Short Note: Isolation and Characterization of Microsatellite Loci in *Castanopsis fissa* in Lower Subtropical China

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Abstract

We report on the development and characterization of ten microsatellite markers from repetitive DNA enriched libraries for *Castanopsis fissa* from lower subtropical China. The number of alleles ranged from three to thirteen. Observed and expected heterozygosities ranged from 0.265 to 0.818, and 0.270 to 0.873, respectively. These microsatellite markers will be used to study fine-scale spatial genetic structure of *C. fissa* in 20 ha Dinghushan plot in lower subtropical China.

Key words: Castanopsis fissa, microsatellite, genetic marker, population genetics, lower subtropical China, reforestation, spatial genetic structure, marker development, DNA enriched libraries, Hardy-Weinberg equilibrium, linkage disequilibrium, Dinghushan.

Castanopsis fissa Rehd. et Wils. (Fagaceae) is a fast-growing broad-leaved evergreen tree, widely distributed in lower subtropical China. It is shade-tolerant when young and need full illumination when mature (Cornelissen, 1993; Tam and Griffiths, 1994). It can adapt a wide variety of soil type, withstand low temperature, and produce a heavy leaf fall (Tam and Griffiths, 1994). These attributes make it ideal for reforestation programms (Tam and Griffiths, 1994). It is also an important economic tree, used for timber, tannin extraction, and paper pulp.

Castanopsis fissa is monoecious, with unisexual staminate and pistillate flowers on the same plant. Flowers of *C. fissa* are wind pollinated. The shape of the seed (nut) ranges from ellipsoid to ovoid. Seeds are animal dispersed. Here, we reported the development of microsatellite markers which will be used to study its spatial genetic structure in 20 ha Dinghushan plot in lower subtropical China.

Genomic DNA was extracted from one dry leaf tissue by using CTAB method (Doyle, 1991). Approximately 250 ng of the total genomic DNA was digested by a restriction enzyme *Mse*I (NEB) and the resulting fragments ligated with *Mse*I adaptor (5'-TACTCAGGACTCAT-3'/5'-GACGATGAGTCCTGAG-3') with T4 ligase (NEB) overnight at 16°C. The digestion-ligation mixture was subsequently diluted 10 times, and 2 µl was used for PCR amplification using adaptor-specific primers (5'-GATGAGTCCTGAGTAAN-3', i.e. *Mse*I-N). PCR products hybridized to a 5' biotin-labeled oligonucleotide probe $(GA)_{15}$ and $(CA)_{15}$. Subsequent probe-bound DNA fragments were enriched for GA or CA repeats using streptavidin-coated magnetic beads (NEB). Enriched fragments were recovered with PCR amplification using *Mse*I-N as primer. PCR products were then ligated into the pGEM-T plasmid vector (Promega), and transformed into the *Escherichia coli* DH5α competent cells (Takara). The PCR-based method described by Lunt et al. (1999) was used to screen the recombinant clones. Identified positive clones were sequenced by United Gene Holdings, LTD (Shanghai, China) with M13R or M13F as primer. Primers were designed using OLIGO 6.54 software (MBI) for the sequences contain microsatellite repeats.

Polymorphisms of these micosatellite loci were assessed by 34 *Castanopsis fissa* individuals collected from Dinghushan, Guangdong Province, China. PCR amplification were performed in 10 µl reaction mixtures, consisting of approximately 5 ng of template DNA, 50 mM KCl, 20 mM Tris-HCl (pH 8.0), 1.5 mM $MgCl_2$, 0.5 µM of each primer, 0.2 mM of each dNTP, and 1U of *Taq* DNA polymerase (Takara). The reaction mixture was subjected to PCR amplification in a PTC-100 (MJ) using a PCR program, 4 min at 95°, followed by 35 cycles of 94°C for 30 s, 52–64°C(depending on locus) annealing temperature for 30s, and 72°C for 30s, followed by 10 min at 72°C. PCR products were then resolved on 6% denaturing polyacrylamide gels and visualized by silver staining. The sizes of PCR products were determined with 20-bp DNA ladder (Dongshen Biotech Company, China).

