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Short Communication

Development and characterization of microsatellite markers for the endangered Chinese yew *Taxus chinensis* var. *mairei* (Taxaceae)

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ABSTRACT. We isolated and characterized 11 microsatellite loci for the Chinese yew, *Taxus chinensis* var. *mairei*, an endangered tree species in China, by constructing a $(CA)_{12}$-enriched library. The number of alleles per locus ranged from 5 to 10. The observed heterozygosities ranged from 0.2500 to 0.8333 and the expected heterozygosities ranged from 0.5196 to 0.8680. No significant linkage disequilibrium was detected at these loci. However, four loci significantly deviated from Hardy-Weinberg equilibrium. The null alleles were found to be present at locus Tach9 and locus Tach11 by the Micro-checker test ($P < 0.001$). These polymorphic loci could be employed in research of gene flow and spatial genetic patterns of *T. chinensis* var. *mairei*.

Key words: *Taxus chinensis* var. *mairei*; Microsatellite; Polymorphism

INTRODUCTION

Taxus chinensis var. *mairei* is a tertiary relict and endemic tree species in China (Zhen and Fu, 1978). Tree species from the genus *Taxus* were economically valuable as the source of Taxol, which has been regarded as one of the most hopeful anti-cancer drugs since its emergence in 1987 (Cragg et al., 1993). It has been confirmed that *T. chinensis* var. *mairei* contains relatively high contents of Taxol, like some other species of the genus (Su et al., 2001). Now, the natural population has been greatly disturbed and overexploited by humans, and its natural resources have also drastically declined due to habitat loss and fragmentation (Liao et al., 1996). Wild populations of *T. chinensis* var. *mairei* are on the edge of extinction. Therefore, the species has been considered as a key protected plant species by the Chinese government and also listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in 1995.

Research on the species' conservation started gradually. Conservation and management strategies for wild population of *T. chinensis* var. *mairei* were suggested at the ecological scale (He et al., 2007). From a micro-perspective, population genetic structure and variation of the species were inferred using random amplified polymorphic DNA (RAPD) markers (Zhang et al., 2003). Four polymorphism microsatellite loci of *T. chinensis* var. *mairei* were also reported to support the research on population genetics (Zhou et al., 2009). In this study, we isolated some more novel loci for the species, since the microsatellites as co-dominant molecular marker are powerful for estimating population genetic parameters because of their high genetic information contents (Morgante and Olivieri, 1993). We used genomic-enriched library protocol that is more effective in the isolation of microsatellite loci (Zane et al., 2002). The novel loci isolated could be used to estimate the gene flow and spatial genetic pattern of wild populations of *T. chinensis* var. *mairei*, and a protection plan could be suggested at the genetics level by the following research.

MATERIAL AND METHODS

Microsatellite isolation was based on the genomic-enriched library. Total genomic DNA (0.2 μg) was extracted from dry leaf tissue by the especially improved protocol for coniferous tissue (Barzegari et al., 2010). DNA was simultaneously digested with the *Hae*III restriction enzyme (MBI) at 37°C overnight. The digested genomic DNA was ligated to double-stranded linkers (SNX-F: 5'-CTAAGGCCTTGCTAGCAGAAGC-3' and SNX-R: 5'-pGCTTCTGCTAGCAAGGCCTTAGAAAA-3'). Linker-ligated fragments were polymerase chain reaction (PCR) amplified with the SNX-F linkers. The PCR products were used for hybridization with biotinylated probes $(CA)_{12}$ and captured with streptavidin-coated beads (Promega). Microsatellite-enriched fragments were amplified again with the SNX-F linker, and the double-stranded products were purified on 1.5% agarose gel, with DNA fragments ranging from 300-800 bp extracted by the gel extraction column kit (TaKaRa). Purified products were ligated into the plasmid pMD18-T vector (Promega) and transformed into DH5α competent cells. PCR using M13F and $(CA)_{12}$ as the primers was to test each clone, and positives were sequenced on 3730 DNA sequencer (Applied Biosystems) using the M13F primer.

Following sequencing, primers were designed in the flanking regions of the repeat motifs (repeating size ≥20 bp) using the Primer 3 software (Rozen and Skaletsky, 2000). Poly-

morphisms were detected on 8% denaturing polyacrylamide gels stained with silver nitrate (Creste et al., 2001) and sized by comparison to a 10-bp ladder standard (Invitrogen). To characterize the polymorphism of the loci, 32 individuals of *T. chinensis* var. *mairei* were genotyped, which were sampled from XianYu Mountain in Anhui Province, China.

