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

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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 x 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_2O. 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).

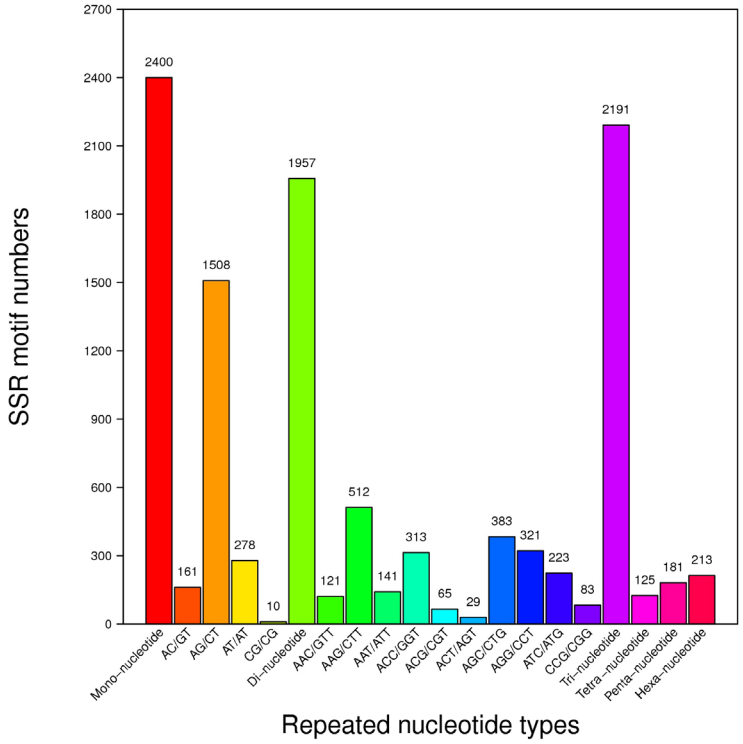

Figure 1. Statistics showing the classification of microsatellite sequences in *Populus cathayana*.

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

Population code	Location	Sample size	Voucher No.	Geographic coordinates	Altitude (m)
LH	Luhuo, Sichuan Province, China	14	Chen2013131	31°10'N, 100°53'E	3080
DL	Dulan, Qinghai Province, China	11	Zhang2014389	36°20'N, 98°8'E	3161
GH	Gonghe, Qinghai Province, China	14	Zhang2014042	36°03'N, 100°06'E	2969
LT	Lintao, Gansu Province, China	9	Zhang2014001	35°13'N, 104°53'E	2050

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).

Table 2. Characteristics of 10 microsatellite markers developed in *Populus cathayana*.

Locus	Primer sequences (5'-3')	Repeat motif	Fragment size (bp)	Ta (°C)	Total No. of alleles	GenBank accession No.
PC9	F: AACTGGAGAAACCCCTGTAATGT	$(CCCATT)_4$	140	57	4	KU220200
	R: AACAGCAACTTCAGATGTCAATG					
PC10	F: CATTCAGTTGTGGAATTCACGTA	$(CT)_8$	107	47	5	KU220199
	R: TGGTGCAGGTTAACAAGTCAATA					
PC14	F: TGGAGTTCTTTCTAGCGGTATTG	$(CT)_9$	160	50	6	KU220198
	R: GCAAGGCCATAATGAATAACTTT					
PC23	F: TGACATCATTCCAAAGGAATACA	$(AT)_{10}$	160	50	5	KU220197
	R: ACTCAACCAGCGTGTCAGAGAAT					
PC26	F: GATCAGCCAATTTATCAGCAATC	$(TC)_6$	118	54	5	KU220196
	R: GCAATATTCCACCTTTACCATCA					
PC30	F: GATGTTCTTGTTGGGTTTGGATA	$(TG)_9$	102	50	6	KU220195
	R: CTCAACTTCACACAACCCAGATT					
PC39	F: AAACAGACCCACTAGTGCAAAAA	$(GA)_9$	154	43	8	KU220194
	R: ATCCAACAGACAACAAAACCATC					
PC53	F: AGATCATCACCTCCTCAAAACAA	$(CAC)_7$	114	57	4	KU220191
	R: TAGTTTCGGTTCGTTAGGGGTAT					
PC57	F: GATTGGTTTAGTTATTGGCGAGA	$(GA)_8$	157	43	6	KU220192
	R: ACAAAACGACAAGATCACAAACA					
PC60	F: AAAGGAAACACATAAGACTTTGGC	$(ATC)_7$	157	51	5	KU220193
	R: GTACCCCAAGAAAGCTGTTGTC					

Ta = annealing temperature.

Table 3. Results of initial primers screening in four population of *Populus cathayana*.

Locus	Population LH (N = 14)				Population DL (N = 11)				Population GH (N = 14)				Population LT (N = 9)			
	A	H_O	H_E	N_A	*A*	H_O	H_E	N_A	*A*	H_O	H_E	N_A	*A*	H_O	H_E	N_A
PC9	5	0.000	0.519	0.333	2	0.000	0.311	0.904	4	1.000	0.765	0.057	3	0.000	0.627	0.531
PC10	5	1.000	0.698*	0.000	4	0.909	0.779	0.000	4	1.000	0.764	0.000	4	0.333	0.467	0.000
PC14	3	0.000	0.635	0.381	4	0.727	0.699	0.000	3	0.857	0.531*	0.000	5	0.889	0.804	0.265
PC23	3	1.000	0.582*	0.000	4	0.727	0.632	0.000	5	0.5	0.720	0.289	5	0.667	0.634	0.213
PC26	5	0.786	0.817	0.121	6	0.455	0.640	0.858	3	0.357	0.415	0.362	4	0.333	0.725	0.603
PC30	4	0.786	0.651*	0.364	3	0.000	0.675	0.393	2	0.000	0.349	0.276	3	0.000	0.523	0.343
PC39	7	0.929	0.857	0.006	3	0.909	0.598*	0.000	2	1.000	0.519*	0.000	5	0.444	0.758	0.377
PC53	2	0.000	0.423	0.302	2	0.000	0.519	0.674	2	0.071	0.389	0.238	2	0.000	0.471	0.315
PC57	4	0.643	0.756	0.421	4	1.000	0.775*	0.000	4	0.714	0.743	0.333	6	0.333	0.817	0.418
PC60	4	0.786	0.621	0.000	5	0.727	0.727	0.232	4	0.714	0.594	0.000	4	0.778	0.739	0.034

A = total number of alleles per locus; H_O = observed heterozygosity; H_E = expected heterozygosity; N_A = null allele frequency. *Significant departure from Hardy-Weinberg equilibrium at $P < 0.01$. See Table 1 for population abbreviations.

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.

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