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PRIMER NOTE

ISOLATION AND CHARACTERIZATION OF 20 POLYMORPHIC MICROSATELLITE MARKERS FOR *JUGLANS MANDSHURICA* (JUGLANDACEAE)[1]

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- *Premise of the study:* Fifty microsatellite loci were developed for the endangered species *Juglans mandshurica* to investigate its genetic diversity and population structure.
- *Methods and Results:* In all, 50 microsatellite markers were isolated from *J. mandshurica*, using the Fast Isolation by AFLP of Sequences COntaining repeats (FIASCO) protocol. Twenty of these polymorphic markers were assessed in samples collected from 98 individuals among five populations in northeastern China. Across all of the *J. mandshurica* samples, the number of alleles per locus ranged from one to 17.
- *Conclusions:* These new microsatellite loci will be useful for conservation genetics studies of *J. mandshurica*.

Key words: genetic conservation; Juglandaceae; *Juglans mandshurica*; microsatellite marker; simple sequence repeat.

Juglans mandshurica Maxim. (Juglandaceae) is a deciduous tree that is widely distributed in northeastern China, as well as in some areas of Korea and the Russian Far East (Lu, 1982). However, the habitat of *J. mandshurica* is now seriously threatened by human activities (Wang et al., 2011). *Juglans mandshurica* has been used in folk medicine for many years, particularly in China and India (Xu et al., 2010). Its leaves, fruits, roots, stem bark, and seeds have also been used as traditional medicine for cancer treatment in Asia and Europe (Kim et al., 1998; Sun, 2004). The therapeutic benefits of *J. mandshurica* have been ascribed to its naphthoquinone content. Naphthoquinone is known to have various physiological properties including the induction of apoptosis effect (Kang et al., 2001).

To date, there have been only a few reports on microsatellites in *J. mandshurica* (Qi et al., 2011). The molecular genetic diversity studies of *J. mandshurica* are limited to the work by Woeste et al. (2002) using dominant markers. Here, we used an improved technique for the isolation of codominant compound microsatellite markers (Lian et al., 2006) to isolate 20 polymorphic microsatellite loci from *J. mandshurica*. These polymorphic markers can be useful for the development of effective conservation programs of the species.

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METHODS AND RESULTS

Total genomic DNA was extracted from silica gel–dried leaves of *J. mandshurica* using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle, 1991). After digestion with *Eco*RI and *Hin*dIII (TaKaRa Biotechnology Co., Dalian, Liaoning, China) at 37°C for 4 h, a fraction containing 200–1000-bp fragments was isolated from total genomic DNA (250 ng) and ligated with *Eco*RI adapters (5′-CTCGTAGACTGCGTACC-3′ and 3′-CTGACGCATGGTTAA-5′) and *Hin*dIII adapters (5′-GACGATGAGTCCTGAG-3′ and 3′-TACTCAGGACTCTCGA-5′) using T4 DNA ligase (Biomanbio, Shanghai, China) at 4°C overnight. Ligated fragments were subsequently PCR amplified with adapter-specific primers. To enrich the fragments containing microsatellite repeats, PCR products were hybridized with biotin-labeled probes, $(AG)_{15}$ and $(GT)_{15}$. Streptavidin-coated magnetic beads (Promega Biotech, Beijing, China) were then used to separate and capture DNA fragments that were hybridized to the probes, according to the manufacturer's instructions. DNA fragments containing simple sequence repeats were eluted from streptavidin-coated magnetic beads in a 1.5-mL tube and put in a freeze-drying machine at –40°C for 3 h. Microsatellite-enriched fragments in eluted solutions were PCR amplified in 20-μL reaction mixtures, consisting of 8.6 μL of DNA template, 10 μL of 2× PCR Mix (Boyouxinchuang Biotech, Beijing, China), 0.3 μL of E00 (10 μM), 0.3 μL of H00 (10 μM), and 0.2 μL of *Taq* polymerase. PCR conditions were as follows: five cycles of denaturation at 94°C for 45 s, annealing at 50°C for 45 s, and extension at 72°C for 45 s; and final extension at 72°C for 10 min. The PCR products were ligated into the pMD-18T vector (TaKaRa Biotechnology Co.) and then transformed into *Escherichia coli* DH5α competent cells to generate the microsatellite sequence–enriched library. Recombinant colonies were selected by white/blue screening on Luria–Bertani agar plates containing ampicillin (60 μg/mL).

