GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Short Communication

Microsatellite markers in *Paulownia kawakamii* (Scrophulariaceae) and cross-amplification in other *Paulownia* species

H.W. Wang[1], J.M. Duan[2], P. Zhang[1], Y.Q. Cheng[1], J.W. Wu[1] and G.Z. Wang[1]

[1]College of Plant Protection, Henan Agricultural University, Zhengzhou, China
[2]College of Life Sciences, Henan Agricultural University, Zhengzhou, China

Corresponding author: Y.Q. Cheng
E-mail: whwcas@163.com

Genet. Mol. Res. 12 (3): 3750-3754 (2013)
Received January 19, 2013
Accepted August 20, 2013
Published September 19, 2013
DOI http://dx.doi.org/10.4238/2013.September.19.6

ABSTRACT. *Paulownia kawakamii* is a fast-growing timber tree. In this study, 21 primer sets were developed using an enriched genomic library. The genetic diversity was measured in one *P. kawakamii* population. The number of alleles per locus ranged from 2 to 19. The observed and expected heterozygosities varied from 0.158 to 0.842 (mean = 0.421) and from 0.376 to 0.952 (mean = 0.771), respectively. All 21 loci were also polymorphic in closely related species (*P. tomentosa*, *P. elongata*, and *P. fortunei*). The described markers will be useful in future population genetic studies and molecular breeding of these *Paulownia* species.

Key words: *Paulownia elongata*; *Paulownia fortunei*; SSR; *Paulownia kawakamii*; *Paulownia tomentosa*

INTRODUCTION

Paulownia Sieb. et Zucc. is an economically important genus in the family Scrophulariaceae, with nine species of fast-growing timber trees (Yaycili and Alikamanoglu, 2005). *Paulownia*, which is native to China, has been introduced in Japan, Australia, Brazil, Europe, and USA. Its wood is strong and light in weight, and it is thus widely used for making furniture, aircraft, plywood, toys, and musical instruments (Ipekci and Gozukirmizi, 2003). *Paulownia* species are attractive trees, with large flowers and colors ranging from white to purple, and are also cultivated as ornamental trees (Yang et al., 1996). Its tolerance to drought and soil extremes makes it important commercially for use in reclamation of surface-mined land (Zhu et al., 1986). As a forestry tree, *Paulownia kawakamii* Ito is planted widely in southern China.

It is well known that simple sequence repeat (SSR) markers are powerful tools for population genetics investigations (Chun et al., 2010; An et al., 2011; Shepherd and Perrie, 2011; Guo et al., 2012). Microsatellite markers are unique in their abundant and random distribution throughout the eukaryotic genome. Here, we report the development of 21 microsatellite markers for *P. kawakamii* and apply them to other *Paulownia* species, including *P. tomentosa* (Thunb.) Steud., *P. elongata* S.Y. Hu, and *P. fortunei* (Seem.) Hemsl. These loci will be important for further analyzing the population genetics and evolutionary history, as well as facilitating molecular breeding of *P. kawakamii* and its related species.

MATERIAL AND METHODS

Genomic DNA (30 µg) was extracted from silica gel-dried leaves of a single individual of *P. kawakamii* using the modified CTAB method (Wang et al., 2011) and digested with *Rsa*I and *Xmn*I enzymes. The digested DNA was linked to forward (5'-GTTTAAGGCCTAGCTAGCAGA ATC-3') and reverse (5'-pGATTCTGCTAGCTAGGCCTTAAACAAAA-3') adapters using 2 units of T4 DNA ligase. After incubation for 16 h at 16°C, the fragments were separated by 2% agarose gel electrophoresis. DNA fragments with lengths between 400 and 1000 bp were recovered using the Qiaquick Gel Extraction Kit (Qiagen, Shanghai, China). Individual fragments were hybridized with three kinds of biotin-labeled probes (New England Biolabs Ltd, Beijing, China): $(AC)_8$, $(AG)_{18}$, and $(ATG)_{12}$. After recovery with streptavidin-coated magnetic beads, fragments were ligated into the pMD 18-T vector (TaKaRa, Dalian, China), and then transformed into *Escherichia coli* strain DH5α. We screened the positive clones by PCR amplification using M13F and M13R primers. Finally, 128 positive clones were selected and sequenced on an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA); 84 of these clones (approximately 67%) contained SSRs.