The number of alleles per locus and observed and expected heterozygosities were estimated with Genetix 4.05 (Belkhir et al., 1996). Hardy-Weinberg equilibrium and linkage disequilibrium tests were performed with Genepop 4.0.1 (Raymond and Rousset, 1995). Micro-checker 2.2.3 (Van Oosterhout et al., 2004) was used to identity null alleles.

RESULTS AND DISCUSSION

In total, 212 positive clones were picked out and sequenced from 672 clones. Thirty-three primer pairs were designed and twenty-one of them amplified clearly interpretable products. Eleven polymorphism microsatellite loci were isolated finally. The number of alleles, PCR product size range and heterozygosity of the loci are summarized in Table 1. The number of alleles per locus ranged from 5 to 10 with an average of 7. The expected and observed heterozygosities ranged from 0.5196 to 0.8680 and from 0.2500 to 0.8333, with a mean of 0.7548 and 0.6186, respectively. No significant linkage disequilibrium ($P < 0.01$) was detected among these loci. However, significant deviations from Hardy-Weinberg equilibrium were detected at loci Tach6, Tach8, Tach9, and Tach11 ($P < 0.05$). Results of the Micro-checker test showed that null alleles could be present at locus Tach9 and locus Tach11 ($P < 0.001$).

Table 1. Characterization of 11 polymorphic microsatellite markers isolated from *Taxus chinensis* var. *mairei*.

Locus	Primer sequence (5'-3')	Repeat motif	Size range (bp)	N_A	Tm (°C)	H_E	H_O	P_{HWE}	GenBank No.
Tach1	CTGTGAAGTCAGCCGAG GAGCCTACCCTTATAGATTG	$(AG)_{34}$	170-204	5	51	0.6852	0.6129	0.1167	JN786105
Tach2	GAACAAGTAGTTTTTCCATG CTCATTCACTTGGTCATATCC	$(AC)_{34}(AG)_{22}$	304-372	7	46	0.7339	0.8333	0.5193	JN786106
Tach3	CCATGTGCAACACTTTAAC GTTTCCAGGTTCCTAGTAATG	$(TG)_9TA(TG)_5\ldots(TG)_{30}$	268-318	6	50	0.7991	0.7857	0.1077	JN786107
Tach4	CCGAAACTAATGTTATTCC GTGTGGTAGTTAGAAAAGATG	$(TG)_{46}$	212-274	9	50	0.8494	0.7667	0.1129	JN786108
Tach5	ATCGGGGGTATTGGAAAC GGTAAGAATACATCATTTTC	$(TG)_{42}$	256-288	5	52	0.7864	0.7143	0.1360	JN786109
Tach6	AGATGACTTGTGATGACCTAC GAAACTCGATTCCATAATGAG	$(TG)_{57}$	282-326	6	50	0.7426	0.5769	0.0062*	JN786110
Tach7	AGTGCTTTAGTGAAAGTGGGTTG AGATCAGTGAGGAAATGATGTG	$(TG)_8TT(TG)_9$	220-255	6	50	0.5196	0.6207	0.9899	JN786111
Tach8	AGCCAAGAAGAATGAACTCAATC TGGCATTCATATTTAGGGGCATC	$(TG)_{33}(AG)_{24}$	304-380	8	52	0.7818	0.6207	0.0032*	JN786112
Tach9	CCTTGGGAGGGGAGGATATAG AAACTGGGGGGTATCCACTTC	$(TG)_{41}TA(TG)_7$	234-302	10	55	0.8680	0.2500	0.0000*	JN786113
Tach10	AAAGTTGAAAACAAACGCAC TGCTTTCCTCATTACACTTG	$(CA)_{15}$	198-254	8	50	0.7485	0.6563	0.1094	JN786114
Tach11	CCCATCCATATTTTTGATGTC AGACAAAGCATTACACAC	$(TG)_{20}$	190-258	7	48	0.7878	0.3667	0.0000*	JN786115

N_A = observed number of alleles; Tm (°C) = annealing temperature; H_E = expected heterozygosity; H_O = observed heterozygosity; P_{HWE} = P values for exact tests for Hardy-Weinberg equilibrium (HWE); *Significant deviation from HWE ($P < 0.05$).

The motif repeat size was relatively large in the newly developed loci, with the locus Tach6 even repeated 57 times. More repeats in the loci may lead to relatively more polymor-

phisms (mean of 7 alleles per locus) because the polymorphism per locus was positively related to the motif repeat size (Weber, 1990). There were four loci deviating from Hardy-Weinberg equilibrium, which could be related to habitat loss and fragmentation of the wild population of *Taxus chinensis* var. *mairei* (Liao et al., 1996). The polymorphic loci isolated could be employed in research of gene flow and spatial genetic pattern of *Taxus chinensis* var. *maire*.

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