Positive clones were then screened using the universal primers of pMD-18T (M13-47 and RV-M, 5′-CGCCAGGGTTTTCCCAGTCACGAC-3′ and 5′-GAGCGGATAACAATTTCACACAGG-3′, respectively), as well as the $(AG)_9$/$(GT)_9$ tandem repeat primers. PCR amplifications were performed in a 15-μL reaction mixture containing DNA template (single colonies), 7.5 μL of 2× PCR Mix (Boyouxinchuang Biotech), 0.1 μL of M13-47 (0.2 μM), 0.1 μL of RV-M (0.2 μM), 0.1 μL of $(AG)_9$/$(GT)_9$ (0.2 μM), 0.2 μL of *Taq* polymerase, and 7 μL of ddH_2O. PCR amplification was performed in the GeneAmp PCR System 9600 Thermal Cycler (Applied Biosystems, Foster City, California, USA) under the following conditions: initial denaturation at 94°C for 10 min; 33 cycles of denaturation

TABLE 1. Primer sequences and characteristics of 50 microsatellite loci in *Juglans mandshurica*.

Locus	Primer sequences (5′–3′)	Repeat motif	Fragment size (bp)	T_a (°C)	GenBank accession no.
P1*	F: CCAAGGGAAATACAAGGTCT	$(AG)_{11}$	186	58	JQ618127
	R: GTTGCCTGAACATCACAGAT				
P2	F: GGTCAAGGCTCTCTGCCTCAA	$(TC)_{15}$	249	57	JQ618128
	R: TAAAATCACCCCCCTCCACTC				
P3*	F: GGAGTTTCGGGTAGGGTTTGA	$(TC)_{15}$	184	58	JQ618129
	R: TAATGGTTTGGAGGAATGGAG				
P4	F: TAGGAGATATTTTCAAGAGGA	$(AC)_8$	136	57	JQ618130
	R: TTGTGTGAGCATGAGTTTTAG				
P5*	F: TATTTCATTGCCAAGACCGAG	$(CA)_8(AT)_5(AG)$	164	57	JQ618131
	R: GTCCCTAAAGAGTGATTTTGTTGT				
P6	F: AAACAGCATCTGAAACCACA	$(CT)_{10}(CA)_8T$	288	56	JQ618132
	R: GCTTTTGCCTCCATAATTAG				
P7	F: AAAAGAGGTGTTGAGGATGG	$(GA)_{13}$	113	55	JQ618133
	R: TTATTTTCACTTGCTTTGCC				
P8*	F: CTGAAAAGTGGGGCAAAGCA	$(GA)_{14}$	280	59	JQ618133
	R: GGGAGACATACCCGACAAGG				
P9	F: CACGACGGCACAACTAAAGG	$(AG)_{20}$	144	57	JQ618134
	R: CACTGAAGGCACACCCAAGA				
P10	F: AAGATTGTTTCTAAGTTTGTC	$(AT)_3(AG)_{15}$	136	58	JQ618135
	R: AATGTGTAGGTCAATAGAGG				
P11	F: TCCACCGTAAAAGATTGTT	$(AG)_{16}$	108	56	JQ618136
	R: GCTTCATAGAGATTCCCAT				
P12*	F: AAACCCTATCTTCCGCAGAA	$(CT)_{11}$	108	57	JQ618137
	R: GATGGAGAGCTAAGGAGTCG				
