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Short Note: Development and characterization of 16 new polymorphic microsatellite loci for *Schima superba* (Theaceae)

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Abstract

Schima superba is a common dominant tree species in evergreen broad-leaved forest in subtropical China. Despite its multiple usages in wood industry, reforestation and traditional Chinese medicine, its genetic diversity is poorly studied. To help studying its genetic diversity and structure in the future, after microsatellite enrichment and screening, we identified 16 microsatellites in *S. superba*. These markers showed polymorphism in three populations. The number of alleles per locus ranged from 3 to 32 with a mean of 14. Within populations, the observed and unbiased expected heterozygosities ranged from 0.048 to 0.926 and from 0.048 to 0.949, respectively. The newly developed 16 microsatellites will be useful for investigating the genetic diversity and structure from large scale patterns to fine-scale structures in this species.

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Key words: DHS plot, DNA enriched libraries, genetic marker, genetic variation, Hardy-Weinberg equilibrium, linkage disequilibrium, marker development, microsatellite, population genetics, reforestation, spatial genetic structure, subtropical China.

Schima superba Gardener & Champ. (Theaceae) is a common evergreen fast growing pioneer tree species in south and east China. Its wood is hard and bug resistant and can be used in furniture and construction. Its root bark has been used as a traditional Chinese medicine, and stem extracts inhibit some cancers (Xu et al., 2010). It has been widely used in reforestation and fire-preventing in plantations because its leaves are thick and contain high water content (Yang et al., 2008; Li et al., 2011). In large scales, it contains high variations in seed mass, leaf morphology and wood color among different sites and populations (Zhang et al., 2004; Wang et al., 2011). In local scales, there were 2290 individuals with DBH ≥ 1 cm recorded in 2005 census in the 20 ha Dinghushan forest dynamics plot (DHS plot, 23°10'09''–23°10'25''N, 112°32'05''–112°32'21''E) in Guangdong province, China, and they occupy different topographies with different age structure. Therefore, it would be interesting to know the contribution of genetic

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Table 1. – Characterization of 16 microsatellites for *Schima superba*. T_a: annealing temperature; N_a: number of alleles.

Locus	Repeat motifs	Primer sequences (5'-3')	Size range (bp)	T_a (°C)	N_a	5'-fluorescence label	GenBank Accession No.
SS37	$(CA)_{10}$	F: AGAGGCTACCCAGCAATG	199-209	52	5	HEX	JX448324
		R: GCACAGGTCTCAGAAAGCA					
SS38	$(AC)_9(TC)_9$	F: TGGATGCTCTGCTTCTTC	278-331	57	23	FAM	JX448325
		R: AGTGGTTGCCATTGACGG					
SS39	$(A)_{12}(CAA)_8$	F: TCCGAAGCAAAACCACAAG	231-238	55	8	FAM	JX448326
		R: AAGGAGGGGATGGTTGTT					
SS40	$CACATT(CACACT)_4CACATT$	F: TCCACCCATCAACCAATAG	252-264	52	3	FAM	JX448327
		R: AGTCACGATCCAAAGCAG					
SS41	Complex [a]	F: GCACCACTCTTCCTCTTT	222-278	55	32	FAM	JX448328
		R: ATCAGCCAAGCATCCGTC					
SS42	$CAAGG(CAA)_2CAG(CAA)_3TGGCAA$	F: TTAGCCTCTGACACCACA	261-282	55	5	FAM	JX448329
		R: GGAACATGAATCCAAACC					
SS43	$(CA)_8(A)_{15}$	F: GTTCCGCTTATCGTCGTG	208-233	52	19	FAM	JX448330
		R: TTAGGGAACAATGCCATC					
SS44	$(CA)_{27}$	F: TCCAACCGATAGATGAGC	251-348	55	22	FAM	JX448331
		R: CAAACCTACCCACGAAGC					
SS45	$CAATAA(CAA)_7$	F: CAATAGCACCACCAGCAG	193-293	52	9	HEX	JX448332
		R: GATTGCCAAGTGGTGTCT					
SS46	$(CAA)_4(AGG)_2$	F: GCGTCTTCCAGTGAGCAT	265-280	55	6	FAM	JX448333
		R: TAACTTGCCTCCCTCCTG					
SS47	$(CA)_{14}$	F: TAGGTAGGTGCTGGAGTAG	212-224	57	8	FAM	JX448334
		R: CAGGTTTACATCATTAGGC					
SS48	$AATAAGAATG(AAG)_5$	F: GATAGAAGTAAACATCAGGC	215-221	52	3	HEX	JX448335
		R: GTTGCACAAGTTGTCCAAT					
SS49	$(ATG)_7(AG)_3$	F: GATGATTCTCGGTTTCTTG	207-219	52	4	FAM	JX448336
		R: GTTATGTTGTTGGCGTTG					
SS50	$(AG)_{24}$	F: CACTGGTGTCACTGCTTCTATG	241-299	57	29	FAM	JX448337
		R: CCTTTAGTGGAACGGCATC					
SS51	$(AG)_{23}$	F: CAACGAGGAGTAAATGATG	263-294	55	19	FAM	JX448338
		R: AAAGAGTGGATACTGGTGT					
SS52	$(AG)_{12}$	F: ATGGAAGAGTAAGGGGAATC	190-296	55	24	FAM	JX448339
		R: CCTTTATTAGTTGGGAGC					

