Development of polymorphic microsatellite markers based on expressed sequence tags in *Populus cathayana* (Salicaceae)

Z.Z. Tian\(^1,2\), F.Q. Zhang\(^3\), Z.Y. Cai\(^1\) and S.L. Chen\(^1\)

\(^1\)Key Laboratory of Adaptation and Evolution of Plateau Biota, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, China
\(^2\)University of Chinese Academy of Sciences, Beijing, China
\(^3\)Key Laboratory of Crop Molecular Breeding, Qinghai Province, Xining, China

Corresponding authors: F.Q. Zhang / S.L. Chen
E-mail: fqzhang@nwipb.cas.cn / slchen@nwipb.cas.cn

Genet. Mol. Res. 15 (3): gmr.15038406
Received January 8, 2016
Accepted February 19, 2016
Published July 15, 2016
DOI http://dx.doi.org/10.4238/gmr.15038406

Copyright © 2016 The Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution ShareAlike (CC BY-SA) 4.0 License.

**ABSTRACT.** *Populus cathayana* occupies a large area within the northern, central, and southwestern regions of China, and is considered to be an important reforestation species in western China. In order to investigate the population genetic structure of this species, 10 polymorphic microsatellite loci were identified based on expressed sequence tags from *de novo* sequencing on the Illumina HiSeq 2000 platform. All microsatellite primers were tested on 48 *P. cathayana* individuals from four locations on the Qinghai-Tibet Plateau. The observed heterozygosity ranged from 0.000 to 1.000, and the null-allele frequency ranged from 0.000 to 0.904. These microsatellite markers may be a useful tool in genetic studies on *P. cathayana* and closely related species.

**Key words:** *Populus cathayana*; Microsatellite; EST; Qinghai-Tibet Plateau
INTRODUCTION

The Qinghai-Tibet Plateau (QTP) is one of the highest and largest plateaus in the world, with a mean elevation of 4500 m and an area of $2.5 \times 10^6$ km$^2$ (Zheng, 1996). Due to its complex geographical and geological history, and dramatic climatic oscillations, the QTP and adjacent highlands have abundant and unique resources of the genus *Populus* (Wu and Petter, 1999; Weisgerber and Han, 2001). *Populus cathayana* Rehd. is a dioecious, fast-growing tree species, widely distributed in the northern, central, and south-western regions of China (Yang et al., 1995). In the southern and eastern areas of the QTP, the vertical distribution of *P. cathayana* ranges from altitudes of 1900 to 3000 m, with some trees occurring at 3900 m (Wang and Fang, 1984). Currently, little is known about the genetic diversity of *P. cathayana* (Lu et al., 2006). Here, we isolated microsatellite loci for *P. cathayana*, based on expressed sequence tags (EST) from Illumina paired-end sequencing, to enable us to obtain a better understanding on the genetic diversity of the species (Rodrigues et al., 2015; Chen et al., 2015). Ten pairs of polymorphic microsatellite primers were developed based on ESTs. These microsatellite markers may be a useful tool in genetic studies on *P. cathayana* and closely related species (Li et al., 2015).

MATERIAL AND METHODS

Microsatellite makers were detected using *P. cathayana de novo* sequencing on the Illumina HiSeq 2000 platform. RNA isolation, cDNA library preparation, and sequencing were performed by BGI-Shenzhen (Shenzhen, China), as previously described (Zhang et al., 2014). In total, 9,481,146,660 nucleotide bases were generated, and 47,521 unigenes were detected after assembly. Microsatellite sequences were detected by Microsatellite (MISA; http://pgrc.ipk-atersleben.de/misa/) using unigenes as a reference. Parameters were consistent with those previously described (Zhang et al., 2015). Finally, 14,346 microsatellite sequences were searched (Figure 1). Sixty microsatellite sequences were selected randomly. All primers were designed using the Primer 3-2.3.4 software (http://primer3.sourceforge.net/).