P13	F: GGTTAGAGTGAGCGAGAGTTG	$(AG)_{15}$	186	58	JQ618138
	R: ATCCTTAGAGTTGAATGGGC				
P14*	F: AAAAAACCTTGCGACCAAAG	$(GA)_{26}$	211	56	JQ618139
	R: CCACTAAAACACACTCCATCA				
P15	F: CTCTCTCGCAAAACCTCTCG	$(CT)_{26}$	171	58	JQ618140
	R: CGTGCATGCTAGGAAACTTA				
P16*	F: CTGCTGATGTGGTGGTGAAG	$(AG)_9$	194	59	JQ618141
	R: GATCTGGTTTGTGGAGGGAA				
P17	F: TGAGTACAGAACTGGCATG	$(TG)_{18}$	187	55	JQ618142
	R: ACTGGGGTGGGCTAAAAAG				
P18*	F: TAGAAACCTCGTGGACTTG	$(AC)_{10}$	214	58	JQ618143
	R: ACTGCTAGAGCCTATGGAA				
P19	F: CCGAATGGAGGAAGGAAGG	$(GT)_{22}$	126	56	JQ618144
	R: ACACGGCTTAGGGCCATAAA				
P20	F: CAGCCACCCCATTACCATTC	$(CT)_9(CA)_7$	283	57	JQ618145
	R: CACAACTCACAAAACCAACAAC				
P21*	F: TGTTACTCTGTTGGGTCGT	$(GA)_{11}(TA)_4$	196	58	JQ618146
	R: CTGGTGTAGCAGTTCATTT				
P22	F: CCTTCTGCTCAGACAAACA	$(AG)_{13}$	241	56	JQ618147
	R: CTAGACCAAAGACCACAACTAT				
P23*	F: CAGGACACGACAACCCCAT	$(AG)_3(AG)_{10}$	200	59	JQ618148
	R: TAACACTCCACGCACGCAC				
P24	F: TATATTGTGGGAGGTGGGT	$(CT)_9$	316	57	JQ618149
	R: TTAAGGGAGTTGTTGAAGC				
P25	F: GGGAGAATGAAAATGACGG	$(AG)_{19}$	124	57	JQ618150
	R: GAAACGAAGGAAAAATGAGG				
P26*	F: CTTCAAATAATGGAACGGT	$(CT)_{17}$	195	56	JQ618151
	R: TAAAGAGATGGAGTACGCT				
P27	F: TTCATAGCACATACCAGTTC	$(CT)_{11}$	283	58	JQ618152
	R: TCCGTAACATCAATCATTC				
P28*	F: GCTTTTGTTACTTTGTGCC	$(CT)_{11}$	196	57	JQ618153
	R: GAGCTGAATTTTTTACCTGA				
P29*	F: GAATAAAAGAAGTTTGAC	$(CT)_{10}$	191	58	JQ618154
	R: TCTGACCCAAATTCCATAG				
P30*	F: ATGGGAATCACAGGTGAC	$(AG)_{13}$	252	57	JQ618155
	R: TGGGAATATCTTCGCAGAG				
P31*	F: CCTTCAACCACTTCAATA	$(CT)_{13}$	244	55	JQ618156
	R: GAGACACACGCACAAAACC				
P32	F: AAGTAAAACCCTAAGTCC	$(CT)_{14}$	241	57	JQ618157
	R: AAAATCCAAACTCAAGCCC				
P33	F: CCAAGGCACCAACACAAT	$(TC)_{15}$	266	56	JQ618158
	R: CCCATGCAATAACAAACCA				
P34	F: TTCCCCATCTCAAATCTG	$(GA)_{17}$	159	58	JQ618159
	R: TTCCTCGTGTAAGTACCCC				
P35*	F: TGCTTTTACCTTCCCTCT	$(AG)_{18}$	296	59	JQ618160
	R: GTCCAACCCAAGTTCCTCC				

TABLE 1. Continued.

