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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*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 Dinhushan 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 markers for black poplar (*Populus nigra* L.). Mol Ecol Notes 1: 188–190.


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 (5'-TACTCAGGACTCAT-3' and 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/H9251 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

<table>
<thead>
<tr>
<th>Locus</th>
<th>Repeat motifs</th>
<th>Primer sequences (5'–3')</th>
<th>Size range (bp)</th>
<th><em>T</em>$_{m}$ (°C)</th>
<th><em>N</em>$_{a}$</th>
<th>5'-Fluorescence labeled</th>
<th>GenBank Accession No.</th>
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</table>
| SS37  | (CA)$_{30}$ | F: AGAGGC AACCAGAACATG  
R: GCACAGCTCCACAAAGCA | 199-209 | 52 | 5 | HEX | JX448324 |
| SS38  | (AC)$_{30}$(1C)$_{3}$ | F: IUGAICICTGCTCTTC  
R: AGTGCCTCCATTGAACGG | 278-331 | 57 | 23 | FAM | JX448225 |
| SS39  | (A)$_{10}$(CAA)$_{4}$ | F: TCCAAGCCAAACCCCAACAG  
R: AGGAAGGGGTAGTTGTT | 231-238 | 55 | 8 | FAM | JX448236 |
| SS40  | CACAT(T)(CACACT)TCACTT | F: TTCAACCCATCAACCAATAG  
R: AGTACAGAACCAAAGCAG | 252-264 | 52 | 3 | FAM | JX448279 |
| SS41  | Complex a | F: GCCACACCTTCTCTCTTT  
R: AGTACAGAACCAAAGCAG | 224-278 | 55 | 5 | FAM | JX448329 |
| SS42  | CAAGG(G)(CAA)$_{2}$CAG(CAA)$_{2}$TGGCAA | F: TTAGAGTTCCACACACCCAAC  
R: GGAACATGAACTCCAACCC | 208-233 | 52 | 19 | FAM | JX448330 |
| SS43  | (CA)$_{12}$(AG)$_{2}$ | F: TTAGAGTTCCAATCAGC | 251-348 | 55 | 22 | FAM | JX448331 |
| SS45  | CAAATAA(CAA)$_{2}$ | F: CAATAGCACACCACGCAG  
R: CAAACCTACCACCCAGAGC | 193-292 | 52 | 9 | HEX | JX448332 |
| SS46  | (CA)$_{12}$(AG)$_{2}$ | F: GGTAGCCTCTCAGGACAT  
R: TAACTCCTCCCTCCCCTC | 265-295 | 55 | 6 | FAM | JX448333 |
| SS47  | (CA)$_{12}$ | F: TTAGAGTTCCAATCAGC  
R: CAGTAGTTAACACATGAGG | 212-224 | 57 | 8 | FAM | JX448334 |
| SS48  | AA7AAGAAATG(AG)$_{2}$ | F: GATGAAAGAATCAACATGAGC  
R: GTTGCAAAAGTTTCCCAAT | 215-221 | 52 | 3 | HEX | JX448335 |
| SS49  | (ATG)$_{2}$(AG)$_{2}$ | F: GATGATTCTGCTGTTTCTTG  
R: TTAGTATTTAGTTGGCGGTT | 207-219 | 52 | 4 | FAM | JX448333 |
| SS50  | (AG)$_{12}$ | F: CACTGGGTCACCTCTCAAGAT  
R: CCTTTTAGTAGAAACGCATC | 241-299 | 57 | 29 | FAM | JX448337 |
| SS51  | (AG)$_{12}$ | F: CAGAGGAGTAAATGAGT  
R: AAAGAGTGAGTAACACGTTG | 263-294 | 55 | 19 | FAM | JX448338 |
| SS52  | (AG)$_{12}$ | F: ATGGAAAGAATTAGGGAATTC  
R: CCTTTTAGTAGTTGGGACG | 190-296 | 55 | 24 | FAM | JX448339 |

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Table 1. – Characterization of 16 microsatellites for *Schima superba*. $T_m$: annealing temperature; $N_a$: number of alleles.
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 (NH₄)₂SO₄, 3 mM MgCl₂, 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 S MOUSE, 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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References