[a] $(TGC)_8CGCCATTGAAGAAGAAACCCC(AC)_4AGACGC(AC)_3TCACCAGAGACAGGAGACAGAAAAC(AG)_{13}$.

diversity to its phenotypic diversity in large scale and to its population persistence in small scale. However, up to now, its genetic variations are poorly known. In this work, we reported newly developed polymorphic microsatellites in order to better study *Schima superba* genetic variation in future.

Total genomic DNA was extracted, using a modified CTAB method, from one dry leaf tissue of *S. superba* in the DHS plot. The 300–1000 bp fragments digested from genomic DNA using restriction enzyme *Mse*I [New England Biolabs (NEB), Massachusetts, USA] were ligated to *Mse*I adaptors (*Mse*I F: 5'-TACTCAGGACTCAT-3' and *Mse*I R: 5'-GACGATGAGTCCTGAG-3') using T_4 DNA ligase (NEB). The digestion-ligation mixture was subsequently diluted 10 times, and 2 µL of the diluted was used for PCR amplification using *Mse*I-adaptor specific primers (5'-GATGAGTCCTGAGTAAN-3', i.e., *Mse*I-N). Amplified products were then hybridized with 5'-biotinylated $(AG)_{15}$, $(AAG)_8$, $(AC)_{15}$ and $(AAC)_8$ probes. Subsequent probe-bound DNA fragments were enriched by streptavidin-coated magnetic beads (NEB). The enriched fragments were recovered with PCR reaction using *Mse*I-N as primer. The selected fragments were then ligated into the pGEM-T plasmid vector (Promega Corp, Wisconsin, USA) and transformed into the *Escherichia coli* DH5α competent cells (Takara Bio Inc., Shiga, Japan). A PCR-based method (Lunt et al., 1999) was used to screen the recombinant clones. Ninety-three positive clones were identified and sequenced by Majorbio Biotech Co., LTD (Shanghai, China) with M13R or M13F as primers. Primers were designed using PREMIER 5.0 (PREMIER Biosoft International, Palo Alto, CA, USA) for the sequences that contained repeats motifs.

To assess the polymorphisms of the microsatellites, leaves were collected from 32, 32 and 28 randomly selected *S. superba* trees in Zhaoqing (23°10'1"N, 112°32'21"E), Dongguan (22°54'39"N, 114°13'21"E) and

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Table 2. – Genetic diversity of 16 loci in three populations of *Schima superba*. *N*: sample size; N_a: number of alleles; H_o: observed heterozygosity; H_e: unbiased expected heterozygosity; *F*: fixation index.