PCR primers were designed for 36 sequences that had a flanking region of adequate size for the design of forward and reverse primers, using the program Primer Premier version 5.0 (http://www.premierbiosoft.com). These primers were tested in 10 individuals from four *Paulownia* species. PCR was performed in of a solution (15 µL) containing approximately 75 ng genomic DNA, 10 µM each primer, and 1X PCR Mix (Tiangen Biotech, Beijing, China). Microsatellites were amplified under the following conditions: 5 min initial denaturation at 95°C; 36 cycles of 30 s at 94°C, 30 s at 51.1 to 57.3°C (Table 1), and 1 min at 72°C; and a final extension at 72°C for 10 min. The products were analyzed on 2.0% agarose gels. Finally, only 21 primer pairs (Table 1) that amplified a clear and single locus were selected, and the forward primer was labeled with one of the fluorescent dyes (FAM or HEX) to detect polymorphisms.

Table 1. Characteristics of 21 microsatellite loci in *Paulownia kawakamii*.

Locus	Primer sequence (5'-3')	Repeat motif	Size range	5'dye	Ta	GenBank ID
PT3	F: TGTTTACCTGCCTGAATGTC R: ATTGCCACCACAGAAGTCCC	$(TC)_{13}$	332-425	HEX	52.6	JX087668
PT7	F: CACATACATTTTAGCAGGAAG R: ATGGCTTGGATTTGAGTTTAC	$(AC)_{15}$	343-411	6-FAM	52.4	JX087669
PT12	F: CAGCAGAAAAGATAGAG R: TCCAAGCGTTCATACT	$(AC)_{18}$	139-253	HEX	52	JX535222
PT13	F: AGAGTGTGTGTGGCGAAT R: GGATTGATGGATTTAGCTCT	$(TG)_{12}(AG)_{15}$	329-402	6-FAM	52	JX535223
PT16	F: TCACAATCCCACCCACTC R: AGATCTTCCTCACCTCGTT	$(TC)_{8}(AC)_{18}$	169-211	6-FAM	52	JX535224
PT36	F: CGTGGTCTGTCTAAGGG R: AACGGAGTAGGTTGAGC	$(TG)_{10}(AG)_{15}$	334-424	HEX	53.2	JX087670
PT44	F: TTAGCTTCCGTGGTGA R: ATGGCTGAGGAGTTTCT	$(TC)_{22}$	276-314	HEX	53.1	JX087671
PT49	F: CATCCCAAACAACGCCAACT R: ACAAAACAGGAAAGAAGAC	$(CT)_{26}(CA)_{17}$	279-302	HEX	50	JX535225
PT50	F: GAACAAAGGAGCAGACCG R: GAAAGGGAATGTGAAATG	$(TG)_{9}$	310-320	HEX	52	JX535226
PT54	F: CCATCTATTTCCAACCTT R: ATCATCGTCTCACCACTA	$(GT)_{11}$	409-486	6-FAM	52.6	JX087672
PT87	F: CTTCAATGTAGAAAGGGTT R: GTTCAGAAAGTAATGGTGC	$(GT)_{12}$	276-314	HEX	53.9	JX087673
PT91	F: CCTCCTTTCACCAACTCC R: TCAAGCCTAAACCAGCAT	$(TG)_{10}(AG)_{9}C(GA)_{4}$	212-262	6-FAM	57.3	JX087674
PT96	F: TTGTTGCCGTCGGAGATT R: GGTGGAAGGTGGTTATGC	$(TG)_{12}$	242-269	6-FAM	55.7	JX087675
PT100	F: TGGGAATACAGGAGGAA R: TTGGCAGTGTTGAAATG	$(AC)_{9}$	346-358	HEX	50.7	JX535227
PT106	F: CTTTCTGCGCTTTTTCTTCT R: CTCGTCCCCATCATTATTAC	$(TG)_{20}$	200-257	HEX	52.6	JX535228
PT150	F: CCTGTAGAAAATGGGGAGT R: GGCTAAAAGGTGTAATCG	$(TG)_{18}$	260-305	6-FAM	49.5	JX535229
PT151	F: ATCACAAGTCATACCACCAT R: CATAACCCAAGCCATACA	$(GT)_{19}$	334-411	6-FAM	51.1	JX087676
PT171	F: TTGGTTTGCCTTTCCTCTG R: ATGGGCGTTCTGTGCTTCT	$(TG)_{19}$	243-265	6-FAM	53.2	JX087677
PT187	F: TGCTTCTCCCTACACT R: AAAACACGCATACAAT	$(TG)_{16}$	210-264	6-FAM	51.7	JX087678
PT196	F: ATCATTGTTCCTCCCTTT R: GCAGCCTAACAGACAGTGAAC	$(TG)_{21}$	150-230	HEX	50	JX535230
PT203	F: GCTGACGCAACAATAGAG R: GCATCAAAGACCAAGAAG	$(TG)_{18}$	409-486	6-FAM	52.4	JX087679

