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Isolation and characterization of microsatellite loci for *Cunninghamia lanceolata* (Lamb.) Hook

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ABSTRACT. As a result of human activities, wild populations of *Cunninghamia lanceolata* (Cupressaceae) have sharply declined in recent years. The development and implementation of a valid conservation strategy require a clear understanding of the genetic makeup of this species. Eleven polymorphic microsatellite loci were isolated and characterized from samples of 52 individuals from the Provenance Test Plantation in Fenyi, Jiangxi Province, China. Among the loci, 10 were polymorphic and 1-34 (average 18.182) alleles per locus were identified. Observed and expected heterozygosities ranged from 0 to 0.750 (mean 0.456) and 0 to 0.968 (mean 0.749), respectively. These microsatellite loci may facilitate further research on the molecular breeding and population genetics of *C. lanceolata* and its relatives.

Key words: *Cunninghamia lanceolata*; Cupressaceae; Microsatellites; Genetic conservation; Population genetics

INTRODUCTION

Cunninghamia lanceolata (Lamb.) Hook is one of the most important commodities in the timber tree industry because of its fast growth, good material, high yield, and wide use (Shi et al., 1993; Yang et al., 2009); moreover, it is widely cultivated in southern China. As a result of human overexploitation of natural populations, wild populations of *C. lanceolata* have sharply declined (Lu et al., 2001). Understanding the genetic structure of a population of *C. lanceolata* will set the foundation for the conservation and utilization of important wild genetic resources. Nevertheless, research on the genetic structure of a population has been impeded because of the lack of high polymorphic genetic markers. Herein, we report a set of high-polymorphic novel microsatellite loci developed specifically for *C. lanceolata*, which will facilitate the exploration of the population genetics and genetic enhancement of *C. lanceolata*.

MATERIAL AND METHODS

Biological material

The biological material was sampled from the Provenance Test Plantation located in Fenyi, Jiangxi Province, China, in 2012, which was constructed in 1981 with seeds collected throughout the country (Duan et al., 2013). The fresh leaves were collected and immediately stored on silica gel. A specimen of this species was deposited in the Key Laboratory of Tree Breeding and Cultivation of the State Forestry Administration, Research Institute of Forestry, Chinese Academy of Forestry.

DNA isolation and marker procedure

Complete genomic DNA was extracted from silica gel-dried leaves using the modified cetyltrimethylammonium bromide (CTAB) procedure (Doyle, 1987).

Total DNA was digested with *Rsa*I and *Xmn*I (New England Biolabs, Ipswich, MA, USA) at 37°C for 12 h. The digested DNA was linked to a double-strand Super SNX-24 linker (reverse: 5'-pGAT TCT GCT AGC TAG GCC TTA AAC AAA-3'; forward: 5'-GTT TAA GGC CTA GCT AGC AGA ATC-3') at 4°C for 12 h. The ligated DNA was then randomly hybridized with 2 single-strand biotinylated microsatellite probes [5'-$(CA)_{15}$-Biotin and 5'-$(GA)_{15}$-Biotin]. Hybridized DNA was captured by streptavidin-coated paramagnetic beads (Dynal Biotech Dynabeads M-280 Streptavidin, Oslo, Norway) and collected with a magnetic particle-collecting unit (MPC, Dynal Biotech Dynal MPC-S, Dynal, Oslo, Norway). The enriched DNA was amplified with superSNX-24 linker-forward as a primer, and the purified polymerase chain reaction (PCR) products were ligated into the pGEM-T Easy vector (Promega Corp., Madison, WI, USA) and then transformed into *Escherichia coli* (TransGen Biotech, Beijing, China) TOP10 cells.

In total, 120 positive colonies were randomly selected for sequencing using the ABI 3730XL DNA analyzer (Applied Biosystems, Foster City, CA, USA); a total of 80 (66.67%) sequences contained repeat motifs. Of the sequences, 65 were selected for primer design with Primer premier 5.0 (Premier Biosoft International, Silicon Valley, CA, USA). Finally, 11 primer pairs (Table 1) were chosen because of their clear single bands of expected size.

