, 15 primer pairs displayed polymorphisms. Standard genetic diversity parameters, departure from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium between pairs of loci were estimated in POPGENE version 1.32 (Yeh et al. 1997). The number of alleles per locus (A) was 2–7 with an average of four, values for observed (H_O) and expected (H_E) heterozygosities ranged from 0.076 to 0.5833 and from 0.156 to 0.6917, with averages of 0.3372 and 0.4724, respectively (Table 1).

For cross-species application, these 15 primer pairs were tested in other three tea plants, *Camellia taliensis*, *C. sinensis var. assamica* and *C. reticulata*. Eleven of them were successfully amplified in all species. These polymorphic SSR markers would be useful tools for population genetics studies and assessing genetic variations to establish conservation strategy and molecular identification and molecular breeding on this tea plant and its allied species and varieties in section Thea genus *Camellia*.

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