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RESEARCH ARTICLE

Microsatellite loci from *Taxus chinensis* var. *mairei* (Taxaceae), an endangered and economically important tree species in China

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Abstract *Taxus chinensis* var. *mairei* is an endangered and economically important tree species in China. Four polymorphic microsatellite markers were developed and characterized using the fast isolation by amplified fragment length polymorphism (AFLP) of sequences containing repeats (FIASCO) protocol. Polymorphism of each locus was quantified for five natural populations from Hubei Province, China. The number of alleles per locus varied from 6 to 24. The ranges of observed and expected heterozygosity were 0.493–1.000 and 0.440–0.845, respectively. These are the first microsatellites reported for the family of Taxaceae and will be helpful for the ongoing population and conservation genetics research of *Taxus chinensis* var. *mairei*.

Keywords *Taxus chinensis* var. *mairei*, genetic variation, microsatellite

1 Introduction

Chinese yew, *Taxus chinensis* var. *mairei* (Lemée et Lévl) Cheng et L.K. Fu is a tertiary relict and endemic tree species in China (Zhen and Fu, 1978). Of the genus *Taxus*, it is the most widespread species mainly distributed along the Yangtze Valley, throughout the Nanling Mountains and the vast areas of southeastern China (Zhen and Fu, 1978). *T. chinensis* var. *mairei* is the characteristic species of subtropical evergreen and deciduous broad-leaved forest occupying humid and shady niches. Plants from the genus *Taxus* are economically important as the source of Taxol (paclitaxel), which has been regarded as one of the most promising anti-cancer drugs since its emergence in 1987 (Cragg et al., 1993). *T. chinensis* var. *mairei* has relatively higher contents of Taxol compared with other species of the genus (Su et al., 2001), which has caused an overexploitation of its natural populations. Apart from this, during the past few decades, its natural resources have also drastically declined due to habitat loss and fragmentation (Liao et al., 1996). In China, wild populations and individuals of *T. chinensis* var. *mairei* are extremely rare, being on the brink of extinction. Therefore, the species has been considered as one of the first-grade state protection plants (The State Council of the People's Republic of China, 1999) and has been listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in 1995.

Information about the level of genetic variation and genetic structure of populations is a prerequisite to make appropriate management strategies for threatened species (Dawson and Powell, 1999). In the last several years, researches related to *T. chinensis* var. *mairei* were mainly focused on plant biochemistry, cell biology and ecology (He et al., 2007; Wang et al., 2007; Yang et al., 2007), while little was known about its population genetics. In a previous study, the population genetic structure and variation of the species were inferred using nuclear random amplified polymorphic DNA (RAPD) markers (Zhang et al., 2003); however, observed heterozygosity and population differentiation could not be detected directly with dominant markers (Zhivotovsky, 1999), which prompted a search for codominant microsatellite markers. Here we report the isolation and characterization of a set of polymorphic microsatellite loci from the genome of *T. chinensis* var. *mairei* and preliminarily assess the genetic diversity using wild individuals from five natural populations in Hubei Province, China.

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2 Materials and methods

Microsatellite markers were developed for *T. chinensis* var. *mairei* using the protocol of fast isolation by amplified fragment length polymorphism (AFLP) of sequences containing repeats (FIASCO) with minor modifications (Zane et al., 2002). Total genomic DNA was extracted from leaf tissue following the modified cetyltrimethylammonium bromide (CTAB) protocols (Su et al., 1998). Approximately 250 ng of genomic DNA was completely digested with three units of *Mse*I (BioLabs) in a 25 μL volume, and then 15 μL of digested DNA was ligated to the *Mse*I AFLP adaptor (5'-GACGATGAGTCCTGAG-3'/5'-TACTCAGGACTCAT-3') in a 30 μL volume with one unit of T_4 DNA ligase (BioLabs) at 20°C for 3 h. The digestion-ligation mixture was diluted (1∶10), and directly amplified using *Mse*I adaptor-specific primers (5'-GATGAGTCCTGAGTAAN-3') in 20 μL with 0.9 μmol/L *Mse*I-N, 0.2 mmol/L dNTPs, 1.5 mmol/L $MgCl_2$, one unit of Taq DNA polymerase (Biomed) and 5 μL diluted digestion-ligation DNA. The PCR reaction was performed using a program of 94°C 30 s, 53°C 1 min, and 72°C 1 min for 20 cycles.

