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Development and characterization of EST-SSR markers for *Taxus mairei* (Taxaceae) and their transferability across species

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Abstract

Taxus is an important genus which is well-known for Taxol. Its genetic analyses were lagged behind those of other conifers due to lack of suitable molecular markers. In this paper, we explored polymorphic loci for *Taxus mairei* and tested their transferability across species based on 150 EST-SSR loci already developed for *Taxus cuspidata* previously. The results showed that 103 loci were polymorphic, the number of alleles per locus ranged from 2 to 11 over 16 individuals. The observed heterozygosity (H_o) and expected heterozygosity (H_e) varied from 0 to 1 and 0.0625 to 0.891, respectively. The *PIC* values ranged from 0.11 to 0.754 with an average of 0.453. The average cross-species transferability was 96.07% among 5 species. Most of these loci can be used as universal markers in *Taxus* genus. The PCA results showed these markers have strong power to identify different species. These markers will be useful for further studies on genetic analysis and conversation of *Taxus mairei*.

Keywords: *Taxus mairei; Expressed sequence tags; Microsatellite markers; Transcriptome sequences; Cross-amplification; Genetic diversity*

Introduction

Taxus mairei is an evergreen and endemic tree species in China. Its seeds have a thick coat and may need more than one year of dormancy before germination (Tan, 1991). Low germination rate is one of the most serious problems for population regeneration (Zhu et al., 1999; Zhang et al., 2012). *Taxus mairei* can produce Taxol for medical purpose. Poor recruitment and overexploitation have caused a serious depletion of natural resources. This species has been considered as the first-class protected plant since 1999 by State Council of People's Republic of China(http://www.gov.cn/gongbao/content/2000/content_60072.htm). Understanding the current genetic patterns and genetic diversity are extremely important for the protection of this species in the future.

In the previous study, different kinds of molecular markers have been used in genetic diversity study of *Taxus mairei*, most of which were dominant markers, such as RAPD, ISSR and AFLP (Zhang et al., 2003; Li et al., 2010; Zhou et al., 2009). Microsatellite marker (also called as simple sequence repeats or SSRs) is an ideal molecular marker to measure genetic diversity. It has a number of advantages over those based on random fingerprinting approaches (Selkoe and Toonen, 2006). With the advent of the next generation sequencing, large amounts of sequences data have been generated and deposited in public databases (NCBI, DDBJ and EMBL). Previously, we have already developed a number of EST-SSR markers for *Taxus cuspidata* from publicly available transcriptome sequences (Ueno et al., 2015). In this work, our aim is to select polymorphic loci for *Taxus mairei* based on these EST-SSR markers, and analyze their cross-transferability among five other *Taxus* species.

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Material and Methods

Sixteen samples of *Taxus mairei* were selected to evaluate the polymorphism of the candidate markers, which are distributed in wide range area of Southern China. A total of 150 candidate loci were used to test PCR amplification and their polymorphism on *Taxus mairei*, which were developed in the previous study (Ueno et al., 2015). Five *Taxus* species other than *T. mairei* including *T. chinensis*, *T. cuspidata*, *T. baccata*, *T. media* and *T. yunnanensis* were used to test the cross-transferability of the polymorphic loci. Polymerase chain reaction and genotyping were the same as Ueno et al. (2015).

Genetic parameters of each locus, such as the number of alleles (N_a), observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficient (F_{IS}) were calculated using FSTAT software (Goudet, 1995). Hardy-Weinberg equilibrium (HWE) testing were assessed by version 4.2 of the online GENEPOP (Rousset, 2008; http://genepop.curtin.edu.au/index.html). PIC (polymorphism information content) values of each locus were calculated using Microsatellite Toolkit package (PARK, 2001). Null alleles were detected by the Micro-checker 2.2.3 software (Oosterhout et al., 2004). The rate of the transferability for each locus was recorded according to the successful number of amplification in five species. The genetic relationship of 6 species (in total 48 individuals) was analyzed with principal component analysis (PCA) using GenALEx 6.3 (Peakall et al., 2006) and phylogenetic tree using the UPGMA method by MEGA 7.0 (Kumar et al., 2016).

Results and Discussion

Characterization of the polymorphic loci

Among 150 candidate loci, 107 (71.33 %) (Table 1) successfully produced PCR fragment patterns, 103 (68.67 %) were polymorphic and 4 were monomorphic. The successful amplification rate and polymorphic rate were higher than that of Li et al. (2014) (53.23 % and 38.71 %). We selected candidate loci that contained nine or more SSRs repeats for evaluation, which benefit for getting higher polymorphic SSR loci (He et al., 2003; Ueno et al., 2012).The number of alleles per locus (N_a) ranged from 2 to 11. The observed heterozygosity (H_o) and expected heterozygosity (H_e) varied from 0 to 1 (average of 0.459) and 0.0625 to 0.891 (average of 0.513), respectively. The *PIC* values ranged from 0.059 to 0.849 with an average of 0.453. It shows that the developed EST-SSR markers have moderately polymorphic in *Taxus mairei*. 44 loci had highly informative scores (*PIC* > 0.50), 32 loci possessed moderately informative scores (0.5 > *PIC* > 0.25) and 27 loci had weakly informative scores (0 < *PIC* < 0.25) (Table 2). 14 loci detected containing null alleles, and the frequency of null alleles were from 0.135 (Ma-8219-1251D) to 0.470 (C-3656-295C). 3 loci (C-3656-295C, C-52641-193B, Me-5700-2160C) showed significantly deviated from Hardy-Weinberg equilibrium (HWE) may due to null alleles, since these loci have the highest frequency, corresponding to 0.470, 0.414 and 0.346, respectively.

