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TECHNICAL NOTE

Isolation and characterization of microsatellite loci in *Fagus longipetiolata* Seem. (Fagaceae)

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Abstract Eleven microsatellite loci have been developed from *Fagus longipetiolata* and the loci were characterized for 21 individuals. All eleven loci were polymorphic, with 2–8 alleles and an average of 4.8 per locus. The observed (H_O) and expected (H_E) heterozygosities were 0.053–0.714 and 0.355–0.856, respectively. There was significant deviation from Hardy–Weinberg equilibrium at two loci. No locus pair had significant linkage disequilibrium. Cross-species amplifications of the markers were also tested in three other congeneric species.

Keywords *Fagus longipetiolata* · Microsatellite primers · Polymorphic · Genetic diversity

The genus *Fagus* (Fagaceae) includes approximately ten species of beeches distributed in the Northern Hemisphere. These monoecious, long-lived trees are wind-pollinated, with seed dispersal by gravity and animal. Unlike their counterparts in North America, Europe and Japan, where beeches are generally continuously distributed, beeches of mainland China are confined to mountains higher than 1,000 m in the subtropical zone (Liu et al. 2003). There have been several studies on genetic structure of some beech populations in China using allozymes (Li et al. 1999). However, due to limited fresh material and low resolution of the markers used, the genetic structure and its maintenance mechanisms in beech populations have not been adequately understood. Microsatellites are a powerful and effective tool for population genetic studies. We chose *F. longipetiolata*, the most importan beech in China, for isolation and characterization of microsatellite loci. Eleven polymorphic microsatellite loci were developed and three other congeneric species were also tested to identify their universality.

Genomic DNA was extracted from leaves of one *F. longipetiolata* individual using a modified CTAB method (Fan et al. 2004). Then a biotin-streptavidin capture method (Zane et al. 2002) was used to construct a microsatellite-enriched library. Briefly, about 250 ng of genomic DNA was digested with MseI restriction enzyme (NEB) and fragments of 200–800 bp in length were fractionated. The DNA fragments were ligated with a MseI-adapter pair (Vos et al. 1995). Five microliter of a 10-times dilution of the adapter-ligated fragments were templates for PCR in a volume of 20 μl, using MseI-N (5′-GATGAGTCCTGAGTAAN-3′) as a primer, with the following temperature profile: 3 min denaturation at 95°C; followed by 30 cycles of 30 s denaturation at 94°C, 60 s annealing at 53°C, and 60 s extension at 72°C; with a final extension 72°C for 5 min. The PCR product was hybridized with 5′biton-labelled oligonucleotide $(AG)_{15}$ and captured with streptavidin-coated magnetic beads (Promega). After stringent washings, the captured DNA fragments were eluted in 50 μl of 1 × TE. The enriched product was amplified as described above. The PCR products, which were purified with a multifunctional DNA Extraction Kit (Bioteke), were ligated into pMD 18-T vector (Takara), and then transformed into *E. coli* strain DH5α. A total of 78 positive clones were chosen and tested by PCR using $(AG)_{10}$ and $M13^+/M13^-$ as primers, respectively. Fifty-six screened clones contained potential microsatellite

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Table 1 Characteristics of 11 microsatellite loci identified from *Fagus longipetiolata*, including locus name, primer sequences, repeat motif, annealing temperature (T_a), size range, no. of alleles (N_A), observed (H_O) and expected heterozygosites (H_E), and accession number of Genbank