After denaturation at 95°C for 5 min, 20 μL amplified DNA fragments with a size range of 200 – 1000 bp were hybridized with 200 pmol of 5′-biotinylated $(AC)_{15}$ probe in a total volume of 250 μL of SSC 4.2×and 0.07% SDS, and incubated at 48°C for 2 h. Hybridization products were selectively captured with 600 μL Streptavidin MagneSphere Paramegnetic Particles (Promega), which had been washed with 150 μL of TEN100 (10 mmol/L Tris-HCl, 1 mmol/L EDTA, 100 mmol/L NaCl, pH 7.5) three times, allowing a selective binding at room temperature for 30 min with constant gentle agitation. The beads-probe-DNA complex was separated by a magnetic field. After removing nonspecific DNA fragments by nonstringent washes (10 mmol/L Tris-HCl, 1 mmol/L EDTA, 1 mmol/L NaCl, pH 7.5) and stringent washes (SSC 0.2×and 0.1% SDS) for three times each, the target DNA was released from the bead-probes with 50 μL TE (Tris-HCl 10 mmol/L, EDTA 1.0 mmol/L, pH 8.0) at 95°C for 5 min, and transferred as soon as possible.

DNA containing repeats were amplified for 30 cycles with *Mse*I-N primers and the same program described above. PCR products were purified using the gel extraction kit (Omega Biotek). They were then ligated into the pMD 19-T plasmid vector (Takara) and were transformed into competent *Escherichia coli* cells DH-5α following the manufacturer's instructions. Recombinant clones were identified by blue/white selection, and then were amplified using M13 universal primers and visualized by agarose gel electrophoresis. Fifty-eight clones with different insert fragments were sequenced, 38 of which contained simple sequence repeats. Subsequently, 22 primer pairs were developed from simple sequence repeats containing eight or more repeats with suitable flanking sequences.

All of the 22 pairs of primers were tested using 85 *T. chinensis* var. *mairei* individuals sampled from five populations located in Hubei Province. The PCRs were performed in a reaction mixture (10 μL) containing 1.5 mmol/L $MgCl_2$, 0.2 mmol/L dNTPs, 0.25 μmol/L each of primer, 1×Taq buffer and 0.5 unit Taq polymerase (Biomed), and 20 ng of genome DNA. The PCR cycling conditions included an initial denaturation at 94°C for 5 min, followed by 35 cycles of 50 s at 94°C, an annealing step for 50 s at the primer's optimized annealing temperature (Table 1), 90 s at 72°C and a final extension step for 10 min at 72°C. Amplified products were electrophoresized in 6% denaturation polyacrylamide gel and visualized by silver staining. A 25 bp DNA ladder (Promega) was used to identify alleles.

3 Results and discussion

Eleven of the 22 primer pairs successfully amplified DNA fragments, but only four yielded clear and polymorphic banding patterns. The number of alleles per locus, the observed and expected heterozygosities, Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were assessed using GENEPOP software, version 3.4 (Raymond and Rousset, 1995). The number of alleles per locus ranged from 6 to 24, the observed heterozygosity ranged from 0.493 to 1.000 and the expected heterozygosity ranged

