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TECHNICAL NOTE

# Isolation and characterization of 12 microsatellite loci for *Michelia coriacea* (Magnoliaceae), a critically endangered endemic to Southeast Yunnan, China

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**Abstract** By using a modified biotin–streptavidin capture method, a total of 12 microsatellite loci were developed and characterized for *Michelia coriacea* (Magnoliaceae), a critically endangered endemic to Southeast Yunnan, China. The number of alleles ($A$) ranged from two to six in 30 samples of this species. The ranges of observed ($H_O$) and expected ($H_E$) heterozygosities were 0.033–0.8000 and 0.033–0.7910, respectively. Cross-species amplification in *M. yunnanensis* showed that a subset of these markers holds promise for congeneric species. These polymorphic SSR markers would be useful tools for population genetics studies on *M. coriacea* and other congeneric species.

**Keywords** Genetic diversity · *Michelia coriacea* · Population structure · SSR markers

*Michelia coriacea* (Magnoliaceae) is a newly described species endemic to Southeast Yunnan, China (Chen 1988). It's scattered on limestone mountain slopes or secondary shrubby woods and on roadsides at altitude of 1400–1700 m (Cicuzza et al. 2007). The natural habitats had been badly degraded and fragmented due to heavy logging and vegetation destruction in the past decade in sub-populations that are isolated from each other. *M. coriacea* has been recently evaluated as a critically endangered tree based on IUCN criteria (Cicuzza et al. 2007). As the species could bear abounding flowers almost every year, but its fruit and seed set are very low in the field, and thus the process of megaspore and microspore genesis and the development of gametophytes were recently observed (Zhao et al. 2008; Zhao and Sun 2009). As a critically endangered plant, the effective and long-team conservation is urgently needed. In present study, we have developed and characterized 12 microsatellite markers for *M. coriacea* that will be used for further studies of genetic diversity, population structure and gene flow of the species.

A microsatellite enriched library was conducted by using a modified biotin–streptavidin capture method (Chen et al. 2008). Briefly, the genomic DNA (about 500–800 ng) was completely digested with *Mse*I restriction enzyme (NEB). And then the digested fragments were ligated to *Mse*I AFLP adaptor following by amplification with adaptor-specific primers (5′-GAT GAG TCC TGA GTA AN-3′) (Huang et al. 2008). For enriching the fragment (about 300–800 bp) contained SSR, the PCR products were hybridized to a mixture of biotinylated probes [$(AAG)_{10}$, $(AC)_{15}$, $(AG)_{15}$] (Zane et al. 2002). The purified PCR products were ligated into PGEM-T vector (Promega), and transformed into *E. coli* strain DH5α (Tiangen). The positive clones were picked out and tested using $(AAG)_7$/$(AC)_{10}$/$(AG)_{10}$ primers and vector primers SP6/T7, respectively. A total of 176 clones were chosen for sequencing with an ABI PRISM 3730XL SEQUENCER. In all, 102 clones (58%) were found to contain microsatellite sequences. Finally, 66 pairs of SSR primers were

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selected for primer designing using Primer Premier 5.0 program (Clarke and Gorley 2001).

Primer pairs were assessed in 30 wild *M. coriacea* samples from Southeast Yunnan, China. Microsatellite loci were amplified in a final volume of 15 µl reaction containing 7.5 µl 2× Taq PCR MasterMix (Tiangen; 0.1 U Taq Polymerase/µl, 0.5 mM dNTP each, 20 mM Tris-HCl (Ph8.3), 100 mM KCl, 3 mM MgCl2), 0.6 µM of each primer, and about 50 ng genomic DNA. The amplification profiles included initial denaturation at 94°C for 3 min; followed by 35–40 cycles of 30 s at 94°C, 30 s at 54–68°C and 1 min at 72°C; then final extension at 72°C for 7 min. The amplified products were then separated on 6% denaturing polyacrylamide gels and visualized by silver staining. A 20 bp DNA ladder standard (Fermentas) was used as standard for scoring.

