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Isolation and characterization of microsatellite loci in *Quercus fabri* (Fagaceae)

Z.Z. Xiao, W.W. Chen, W. Bao, R. Wang and Y.Y. Li

School of Ecological and Environmental Sciences,
Tiantong National Station of Forest Ecosystem,
Shanghai Key Laboratory for Urban Ecology and Restoration,
East China Normal University, Shanghai, China

Corresponding author: Y.Y. Li
E-mail: yyli@des.ecnu.edu.cn

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ABSTRACT. *Quercus fabri* is a pioneer species of secondary succession in evergreen broadleaved forests in China. In this study, we isolated and developed 12 polymorphic and 2 monomorphic microsatellite loci for *Q. fabri* using the biotin-streptavidin capture method. We characterized 12 polymorphic loci in 52 individuals from two populations. The number of alleles per locus ranged from 3 to 23. The observed and expected heterozygosities per locus were 0.033-0.773 and 0.138-0.924, respectively. These microsatellite loci will facilitate the studies on genetic variation, mating system, and gene flow of *Q. fabri*.

Key words: Ecological restoration; Fagaceae; Genetic diversity; *Quercus fabri*; Simple sequence repeat

INTRODUCTION

Quercus fabri Hance (Fagaceae) is a common deciduous and nut-bearing tree in subtropical forests in China. Due to its ability to grow in barren soils, high capacity to sprout, and tolerance to disturbance, *Q. fabri* is not only a pioneer species for secondary succession of evergreen broadleaved forests (EBLFs) but also plays an important role in the recovery of EBLFs, which have suffered from serious anthropogenic destructions in China and need to be restored and managed efficiently (Song and Chen, 2007). It is therefore necessary to identify the genetic variation of *Q. fabri* because a better understanding of the genetic background of early successional species will greatly contribute to the success of preliminary stages in forest recovery (Davies et al., 2015).

Microsatellites are co-dominant and highly polymorphic markers that provide an outstanding tool to detect genetic variation and have been widely used in the study of population genetics (Liu et al., 2009). In this study, 12 polymorphic and two monomorphic microsatellites in *Q. fabri* were isolated and characterized for further study of the population genetic diversity, gene flow, and parentage relationship for this species.

MATERIAL AND METHODS

Genomic DNA was extracted from silica-gel-dried leaves of one *Q. fabri* individual using the Plant Genomic DNA Extraction Kit (Tiangen, Beijing, China). This individual was collected from Tiantong, Zhejiang Province, China. Microsatellite loci were developed according to the protocol of Tong et al. (2012). A total of 250 ng genomic DNA was digested with the *Mse*I restriction enzyme (New England Biolabs, Beverly, MA, USA) and fragments 200-800 bp in length were produced. An *Mse*I-adapter pair (forward: 5'-TACTCAGGACTCAT-3', reverse: 5'-GACGATGAGTCCTGAG-3') was then attached to the DNA fragments instantly and the fragments were then amplified with an *Mse*I-N primer (5'-GATGAGTCCTGAGTAAN-3') in a 20-µL reaction system using the following conditions: 95°C (3 min), followed by 23 cycles at 94°C (30 s), 53°C (60 s), and 72°C (60 s). After samples were denatured at 95°C for 5 min, the PCR products were hybridized with a 5'-biotinylated oligonucleotide probe $(AG)_{15}/(AC)_{15}$ in a 250-µL solution at 48°C for 2 h. The products were captured with streptavidin-coated magnetic beads (Promega Corporation, Madison, WI, USA), and then amplified with the *Mse*I-N primer again and purified with a multifunctional DNA Extraction Kit (BioTeke, Beijing, China). Following ligation into the pMD 19-T vector (TaKaRa Biotechnology Co., Dalian, China), the products were transformed into the *Escherichia coli* strain JM109 by transient thermal stimulation. A total of 943 clones were tested by PCR using $(AG)_{10}/(AC)_{10}$ and $M13^{+}/M13^{-}$ as primers, respectively. Among these, 242 positive clones were chosen and sequenced on an ABI 3730 DNA Sequence Analyzer (Applied Biosystems, Foster City, CA, USA). Finally, 21 sequences were selected for use in the design of microsatellite primers with the Primer Premier 5.0 program (PREMIER Biosoft International, Palo Alto, CA, USA).