Cross transferability of the 107 polymorphic markers

Among the 107 polymorphic markers, the average cross transferability rate was 96.07 % (Table 3), only the loci that Ma-25497-83A did not successfully amplified in five species, four loci (C-52641-193B, Ma-1402-721B, Ma-186-234D, Ma-6383-968B) had the success rate lower than 50%. Compared with our previous study of *Taxus*, the cross-amplification success of EST-SSR markers was much higher than that of genomic SSRs (48.6%) (Wen et al., 2012) . EST sequences originated from the transcriptional region, which was more conservative compared with non-coding genomic DNA (Thiel et al., 2003; Cordeiro et al., 2001; Gupta et al., 2003). The high transferability rate indicated these new markers could be used as universal markers in *Taxus* genus.

Assessing the power of the EST-SSR markers

The principal component analysis (PCA) showed that 48 individuals (6 species) were clustered into 4 looser groups (Figure 1). *T. mairei*, *T. chinensis* and *T. baccata* had relatively far genetic distance with each other. For *T. cuspidata*, *T. media* and *T. yunnanensis*, 22 individuals were clustered into the same group, showing close genetic relationship. The dendrogram for six species (Figure 2) also showed the same results. The clustering pattern, except for *T. yunnanensis*, was similar with the phylogenetic relationship of genus *Taxus* inferred from chloroplast intergenic spacer and nuclear coding DNA (Hao et al., 2008). In the Flora of China (FOC) (http://foc.eflora.cn/), *T. yunnanensis* was considered as *T. wallichiana*, *T. mairei* and *T. chinensis* were the varities of *T. wallichiana*. It was surprised that *T. yunnanensis* had very far genetic distance with *T. mairei* and *T. chinensis*. This issue needs more in-depth study to confirm the taxonomical status of *T. yunnanensis*.

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Data Archiving Statement

All primer sequences (EST-SSR loci) were deposited in DDBJ with Accession numbers AB786925-AB787148

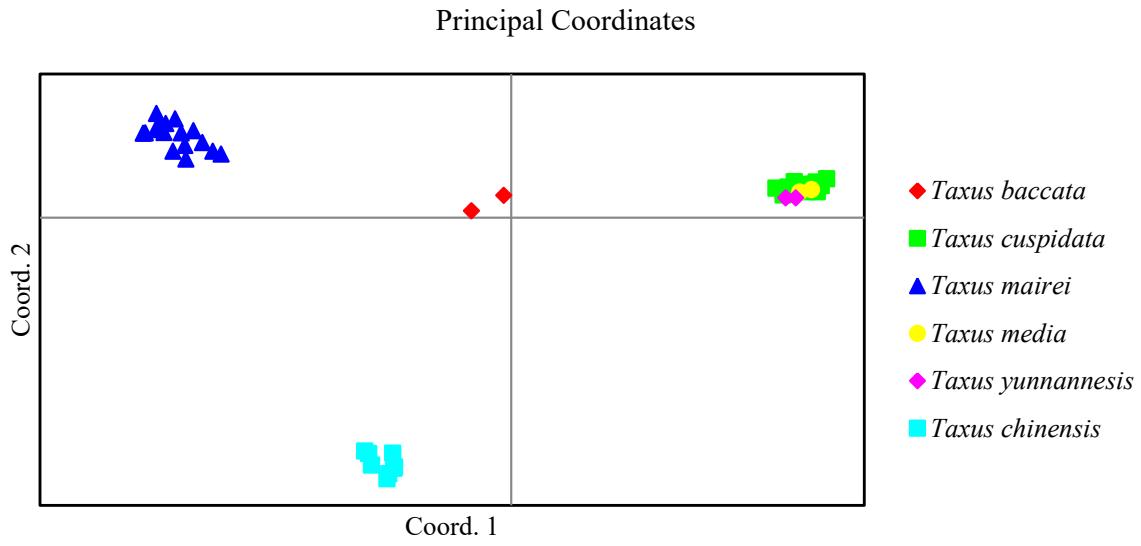

Figure 1
PCA result of 48 individuals from 6 *Taxus* species

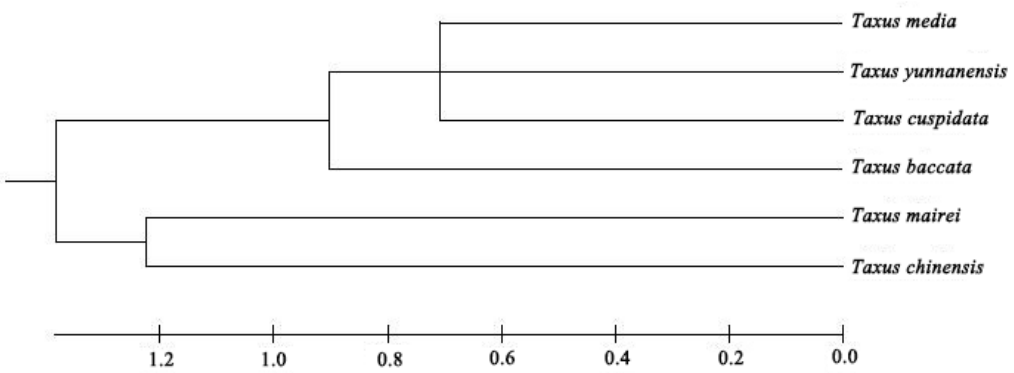

Figure 2
Dendrogram of *Taxus* species based on amplification success of EST-SSR markers

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