The variability at each locus was measured in terms of number of alleles ($A$), observed ($H_O$) and expected heterozygosity ($H_E$) for the 12 microsatellite loci using GENEPOP version 4.0 (Raymond and Rousset 1995). The same program was used to test the deviations from Hardy–Weinberg equilibrium (HWE) and pair-wise linkage disequilibrium. The number of alleles per locus ($A$) was 2–6 with an average of 3.667, values for observed ($H_O$) and expected ($H_E$) heterozygosities ranged from 0.0333 to 0.8000 (averaged at 0.3861) and from 0.0333 to 0.7910 (averaged at 0.4533), respectively (Table 1). Four loci (MC-8, MC-45, MC-49, and MC-66) deviated significantly from the Hardy–Weinberg equilibrium (HWE) ($P < 0.01$). No significant linkage disequilibrium was detected between locus pairs except for locus pair MC-8 and MC-64. Cross-species amplification was further investigated using 8 wild individuals from one population of *M. yunnanensis*. About eight of 12 loci revealed polymorphism (Table 1).

These primers amplifying microsatellites may provide a useful tool for understanding population genetic structure

**Table 1** Characteristics of 12 microsatellite loci developed for *Michelia coriacea* and cross-species amplification of one congeneric species, *M. yunnanensis*

| Locus | Primer Sequence (5′–3′) | Repeat motif | Size range (bp) | *Ta* (°C) | *A* | $H_O$ | $H_E$ | *M. yunnanensis* |
|---|---|---|---|---|---|---|---|---|
| MC-2 | F CCATTTCTTTGGCTTTTTC<br>R ATCCTTGGTCGTATTCGTG | $(CA)_8$ | 192–206 | 60 | 3 | 0.4333 | 0.6119 | P |
| MC-3 | F CCGCAGCAAACCCTACGC<br>R GCACACGCCAACAATGGAAAG | $(CT)_{16}$ | 292–306 | 68 | 3 | 0.5333 | 0.6333 | P |
| MC-8 | F GAGTTCCGTGAGTCCCA<br>R CATAATAGAAGTCATAAATCCC | $(TC)_{15}$ | 189–211 | 58 | 6 | 0.8000 | 0.7910* | W |
| MC-11 | F TCAACAGCACAAACCGAC<br>R AGAACAACAGAACCAGGG | $(CT)_4$–$(CT)_3$–(CT)–$(CT)_2$ | 181–201 | 55 | 2 | 0.0333 | 0.0333 | M |
| MC-34 | F GAAATCATCGGAAAAGCG<br>R TCTCTCATTCAGCCCATACA | $(TC)_4T_3(TC)_3$–$(TC)_4$ | 156–160 | 62 | 4 | 0.6333 | 0.6412 | M |
| MC-35 | F TACATCTAAAGTCCCCACAT<br>R CTCATTTCCAGCCCATAC | $(CT)_3$–$(CT)_4$–$(CT)_3$ | 375–381 | 62 | 3 | 0.2667 | 0.3712 | P |
| MC-41 | F TTGGACCTGAACTCAACTCG<br>R GCCAGCCTACCCTTTACG | $(TTTC)_4$–$(CT)_{10}$ | 216–232 | 60 | 3 | 0.6000 | 0.5215 | P |
| MC-45 | F CTCCACCACCACTGATAC<br>R GGATGGGACGACATAGAT | $(CT)_{10}T_2(CT)_2$ | 116–124 | 62 | 3 | 0.1000 | 0.2164* | P |
| MC-48 | F TACAGCCACCCTTTCCA<br>R CTTGGATGTGGGTTGCTT | $(TC)_8$–$(TC)_5$–$(TC)_6$ | 137–157 | 58 | 4 | 0.3667 | 0.4847 | P |
| MC-49 | F TCAAGACCTCCCCTTACCT<br>R GGTTGGACATCAATGGTTC | $(TC)_8$ | 140–158 | 64 | 5 | 0.2000 | 0.4090* | P |
| MC-64 | F TCTAACCCTACCCCGACC<br>R GGACTGAGTCTGATCCCGTA | $(CT)_4$–$(CT)_4$–$(CT)_2$ | 248–252 | 60 | 2 | 0.4000 | 0.3254 | M |
| MC-66 | F AGGGAGATATGGGCTGGC<br>R CTTAGGAAAGGCTTGGTTGTG | $(CT)_8$ | 295–305 | 54 | 6 | 0.2667 | 0.4011* | P |

$T_a$ PCR annealing temperature; $A$ number of alleles; $H_O$ observed heterozygosity; $H_E$ expected heterozygosity; $W$ weak amplifications; $M$ monomorphic amplification

$P$ polymorphic amplification

* Indicated that the observed heterozygosity is significantly different from the expected heterozygosity under Hardy–Weinberg equilibrium ($P < 0.01$)

and assessing genetic variations to establish conservation strategy on *M. coriacea* and other congeneric members.

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