To test the performance and polymorphism for each locus, 24 *Q. fabri* individuals that were randomly selected from two populations in Tiantong, and Gutian, Zhejiang

Province, China, were assessed following the method proposed by Schuelke (2000). For fluorescent labeling of PCR fragments manually, three primers were prepared: a sequence-specific forward primer with an M13(-21) tail (5'-TGTAAAACGACGGCCAGT-3') at its 5'-end, a sequence-specific reverse primer, and a fluorescently (6-FAM, HEX, or ROX) labeled M13(-21) primer (Sangon Biotech, Shanghai, China). The PCR method was performed in a 15-µL reaction system, including 50 ng genomic DNA, 0.2 mM each dNTP, 0.2 µM reverse primer, 0.2 µM fluorescent-labeling M13(-21) sequencing primer, 0.05 µM forward primer with M13(-21) tail, 1X PCR buffer, 1.5 mM Mg^{2+}, and 0.4 U DNA *Taq* polymerase (Sangon Biotech). The PCR amplification conditions were as follows: 94°C (5 min); 30 cycles at 94°C (30 s), 56°-63°C (45 s) (depending on the specific locus; Table 1), and 72°C (45 s); followed by 8 cycles 94°C (30 s), 53°C (45 s), and 72°C (45 s); and a final extension at 72°C for 10 min on a Mastercycler® Pro thermal cycler gradient (Eppendorf, Hamburg, Germany). The PCR products were then scanned on an ABI 3730 automated sequencer (Applied Biosystems) using the internal lane standard (GS-500 LIZ) and analyzed by the GeneMapper 4.0 software (Applied Biosystems).

All polymorphic loci were further characterized in the 52 individuals collected from Tiantong and Gutian using the method proposed by Schuelke (2000). Voucher specimens were deposited at East China Normal University, China. We performed the PCR amplification for each polymorphic locus separately and scanned the products using an ABI 3730 genetic analyzer. The alleles were then binned using GeneMapper 4.0 (Applied Biosystems).

For each polymorphic locus, the number of alleles was estimated using the GENEPOP v4.0 software (Rousset, 2008). The observed (H_O) and expected (H_E) heterozygosities, Hardy-Weinberg equilibrium (HWE), and linkage disequilibrium were analyzed using the TFPGA version 1.3 software (Miller, 1997).

RESULTS AND DISCUSSION

We obtained clear PCR products from 14 of the 21 primer pairs tested, among which 12 loci were polymorphic and 2 loci were monomorphic (Table 1). In the 12 primer pairs tested, the number of alleles per locus varied from 3 to 23, with an average of 9.2 (Table 1). At the population level, H_O and H_E were 0.033-0.773 and 0.138-0.924, respectively (Table 1). No significant linkage disequilibrium ($P > 0.05$) was found for any pair of loci across either population. After sequential Bonferroni adjustment (Rice, 1989), five loci (QF04, QF05, QF14, QF17, and QF20) in the Tiantong population and five loci (QF01, QF05, QF10, QF14, and QF17) in the Gutian population significantly deviated from HWE ($P < 0.05$) (Table 1), most likely due to heterozygote deficiency or population structure within the samples.

We successfully developed 12 polymorphic and two monomorphic microsatellite loci for *Q. fabri*. These primers will facilitate the analysis of population genetic structure and gene flow in *Q. fabri* (Liu et al., 2015). The use of these markers in parentage analyses will provide key information on mating system, seed dispersal, and seedling recruitment of *Q. fabri* for a better understanding of the succession of EBLFs.

Table 1. Characterization of 12 polymorphic and 2 monomorphic microsatellite loci in two *Quercus fabri* populations.