Polymorphisms of all of the primer pairs were then surveyed in 48 individuals collected from four *Paulownia* species: *P. kawakamii* (N = 19, one population, 25°37.645N, 109°54.705E), *P. elongata* (N = 19, one population, 34°52.317N, 113°35.560E), *P. tomentosa* (N = 5, one populations, 34°47.335N, 113°39.729E), and *P. fortunei* (N = 5, one population, 34°47.356N, 113°39.718E). The number of alleles per locus (N_A), the observed and expected heterozygosities (H_O and H_E), and the deviation from Hardy-Weinberg equilibrium (HWE) were analyzed using the computer program package Arlequin version 3.1 (Excoffier et al., 2005).

RESULTS AND DISCUSSION

We produced 21 polymorphic microsatellite loci that produced clear and reliable bands, and individual loci were assessed in 48 plant samples. Twenty-one loci were

successfully amplified for all samples drawn from the four *Paulownia* species (Table 2). The number of alleles per locus ranged from 2 to 19 and 3 to 20, respectively, for *P. kawakamii* and *P. elongata*. The estimated average heterozygosity value of the microsatellite loci was high; values of 0.421 (0.15 to 0.842) (H_O) and 0.771 (0.376 to 0.952) (H_E) were found in the *P. kawakamii* population, and 0.519 (0 to 0.947) and 0.812 (0.653 to 0.957) in the *P. elongata* population. The exact tests for HWE revealed that 16 of the 21 microsatellites showed significant deviation from HWE ($P < 0.05$) within the *P. kawakamii* population. The reason for this may be that the small population and the significantly high frequency of asexual reproduction maintained the homozygotes (Lee et al., 2012).

Table 2. Results of initial microsatellite marker screening in four species of *Paulownia*.