Observed heterozygosity (H_O), the unbiased expected heterozygosity (H_E) and fixation index (F_{IS}) were calculated using GDA 1.1 (Lewis and Zaykin, 2001). Deviations from Hardy-Weinberg equilibrium (HWE) for each locus and genotypic linkage disequilibrium (LD) between all pairs of loci were tested using GENEPOP 4.0.7 (Raymond and Rousset, 1995; Rousset, 2008).

The number of allele varied from 3–13 with an average of 7.2 alleles pre locus. The observed and expected heterozygosities ranged from 0.265 to 0.818 and from 0.270 to 0.873, respectively (*Table 1*). One loci (Ms07) exhibited significant deviation from HWE after Bonferroni correction, which could be due to the occurrence of null alleles. Only one locus, Ms08, showed significant LD with MS03, Ms09 and Ms10 after Bonferroni correction.

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Table 1. – Details of microsatellite loci in *Castanopsis fissa* including locus name, forward and reverse primer sequences, repeat motif, annealing temperature (T_a), numbers of alleles (A), observed/expected heterozygosities (H_O/H_E), fixation index (F_{IS}) by WEIR and COCKERHAM's (1984), size range and GenBank accession number.

Locus	Primer sequence (5'-3')	Repeat motif	T_a (°C)	A	H_O	H_E	F_{IS}	Size range (bp)	GenBank accession number
Ms01	F: GTAAAGGAGGGGGCTAAGA R: CGCATACATCACTTCCGAACCCAC	$(GA)_{11}$……$(GA)_{17}$	64	6	0.471	0.604	0.223	242-263	GU097384
Ms02	F: GGGCTAGGTAAGTGGATAAT R: GCTGCAAAAGTTTCATAATATACG	$(TC)_{20}$	62	8	0.559	0.745	0.252	260-285	GU097385
Ms03	F: CCCTTCCCATAAATCGATA R: GTTGACCTAGTTCACTATTCCTCT	$(AG)_{22}$	62	13	0.727	0.873	0.169	203-242	GU097386
Ms04	F: CAGGCTCAAGGGGTCGC R: GTGGGATCACGCACTTGCTCG	$(CT)_5$ (TT) $(CT)_{12}$	64	3	0.617	0.549	-0.127	219-223	GU097387
Ms05	F: TAAGGCCATCGAGAAA R: CAAATGAAGCCATAATAGAGG	$(TG)_{13}$ (TT) $(TG)_4$	54	5	0.265	0.270	0.020	242-270	GU097388
Ms06	F: TCCCTTCTCTTTTTATCCATC R: GGTGACAATTCCAAGTCCC	$(CA)_{12}(CT)_{13}(TC)(TTC)_9$	54	11	0.818	0.793	-0.032	168-233	GU097389
Ms07	F: TATTTGGCCATTGAGC R: TTCCACCAAGAGCCTGTTGAT	$(AC)_{18}$	52	9	0.471	0.849	0.450*	139-168	GU097390
Ms08	F: CAATTCCACTAGGGCGTCTT R: GCGGGCAGGGAGTGAATGATA	$(AC)_{13}$	64	6	0.697	0.757	0.081	236-252	GU097391
Ms09	F: CCTCTGCTCCACAGGTAATCA R:CCCGTAGCAAGGAGTATC	$(TTC)_{10}$ (CT) $(CTT)_2$	64	3	0.441	0.532	0.173	261-276	GU097392
Ms10	F: CAAGTCGCAATTCTACCA R: CGCATATTAGGGAGATTAGTT	$(CA)_6$ (TA) $(GA)_{15}$	60	8	0.727	0.730	0.003	218-263	GU097394

* $P < 0.05$ after Bonferroni correction.

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