Locus	Primer sequence (5'-3')	Repeat motif	Size range (bp)[a]	Ta (°C)	GenBank accession No.	Total			Tiantong (29°48'N, 121°47'E)				Gutian (29°14'N, 118°06'E)			
						N_A	H_O	H_E	N	N_A	H_O	H_E	N	N_A	H_O	H_E
QF01	F: <FAM>AATAATCATAACGCCTCCAC	$(AG)_{11}$	167-197	60	KU194476	15	0.5000	0.8947	27	12	0.5185	0.8931	21	13	0.4762*	0.8479
	R: CCAATGAACACCCTTACTCT															
QF02	F: <ROX>AGCAAGACCACAACAATGAC	$(AG)_9$	113-141	61	KU194477	5	0.6538	0.7093	30	5	0.6000	0.6791	22	5	0.7273	0.7516
	R: GCGGTTGGATTAGTAAAGCT															
QF03	F: <FAM>TTACTGGCTAGGACAATTCA	$(CT)_{13}$	178-202	56	KU194478	10	0.7500	0.8236	30	10	0.7667	0.8085	22	8	0.7273	0.8446
	R: GTAGGGAAATTGTTAGTGGG															
QF04	F: <FAM>CTAAGTAATCCTCACCACCG	$(CA)_{5-}(TC)_7$	123-151	63	KU194479	14	0.5385	0.8926	30	13	0.6000*	0.8802	22	13	0.4545	0.9070
	R: AAGTTTTGTCAGTGTCAGGC															
QF05	F: <HEX>ACCAAAAGGAGGGGATACAT	$(AC)_6$	206-212	59	KU194480	4	0.1200	0.5269	30	4	0.1000*	0.5023	21	4	0.1905*	0.5726
	R: TGAAAATAGGGGCAGAAAGT															
QF06	F: <HEX>AGATCAGCTCACTTCATCCCTT	$(GA)_{19}$	196-298	63	KU194481	23	0.7000	0.9337	29	17	0.6897	0.9244	21	16	0.7143	0.9164
	R: CACGCTTCTGACTATTATCAACTC															
QF08	F: <FAM>TACCCAACCTATTCCACCAC	$(CT)_8$	100-126	63	KU194482	9	0.3922	0.7166	29	8	0.3448	0.7562	22	6	0.4545	0.6617
	R: AGACCTCGAAACTTTGCCTC															
QF10	F: <ROX>ATTCCACCTCAGCCCTCA	$(CA)_5$	129-133	62	KU194483	3	0.6538	0.4563	30	3	0.5667	0.4220	22	3	0.7727*	0.5021
	R: CGGAAAGCGTGATTCATAG															
QF14	F: <ROX>CAGACATACAATTCAGCACA	$(AG)_{10}$	154-182	56	KU194484	13	0.3265	0.8662	28	12	0.3571*	0.8643	21	10	0.2857*	0.8688
	R: CCCTATGTAAGGAAAGAGTG															
QF15	F: <FAM>GCGGTGTAACTCACATGGAC	$(AG)_7$	125-129	62	KU194485	3	0.4038	0.5267	30	3	0.3667	0.4797	22	3	0.4545	0.5539
	R: CGTGAGGCGTGAGATATGGT															
QF17	F: <ROX>GGTTTTGAAACACTGGAAGA	$(AG)_5$	155-191	56	KU194486	8	0.2692	0.6645	30	6	0.2333*	0.6836	22	6	0.3182*	0.6300
	R: CCACTTGGAAACAACTCATG															
QF18[#]	F: <ROX>AGCCATTGTCAACCCTAAC	$(GA)_5$	142	59	KU194487	1	-	-	-	-	-	-	-	-	-	-
	R: TTCTAGTCGATGCAACCTC															
QF20	F: <FAM>ACAAGCTGCCCATGACTC	$(TC)_3GT(AC)_5$	124-128	63	KU194488	3	0.0196	0.0577	30	3	0.0333*	0.1588	21	3	0.0476	0.1382
	R: CCGTGGTGGTTTGGAGTA															
QF21[#]	F: <ROX>CCAAACCCAAACCAATGAG	$(GA)_5$	133	61	KU194489	1	-	-	-	-	-	-	-	-	-	-
	R: TCACCATATCAGAGCAAGC															

N_A = number of alleles; N = number of samples genotyped; Ta = annealing temperature using the genotyping protocol of Schuelke (2000); H_E = expected heterozygosity; H_O = observed heterozygosity; [a]the allele sizes of the PCR products including the length of the fluorescent-labeling M13 (-21) sequencing primer (18 bp). *Significant deviation from Hardy-Weinberg equilibrium ($P < 0.05$). [#]Monomorphic microsatellite loci.

Conflicts of interest

The authors declare no conflict of interest.

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