Locus	Primer sequences (5′–3′)	Repeat motif	Fragment size (bp)	T_a (°C)	GenBank accession no.
P36	F: ACCAAACAGAACGAGTAA	$(GA)_{13}(GT)_4$	240	57	JQ618161
	R: AGGGGATAGATTGTGATAC				
P37	F: CGTTTTGAGTTTCTGCCT	$(GA)_{33}$	291	58	JQ618162
	R: CACACACTACATGGATGTC				
P38	F: ATGTGTAACCAGATAAGG	$(GA)_{18}$	158	57	JQ618163
	R: ACAAATTGGTCATCCTAGA				
P39	F: GATCTGAAGAGCCTGCCT	$(AG)_{30}$	198	56	JQ618164
	R: ATCCACCAAAACCCTAAAA				
P40	F: CCCTCGTTACTCTCCCTT	$(CT)_{24}$	170	58	JQ618165
	R: ATGTGGGTCGTGGGTTTGTC				
P41*	F: AGCATACCTTCAATGGAT	$(AG)_{18}$	206	59	JQ618166
	R: CGCAGAATACACGCAAATAG				
P42	F: ACAGAATGTGAGGTTTACTACG	$(CT)_{10}$	250	57	JQ618167
	R: GTGCTACTCTTTTTGTGGAT				
P43	F: TACAGCACACCCCTGAAAT	$(AG)_{18}$	234	58	JQ618168
	R: AACGACGCCGACCAACAAC				
P44*	F: AAGCATCATCTCTATTTCTC	$(CT)_{11}$	200	57	JQ618169
	R: ACTTTGTGGGTGTTTCTAT				
P45*	F: TTGATGCCTGTAGGTGAATG	$(CT)_{10}(CA)_{13}$	252	57	JQ618170
	R: TAATGCTATGGAAGTATGGA				
P46	F: ATTCTAATGCCGTCACTTG	$(ACC)_3(AC)_8$	234	58	JQ618171
	R: AGGATCTGTTGACACATACAT				
P47*	F: TGTGAAAAGGACTTCACAT	$(AT)_4(AG)_2(AC)$	217	57	JQ618172
	R: GGATCTCAAGACTGGCTAG				
P48	F: TTATTATGATGGGGTCTTTG	$(TG)_{20}$	198	56	JQ618173
	R: TTGATACCAACTGTAACGC				
P49	F: CAAAGCAGAGGCTGAGATA	$(GC)_4(AC)_5$	180	57	JQ618174
	R: CTAAGCAAAGGAACGAAAG				
P50	F: GGGGAAACAGCAACAATAG	$(AC)_{18}$	268	58	JQ618175
	R: TCCAGGAAGTTAGGGTGAG				

Note: T_a = annealing temperature when run individually.
* Indicates polymorphic primers; all other loci were monomorphic.

at 94°C for 45 s, annealing at 58°C for 45 s, and extension at 72°C for 45 s; and final extension at 72°C for 10 min. A total of 90 colonies were randomly selected and sequenced on an ABI Prism ABI 3730xL automated DNA sequencer (Applied Biosystems); 82 of these contained repeats. Primer Premier 5 software (PREMIER Biosoft International, Palo Alto, California, USA) was used to design primer pairs for 50 of the 82 repeat-containing samples. The forward polymorphic primer of each pair was also labeled with a fluorochrome, 6-FAM (Applied Biosystems).

These 50 primer pairs were then tested for amplification in 20 samples from five different populations (four individuals each) from the mountains of northeastern China (Appendix 1). PCR products were electrophoresed on 8% denaturing polyacrylamide gels and visualized by silver staining. We found that all 50 primer pairs successfully amplified products after PCR optimization (denaturation at 94°C for 45 s, at 55–60°C for 45 s, and 32 cycles at 72°C for 45 s, with a final extension of 10 min at 72°C) (Table 1), and that 20 of these primer pairs amplified high-quality, polymorphic PCR products. These 20 polymorphic primer pairs were characterized in 98 individuals among five natural populations of *J. mandshurica* (Appendix 1). The distances between these individuals were more than 100 m. PCR products were analyzed by capillary electrophoresis, visualized using the ABI 3730xL sequencer with a GeneScan 500 ROX Size Standard (Applied Biosystems), and scored using GeneMarker (SoftGenetics, State College, Pennsylvania, USA).