Amplification and sequencing

The forward primer of each primer pair was labeled with one of the 2 fluorescent dyes (i.e, FAM or HEX) at the 5'-end. PCR was performed in 10-μL reactions containing 5-50 ng template DNA, 0.4 μL 10 μM of each primer, 1 μL 10X PCR buffer (Mg^{2+} Plus), 0.8 μL 2.5 mM dNTP mixture, and 0.05 μL 5 U/μL rTaq DNA polymerase (TaKaRa, Dalian, Liaoning, China). The reactions were then placed in a temperature gradient 96 U thermocycler (Applied Biosystems).

PCR was performed using an initial denaturation step at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at a specific temperature for each locus (optimized for each locus; Table 1) for 30 s, extension at 72°C for 70 s, and a final extension at 72°C for 8 min. PCR products were screened on an ABI 3130xl genetic analyzer with the GeneScan LIZ500 size standard (Applied Biosystems).

Data analysis

Microsatellite profiles were analyzed with the aid of GeneMapper v4.0 (Applied Biosystems). The N_A, H_O, and H_E were calculated using Arlequin v3.0 (Excoffier et al., 2005).

RESULTS

In total, 10 of the 11 loci displayed polymorphisms, and the number of alleles per locus ranged from 1 to 34, with an average of 18.182 per locus. The H_O and H_E per locus ranged from 0 to 0.750 and 0 to 0.968, respectively (Table 1). All the DNA sequences in this study are available under GenBank accession Nos. KF873003-KF873013.

Table 1. Characteristics of 11 microsatellite loci developed for *Cunninghamia lanceolata*.

Locus	Primers (5'-3')	Repeat motif	Size range (bp)	Ta (°C)	N_A	H_O	H_E	GenBank accession No.
SM19	F: CACTTTGAATACGGTGCCTTTG R: CACGCAACCAAAACATGTAAAA	$(TG)_8$	223-241	57	6	0.385	0.604	KF873006
SM37	F: TAGGGTGAAAGGAGGATTTTGACAT R: ACATAGAGTGAGTGCCAATGCTCAT	$(TG)_{13}$	345-397	66	17	0.462	0.878	KF873008
SM2	F: CTCATCATTCTTGAACCCATCC R: AACCTGACCAATCCTTGCTCT	$(GT)_9$	231-259	58	16	0.750	0.874	KF873003
SM51	F: TCCTCTATCTTGTCAGTTTGGGTT R: TATTGGTGTTGCTCGTCATTCC	$(AC)_{12}$	291-303	66	6	0.442	0.511	KF873009
SM14	F: ATTTTCATTTTGAAGATCATCCGC R: CTTGTTTGGGTTTTGTTCTTCATC	$(AC)_{33}$	244-327	60	34	0.673	0.968	KF873005
SM13	F: TCGTGAGTTTCTTGGTCATTTCG R: CATAAGGGTTTTCCCCACGTATA	$(AG)_8$	389-395	61	6	0.404	0.603	KF873004
XSM22	F: AGATAGTTCTCCCAGGCTATCCAAGAT R: CCACCTACATACAACATAAGCGACCAA	$(AG)_{33}$	207-289	69	31	0.596	0.961	KF873007
SM53	F: CACATGAAGTGAATGCCAAAGTTA R: TTCCAGATAGTGTTGAATGGGGTA	$(CA)_6$	354	62	1	0	0	KF873010
XSM8	F: TCAGGGCATTTGAAACGAATAG R: TCAAGTGGGAGATTGTTGGGTA	$(CA)_{28}$	412-461	58	25	0.692	0.926	KF873011
XSM24	F: TTTCCCCTTAATGAACTTGTCTT R: ATTCAGATGGAGGCAACAGAG	$(CT)_{27}$	272-367	57	32	0.288	0.967	KF873013
SM9	F: TAGTGGCAAAGAAGGGAGAAAGG R: TGGTCTAAAAGTGCAAGTTGAGGA	$(GT)_{41}$	256-334	63	26	0.327	0.953	KF873012
Average					18.182	0.456	0.749	

Ta = annealing temperature.

CONCLUSIONS

As high-resolution and codominant-inheritance genetic markers (Vendramin et al., 1996), microsatellite analysis is widely applied to population genetic studies. The 11 novel microsatellite loci developed herein will play an important role in further genetic studies, including population genetic diversity and differentiation, gene flow, and population demography of *C. lanceolata*.

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