Locus	POP-Zhaoqing (*N* = 32)				POP-Dongguan (*N* = 32)				POP-Shaoguan (*N* = 28)			
	N_a	H_o	H_e	*F*	N_a	H_o	H_e	*F*	N_a	H_o	H_e	*F*
SS37	2	0.048	0.048	-0.024	5	0.250	0.285	0.110*	5	0.560	0.598	0.044
SS38	12	0.880	0.837	-0.073	14	0.759	0.878	0.121	19	0.889	0.927	0.023
SS39	6	0.148	0.418	0.639*	5	0.172	0.575	0.695*	5	0.370	0.766	0.507*
SS40	2	0.393	0.431	0.071	3	0.281	0.426	0.329	3	0.357	0.309	-0.176
SS41	12	0.320	0.863	0.622*	20	0.679	0.949	0.272	20	0.333	0.937	0.638*
SS42	4	0.179	0.260	0.302*	2	0.563	0.411	-0.391	3	0.643	0.473	-0.383
SS43	15	0.467	0.853	0.444*	10	0.217	0.860	0.742*	10	0.304	0.887	0.649*
SS44	12	0.500	0.802	0.365*	15	0.357	0.916	0.603*	9	0.292	0.839	0.645*
SS45	7	0.519	0.628	0.159	8	0.813	0.804	-0.027	7	0.769	0.766	-0.024
SS46	5	0.667	0.730	0.070	6	0.613	0.797	0.218	6	0.750	0.807	0.054
SS47	7	0.640	0.763	0.144*	6	0.806	0.682	-0.202	5	0.520	0.699	0.241
SS48	3	0.577	0.551	-0.067	3	0.813	0.637	-0.296	3	0.692	0.541	-0.305
SS49	4	0.679	0.588	-0.176	4	0.769	0.645	-0.216	4	0.880	0.633	-0.419
SS50	17	0.926	0.931	-0.014	20	0.813	0.920	0.103	19	0.875	0.936	0.045
SS51	13	0.571	0.894	0.349*	12	0.281	0.891	0.679*	12	0.320	0.869	0.624*
SS52	12	0.704	0.898	0.202	19	0.581	0.911	0.352*	17	0.808	0.925	0.109

* ($P < 0.05$) indicates a significant deviation from Hardy-Weinberg equilibrium after Holm's sequential Bonferroni correction.

Shaoguan (24°46'53"N, 113°36'16"E) populations in Guangdong province, respectively. PCR amplifications were performed in a 20 µL reaction mixture containing 20 mM Tris-HCl (pH 8.4), 100 mM $(NH_4)_2SO_4$, 3 mM $MgCl_2$, 0.4 mM dNTPs, 0.4 µM each primer (5' end fluorescent dye labeled with FAM or HEX), 50 ng of genomic DNA, and 1 U Taq polymerase (Takara). The amplification program was 95 °C for 5 min, 35 cycles of 94 °C for 30 s, optimized annealing temperature for 30 s and 72 °C for 45 s, and a final extension at 72 °C for 10 min. PCR products were electrophoresis-analyzed on ABI 3730 sequencer (Applied Biosystems Inc., California, USA) and the lengths of them were analyzed by ABI GeneMapper Software Version 3.7. Number of alleles (N_a), observed and unbiased expected heterozygosities (H_o, H_e) and fixation index (*F*) were obtained using GenAlEx 6.2 (Peakall and Smouse, 2006). Deviation from Hardy-Weinberg equilibrium (HWE) and genotypic linkage disequilibrium among all pairs of loci in each population were analyzed with GENEPOP 3.4 (Raymond and Rousset, 1995). Significance levels were adjusted using Holm's sequential Bonferroni correction (Holm, 1979) implemented in R "stats" package (R Development Core Team, 2011).

A total of 16 polymorphic microsatellite loci were amplified with clear and stable polymorphism (*Table 1*). Overall, alleles per locus varied from 3 to 32 with a mean of 14 (*Table 1*). Within populations, H_o ranged from 0.048 to 0.926, and H_e from 0.048 to 0.949 (*Table 2*). Four loci showed significant deviation from HWE in one population each, one locus in two and four loci in all three populations (*Table 2*). No consistently significant linkage disequilibrium was detected among all pairs of loci within each population, indicating the independence of these 16 microsatellite markers.

The polymorphic microsatellites developed here will be powerful genetic tools for studying the genetic diversity and structure of *S. superba*. Such genetic information will help to classify the germplasm resource, understand adaptation, and then establish conservation policy for *S. superba*. They will also be useful in studying fine-scale spatial genetic structure in DHS plot, which is important in analyzing population dynamics and persistence in the local population (Epperson, 1992).

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