Genetic diversity statistics, including the number of alleles, observed heterozygosity, and expected heterozygosity, were calculated based on 98 samples using the software POPGENE32 (version 1.31; Yeh et al., 1999). The number of alleles ranged from one to 17 (mean = 6), whereas the expected and observed levels of heterozygosity were 0–0.925 and 0–1.000 (mean = 0.358 and 0.627), respectively. The large number of alleles per locus indicates the potential usefulness of these primers to characterize the population genetic structure of *J. mandshurica*. Moreover, nine loci (P8, P12, P18, P23, P26, P30, P41, P44, P45) in *J. mandshurica* were found to show significant deviations from Hardy–Weinberg equilibrium ($P < 0.01$ in χ^2 test) (Table 2) due to heterozygote deficiency. No significant linkage disequilibrium was detected between any pairs of loci.

CONCLUSIONS

Twenty polymorphic microsatellite markers were identified in *J. mandshurica* and used to investigate its genetic diversity and population structure. The microsatellite loci described here (including both monomorphic and polymorphic primers) can be useful for conservation genetic studies of *J. mandshurica.*

LITERATURE CITED

DOYLE, J. J. 1991. DNA protocols for plants: CTAB total DNA isolation. *In* G. M. Hewitt and A. Johnston [eds.], Molecular techniques in taxonomy, 283–293. Springer-Verlag, Berlin, Germany.

KANG, K. H., K. H. LEE, M. Y. KIM, AND K. H. CHOI. 2001. Caspase-3-mediated cleavage of the NF-κB subunit p65 at the NH_2 terminus potentiates naphthoquinone analog-induced apoptosis. *Journal of Biological Chemistry* 276: 24638–24644.

KIM, S. H., K. S. LEE, J. K. SON, G. H. JE, J. S. LEE, C. H. LEE, AND C. J. CHEONG. 1998. Cytotoxic compounds from the roots of *Juglans mandshurica. Journal of Natural Products* 61: 643–645.

LIAN, C. L., M. A. WADUD, Q. F. GENG, K. SHIMATANI, AND T. HOGETSU. 2006. An improved technique for isolating codominant compound microsatellite markers. *Journal of Plant Research* 119: 415–417.

LU, A. M. 1982. The geographical dispersal of Juglandaceae. *Acta Phytotaxonomica Sinica* 20: 257–274.

QI, J. X., Y. B. HAO, Y. ZHU, C. L. WU, W. X. WANG, AND P. LENG. 2011. Studies on germplasm of *Juglans* by EST-SSR markers. *Acta Horticulturae Sinica* 38: 441–448.

TABLE 2. Results of PCR screening of 20 polymorphic microsatellite loci in *Juglans mandshurica*.[a]

	Xiaoxing'anling (N = 19)			Zhangguangcailing (N = 20)			Changbai Mountain (N = 20)			Laoyeling (N = 19)			Wanda Mountain (N = 20)			
Locus	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	HWE P value[b]
P1	5	0.313	0.720	6	0.688	0.470	6	0.250	0.728	5	0.250	0.754	6	0.125	0.758	0.128
P3	5	0.250	0.780	5	0.625	0.474	7	0.375	0.798	5	0.438	0.760	6	0.250	0.841	0.012
P5	5	0.375	0.726	6	0.000	0.712	8	0.125	0.758	5	0.188	0.601	6	0.125	0.677	0.011
P8	7	0.438	0.798	4	0.188	0.591	8	0.250	0.768	6	0.063	0.750	7	0.313	0.802	0.000
P12	5	0.500	0.595	3	0.063	0.619	7	0.563	0.633	6	0.313	0.534	3	0.625	0.542	0.000
P14	12	0.400	0.869	8	0.267	0.770	9	0.438	0.605	8	0.125	0.794	9	0.077	0.846	0.010
P16	5	0.188	0.565	3	0.063	0.542	5	0.500	0.559	4	0.438	0.569	4	0.375	0.595	0.113
P18	5	0.688	0.750	4	0.688	0.466	7	0.750	0.651	5	0.438	0.655	7	0.375	0.813	0.000
P21	7	0.125	0.802	6	0.500	0.629	11	0.125	0.865	8	0.188	0.728	7	0.250	0.710	0.011
P23	3	0.563	0.365	4	0.063	0.625	2	0.625	0.315	3	0.688	0.365	2	0.438	0.466	0.000
P26	8	0.188	0.843	5	0.125	0.649	6	0.063	0.790	5	0.000	0.665	7	0.000	0.702	0.000
P28	7	0.313	0.633	4	0.813	0.236	6	0.125	0.748	4	0.625	0.375	5	0.313	0.712	0.081
P29	4	0.250	0.579	4	0.313	0.591	3	0.500	0.486	4	0.250	0.659	4	0.813	0.236	0.046
P30	7	0.500	0.768	5	0.188	0.623	17	0.125	0.925	6	0.250	0.750	11	0.375	0.857	0.000
P31	2	0.938	0.063	2	0.875	0.121	2	0.938	0.063	1	1.000	0.000	2	0.938	0.063	0.066
P35	6	0.375	0.786	7	0.063	0.702	6	0.313	0.714	7	0.188	0.756	6	0.375	0.655	0.794
P41	6	0.375	0.849	7	0.063	0.726	6	0.250	0.843	6	0.125	0.827	8	0.250	0.798	0.000
P44	5	0.000	0.768	4	0.000	0.599	5	0.125	0.730	5	0.000	0.726	5	0.438	0.508	0.000
P45	6	0.133	0.821	6	0.286	0.725	8	0.063	0.819	5	0.438	0.736	6	0.273	0.823	0.000
P47	2	0.875	0.315	2	0.813	0.272	3	0.688	0.401	3	0.625	0.325	3	0.750	0.232	0.316
Average	6	0.389	0.670	5	0.334	0.557	6.6	0.359	0.660	5	0.331	0.616	6	0.374	0.632	