	P. kawakamii (N = 19)			*P. elongata* (N = 19)			*P. tomentosa* (N = 5)	*P. fortunei* (N = 5)
Locus	N_A	H_O	H_E	N_A	H_O	H_E	N_A	N_A
PT3	19	0.842	0.952*	20	0.737	0.957*	7	8
PT7	10	0.579	0.861*	8	0.632	0.839*	4	6
PT12	10	0.368	0.798*	7	0.000	0.791*	4	6
PT13	8	0.632	0.818	5	0.000	0.745*	4	4
PT16	4	0.421	0.559	8	0.947	0.707	2	7
PT36	13	0.579	0.912*	15	0.579	0.909*	7	9
PT44	9	0.316	0.809*	9	0.368	0.869*	5	3
PT49	6	0.368	0.681*	5	0.053	0.696*	3	4
PT50	2	0.368	0.508	3	0.368	0.653*	2	2
PT54	12	0.368	0.869*	10	0.211	0.817*	4	4
PT87	9	0.474	0.886*	12	0.526	0.915*	5	6
PT91	8	0.412	0.838*	10	0.474	0.839*	6	4
PT96	11	0.579	0.849*	10	0.737	0.872*	5	7
PT100	4	0.222	0.376	4	0.895	0.687	2	2
PT106	11	0.263	0.836*	8	0.947	0.846*	4	7
PT150	7	0.263	0.555*	4	0.895	0.667*	4	3
PT151	9	0.421	0.836*	10	0.632	0.875*	6	5
PT171	5	0.158	0.676*	8	0.368	0.775*	5	4
PT187	10	0.316	0.863*	13	0.632	0.848*	6	5
PT196	10	0.684	0.838	11	0.632	0.885*	2	7
PT203	7	0.211	0.878*	8	0.263	0.868*	3	5

*Significant Bonferroni-corrected ($P < 0.05$) departures from Hardy-Weinberg equilibrium. N_A, number of alleles; H_O, observed heterozygosity; and H_E, expected heterozygosity.

The 21 microsatellite markers developed in this work are suitable for further molecular breeding and genetic studies of *P. kawakamii*. Cross-amplification of these loci in related species suggests that they may be broadly applicable across the genus *Paulownia*.

ACKNOWLEDGMENTS

Research supported by the National Science Foundation of China (#30770325, 31170351) and the Natural Science Foundation of Henan (#2008A180012).

REFERENCES

An HS, Byun SG, Kim YC, Lee JW, et al. (2011). Wild and hatchery populations of Korean starry flounder (*Platichthys stellatus*) compared using microsatellite DNA markers. *Int. J. Mol. Sci.* 12: 9189-9202.

Chun YJ, Fumanal B, Laitung B and Bretagnolle F (2010). Gene flow and population admixture as the primary post-invasion processes in common ragweed (*Ambrosia artemisiifolia*) populations in France. *New Phytol.* 185: 1100-1107.

Excoffier L, Laval G and Schneider S (2005). Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evol. Bioinformat. Online* 1: 47-50.

Guo J, Liu Y, Wang Y, Chen J, et al. (2012). Population structure of the wild soybean (Glycine soja) in China: implications from microsatellite analyses. *Ann. Bot.* 110: 777-785.

Ipekci Z and Gozukirmizi N (2003). Direct somatic embryogenesis and synthetic seed production from *Paulownia* elongata. *Plant Cell Rep.* 22: 16-24.

Lee JY, Lee DH and Choi BH (2012). Isolation and characterization of 13 microsatellite loci from a korean endemic species, *Sophora koreensis* (Fabaceae). *Int. J. Mol. Sci.* 13: 10765-10770.

Shepherd LD and Perrie LR (2011). Microsatellite DNA analyses of a highly disjunct New Zealand tree reveal strong differentiation and imply a formerly more continuous distribution. *Mol. Ecol.* 20: 1389-1400.

Wang H, Fang X, Ye Y and Cheng Y (2011). High genetic diversity in *Taihangia rupestris* Yu et Li, a rare cliff herb endemic to China, based on inter-simple sequence repeat markers. *Biochem. Syst. Ecol.* 39: 553-561.

Yang JC, Ho CK, Chen ZZ and Chang SH (1996). Paulownia x taiwaniana (Taiwan Paulownia). *Biotech. Agr. Forest.* 35: 269-290.

Yaycili O and Alikamanoglu S (2005). The effect of magnetic field on *Paulownia* tissue cultures. *Plant Cell Tissue Organ Cult.* 83: 109-114.

Zhu ZH, Chao CJ, Lu XY and Xiong DY (1986). *Paulownia* in China: Cultivation and Utilization. Asia Netmork of Biological Sciences. Singapore and International Developmental Research Center, Canada.