Fresh leaves were collected and dried on silica gel. For each population, 9-14 individuals were sampled and voucher specimens were deposited in the Herbarium of the Northwest Institute of Plateau Biology (HNWP), Xining, Qinghai Province, China. Forty-eight *P. cathayana* individuals from four populations (LH, DL, GH, and LT) were sampled in total (Table 1). Total genomic DNA was extracted using a modified cetyltrimethylammonium bromide method (Doyle and Doyle, 1987). PCR was performed in a 50-µL reaction mixture containing: 20-30 ng template DNA, 5 µL 10X PCR buffer (15 mM MgCl$_2$), 1.5 µL each primer (5 pM), 1.0 µL Taq DNA polymerase (Takara, Dalian, China), 0.5 µL dNTP mix (10 mM), supplemented with ddH$_2$O. The PCR program included the following steps: 94°C for 5 min, followed by 35 cycles of 94°C for 45 s, relevant annealing temperatures (Table 2) for 45 s, 72°C for 50 s, with a final extension for 10 min at 72°C. Amplification products were visualized on 0.7% agarose gels stained with ethidium bromide, then separated on 12% w/v non-denaturing polyacrylamide gels electrophoresis (PAGE). The total number of alleles, observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), null-allele frequency ($N_a$), deviation from Hardy-Weinberg equilibrium, and linkage disequilibrium were calculated using the GENEPOP version 4.4 software (Rousset, 2008).
Voucher specimens are deposited in the Herbarium of the Northwest Institute of Plateau Biology (HNWP), Xining, Qinghai Province, China.

Table 1. Locality information for populations of *Populus cathayana*.

<table>
<thead>
<tr>
<th>Population code</th>
<th>Location</th>
<th>Sample size</th>
<th>Voucher No.</th>
<th>Geographic coordinates</th>
<th>Altitude (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>Luhuo, Sichuan Province, China</td>
<td>14</td>
<td>Chen2013131</td>
<td>31°10’N, 100°53’E</td>
<td>3080</td>
</tr>
<tr>
<td>DL</td>
<td>Dulan, Qinghai Province, China</td>
<td>11</td>
<td>Zhang2014389</td>
<td>36°20’N, 98°38’E</td>
<td>3161</td>
</tr>
<tr>
<td>GH</td>
<td>Gonghe, Qinghai Province, China</td>
<td>14</td>
<td>Zhang2014042</td>
<td>36°03’N, 100°06’E</td>
<td>2969</td>
</tr>
<tr>
<td>LT</td>
<td>Lintao, Gansu Province, China</td>
<td>9</td>
<td>Zhang2014001</td>
<td>35°17’N, 104°55’E</td>
<td>2050</td>
</tr>
</tbody>
</table>

Voucher specimens are deposited in the Herbarium of the Northwest Institute of Plateau Biology (HNWP), Xining, Qinghai Province, China.

RESULTS

A total of 14,346 microsatellite sequences were searched, and 60 loci were randomly selected for PCR amplification, 24 of which were successfully amplified. Following PAGE analysis, 10 microsatellite loci proved to be highly polymorphic in *P. cathayana* (Table 2). Among the four populations of *P. cathayana*, the total number of alleles per locus ranged from 2 to 7. The $H_o$ ranged from 0.000 to 1.000, and $H_e$ ranged from 0.311 to 0.857. The $N_A$ ranged from 0.000 to 0.904 (Table 3).

Figure 1. Statistics showing the classification of microsatellite sequences in *Populus cathayana*.
DISCUSSION

In this study, we isolated 10 microsatellite markers, which were polymorphic among populations of *P. cathayana*. These microsatellite markers may be a useful tool in genetic studies on *P. cathayana* and closely related species.

Conflicts of interest

The authors declare no conflict of interest.
ACKNOWLEDGMENTS

Research supported by the National Natural Science Foundation of China (Grants #31270270 and #31400322), CAS “Light of West China” Program, Youth Innovation Promotion Association CAS, and the International Scientific and Technological Cooperation Projects of Qinghai Province (#2014-HZ-812).

REFERENCES