Note: A = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; HWE = Hardy–Weinberg equilibrium; N = sample size.
[a]All values are based on 98 samples located in Xiaoxing'anling, Zhangguangcailing, Changbai Mountain, Laoyeling, and Wanda Mountain (Appendix 1).
[b]Values shown are χ^2 test results for each locus across all populations sampled.

SUN, M. L. 2004. Chemical components of manchurian walnut and the research development of its bio-activity. *Journal of Northeast Forestry University* 32: 85–86.

WANG, D. N., C. C. MU, Z. GAO, AND F. J. FENG. 2011. ISSR analysis of genetic diversity of *Juglans mandshurica* Maxim. populations. *Nonwood Forest Research* 29: 22–29.

WOESTE, K., R. BURNS, O. RHODES, AND C. MICHLER. 2002. Thirty polymorphic nuclear microsatellite loci from black walnut. *Journal of Heredity* 93: 58–60.

XU, H. L., X. F. YU, S. C. QU, R. ZHANG, X. R. QU, Y. P. CHEN, X. Y. MA, AND D. Y. SUI. 2010. Anti-proliferative effect of Juglone from *Juglans mandshurica* Maxim. on human leukemia cell HL-60 by inducing apoptosis through the mitochondria-dependent pathway. *European Journal of Pharmacology* 645: 14–22.

YEH, F. C., R. C. YANG, AND T. BOYLE. 1999. POPGENE version 1.32: Microsoft Windows–based freeware for population genetic analysis, quick user guide. Center for International Forestry Research, University of Alberta, Edmonton, Alberta, Canada.

APPENDIX 1. Geographic localities and sample sizes of the *Juglans mandshurica* populations in this study. Voucher specimens were deposited at the Northeast Forestry University Herbarium (Heilongjiang; NEFI).

Locality	Latitude (°N)	Longitude (°E)	Altitude (m)	n	Population code; herbarium voucher accession code
Xiaoxing'anling	46.8927	128.4209	275.0	19	XXAL; jmXXAL2010-Yu
Zhangguangcailing	44.0133	127.0052	357.5	20	ZGCL; jmZGCL2010-Yu
Changbai Mountain	41.1009	126.1315	332.0	20	CBS; jmCBS2010-Yu
Laoyeling	43.0405	130.3383	364.0	19	LYL; jmLYL2010-Yu
Wanda Mountain	44.5802	131.1351	115.0	20	WDS; jmLYL2010-Yu

Note: n = sample size.