Table 1 Characterization of 4 polymorphic microsatellite loci in *Taxus chinensis* var. *mairei*

locus	GenBank accession no.	repeat motif	primer sequence (5'–3')	T_a/°C	size range/bp	N_A	H_O	H_E
Tc1	EU391178	$(CA)_{11}$	F: ATTATGCTAGGCTCAACT R: GTTTGGATGTCCATGTGA	55	130–143	6	0.512	0.440
Tc2	EU391177	$(CA)_{15}$C G$(CA)_{14}$	F: GAATGGATTTGGGATGTA R:TCATACTGACCTTGGTGG	55	225–295	24	0.493	0.845
Tc3	EU391176	$(TC)_8$	F: GCTATGGAATGAAGAATC R:CGTCTTTACTTTCCGTGT	50	64–75	11	1.000	0.737
Tc4	EU391175	$(GT)_{11}$	F: GAATGCTTCCCACAATAG R: AAACATGGTGGCTACACT	53	112–152	12	0.699	0.778

F and R: left and right primers, respectively; T_a: annealing temperature; N_A: number of alleles; H_O: observed heterozygosity; H_E: expected heterozygosity.

from 0.440 to 0.845 (Table 1). Deviation from HWE was detected only in Tc3 locus, showing highly significant heterozygote excess ($P < 0.001$). Two pairs of loci (Tc1 and Tc3, Tc1 and Tc4) showed significant LD, as indicated by Fisher's exact tests ($P < 0.05$).

The four polymorphic loci presented here provided the first set of codominant markers for the population genetic study of *T. chinensis* var. *mairei*. We believe that these markers will be useful for the ongoing population and conservation genetics studies of the other remaining populations, which should provide us valuable information for developing conservation strategies of this endangered and economically important species.

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References

Cragg G M, Schepartz S A, Suffness M, Grever M R (1993). The taxol supply crisis-new NCI policies for handling the large-scale production of novel product anticancer and anti-HIV agents. J Nat Prod, 56: 1657–1668

Dawson I K, Powell W (1999). Genetic variation in the afromontane tree *Prunus africana*, an endangered medicinal species. Mol Ecol, 8: 151–156

He S A, Li X H, Peng F, Sheng N, Yu H, Gu Y (2007). Study on dynamic of small ex-situ conservation population of *Taxus chinensis* var. *mairei*. J Plant Resour Environ, 16: 35–39 (in Chinese)

Liao W B, Zhang Z Q, Su Z Y (1996). Importance and exigency on study of protection biology of anticancer plant *Taxus mairei*. Ecol Sci, 15: 17-20 (in Chinese)

Raymond M, Rousset F (1995). Genepop (version 1.2): population genetics software for exact tests and ecumenicism. J Hered, 86: 248–249

Su Y J, Wang T, Li X Y, Fan G K, Ke Y Y, Zhu J M, Liao W B, Shi Z Q, Wu H Q, Yang L X, Ge F H (2001). Studies on the chemical constituents of the stem of *acanthopanax setchuenensis*, Nat Prod Devel, 13: 19–20 (in Chinese)

Su Y J, Wang T, Yang W D, Huang C, Fan G K (1998). DNA extraction and RAPD analysis of *Podocarpus*. Acta Sci Nat Univ Sunyatseni, 37: 13–18

Wang Y D, Wu J C, Yuan Y J (2007). Salicylic acid-induced taxol production and isopentenyl pyrophosphate biosynthesis in suspension cultures of *Taxus chinens*is var. *mairei*. Cell Biol Int, 31: 1179–1183

Yang S, Qiao B, Lu S H, Yuan Y J (2007). Comparative lipidomics analysis of cellular development and apoptosis in two *Taxus* cell lines. Biochim Biophys Acta, 1171: 600–612

Zane L, Bargelloni L, Patarnello T (2002). Strategies for microsatellite isolation: a review. Mol Ecol, 11: 1–16

Zhang H Y, Chen Y Q, Liao W B (2003). RAPD analysis of population genetic diversity of *Taxus mairei*. Acta Bot Boreal-Occident Sin, 23: 1194–1197 (in Chinese)

Zhen W J, Fu L G (1978). *Flora Reipublicae Popularis Sinicae* Tomus 7. Beijing: Science Press, 443–445 (in Chinese)

Zhivotovsky L A (1999). Estimating population structure in diploids with multilocus dominant DNA markers. Mol Ecol, 8: 907–913