Locus	Primer sequences (5′-3′)	Repeat motif	Ta(°C)	Size range (bp)	N_A	H_O	H_E	Genbank accession number
*Fl*05	F: GGCACATTACACCTAAATA	$(TC)_{12}$	59	153–189	6	0.667	0.792	FJ377320
	R: GCATAATAAAGAGAGGAGAC							
*Fl*13	F: TCATGGCTGAGCTTAGACAT	$(GA)_4A(AG)_7G(GA)_6$	60	136–158	4	0.650	0.675	FJ377321
	R: ACAGACCCTCATTTACATCG							
*Fl*15	F: TCTTTCATACTCATATCATCTAC	$(AG)_{12}$	56	137–179	7	0.619	0.743	FJ377322
	R: TGTCCTTATCTCTGGAACTT							
*Fl*16	F: GAATCTGGGGTTTTGAAGTG	$(GA)_{15}$	56	113–161	8	0.714	0.856	FJ377323
	R: ATCAACATCCTCTTTTTTCTCA							
*Fl*20	F: CCACTCATAACTAAATAGCCAC	$(GA)_{12}$	63	216–280	5	0.571	0.648	FJ377324
	R: GGTGAAAAGTGTAATTTACCCTTCT							
*Fl*44	F: TAAGCAGAGGATAAGAAGCC	$(TC)_{10}(AC)_6$	60	188–228	3	0.100*	0.355	FJ377325
	R: GTGACAATAAGCGATAGGAT							
*Fl*47	F: GGTTTGGGTTTGAATCGTTT	$(CT)_3(TC)_{31}$	60	112–156	5	0.619	0.775	FJ377326
	R: TGATTTGTGACTCTGTGACTTG							
*Fl*49	F: AAACAGTGGCACATACGAAT	$(CT)_{11}$	63	215–269	4	0.500	0.699	FJ377327
	R: TGGGGAGTAGAAGAGTCAGA							
*Fl*54	F: ATCAATATAAGCCTTTCCAACTG	$(TC)_{13}$	62	177–225	5	0.571	0.748	FJ377328
	R: GGTCAGCATAACCACTGTAAA							
*Fl*68	F: AACTTTGTTGTCCACTTCG	$(AG)_8$	56	203–223	2	0.053*	0.433	FJ377329
	R: GCCTTCCATCACTGGTTAGA							
*Fl*76	F: GAATCCAAAATCTTATATTCCAG	$(TC)_8A(TA)_3$	61	115–129	4	0.500	0.725	FJ377330
	R: ACACCCTTCAAATCCGTTAG							

* Indicates significant departure from Hardy–Weinberg expectation

Table 2 Cross-species amplication of the 11 microsatellites in *F. hayatae* (FH), *F. engleriana* (FE) and *F. lucida* (FL)

Species	Locus										
	*Fl*05	*Fl*13	*Fl*15	*Fl*16	*Fl*20	*Fl*44	*Fl*47	*Fl*49	*Fl*54	*Fl*68	*Fl*76
FH	–	1	2	3	1	3	2	2	4	2	3
FE	3	–	3	1	2	3	3	3	3	1	4
FL	5	4	4	7	7	4	4	3	5	3	4

The Arabic numerals are the number of alleles detected in each species at each locus

– No specific product

motifs. Sequence analysis was performed on an ABI 3730 DNA Sequence Analyzer.

PCR primers were designed for 25 sequences using program PRIMER3.0 (Rozen and Skaletsky 2000). These primers were tested for polymorphism in 21 *F. longipetiolata* individuals collected from Leigong Mountain (26°22.400′N, 108°12.350′E) of Guizhou Province, China. PCR was performed in a 20 μl final volume, which included approximately 50 ng of genomic DNA, 0.2 mM each dNTPs, 0.1 μM of each primer, 1× PCR buffer, 1.5 mM Mg^{2+}, 0.4 U of DNA *Taq* polymerase (Sangon). Microsatellite loci were amplified under the following conditions: 3 min denatuation at 94°C; 35 cycles of 30 s at 94°C, 30 s at 56–63°C (Table 1), 30 s at 72°C; and a final extension of 72°C for 5 min. Amplified PCR products were resolved on 8% polyacrylamide denaturing gel and visualized by silver staining using pUC19 DNA/MspI (*Hpa*II) (Fermentas) as the ladder.

Finally, 11 loci were found to be polymorphic (Table 1). The other 14 primers had no amplified products (4 primers), no polymorphism (1 primer), multiple bands difficult to interpret (7 primers) or failure in some individuals (2 primers). The number of alleles present at each locus was 2–8, with an average of 4.8. For each locus, we inferred observed (H_O) and expected (H_E) heterozygosities with the software GENEPOP v4.0 (Rousset 2008). Hardy–Weinberg equilibrium and linkage disequilibrium (LD) were tested using GENEPOP v4.0 (Rousset 2008) followed by the sequential Bonferroni correction (Rice 1989). The H_O and H_E were 0.053–0714 and 0.355–0.856, respectively. There was significant deviation from Hardy–Weinberg equilibrium at two loci due to heterozygote deficits (Table 1). No primer pair had significant linkage disequilibrium.

For cross-species amplification, nine of them were successfully amplified in the three other beeches. *Fl*05 failed to amplify in *F. hayatae* and *Fl*13 failed to amplify in *F. engleriana* (Table 2). These polymorphic microsatellite loci would be useful tools for studying the demography and population structure of genus *Fagus*.

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