Characterization of novel polymorphic genomic microsatellite markers of *Boehmeria tricuspis* (Hance) Makino

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Genet. Mol. Res. 15 (2): gmr.15027882

Received October 22, 2015

Accepted January 18, 2016

Published April 26, 2016

DOI <http://dx.doi.org/10.4238/gmr.15027882>

ABSTRACT. In the present study, 59 polymorphic microsatellite loci of *Boehmeria tricuspis* (Hance) Makino were developed from the specific length amplified fragment sequencing data library of genome. The number of alleles per locus ranged from two to five, and the observed and expected heterozygosities ranged from 0.0000 to 1.0000 and from 0.0769 to 0.6751, respectively. Among the 59 loci, 25 displayed significant deviations from Hardy-Weinberg expectations ($P < 0.05$). The developed simple sequence repeat markers should be useful for studying population genetics in *B. tricuspis* (Hance) Makino, for providing further knowledge on its population differentiation, breeding system, and dispersal ability, as well as quantitative trait locus mapping. These markers could also be valuable genetic resources for closely related species.

Key words: *Boehmeria tricuspis* (Hance) Makino; SSR; SLAF

INTRODUCTION

Boehmeria tricuspis (Hance) Makino, which is naturally distributed in China, Japan, and Korea, has traditionally been used for fabric production. Presently, it is also used as a material for apomictic research in the field of basic science (Chen et al., 2011b). However, in the past decade, natural populations of *B. tricuspis* (Hance) Makino have decreased, with some populations having disappeared, due to human activities as well as climatic and environmental changes. Therefore, it is essential to protect natural resources of *B. tricuspis* (Hance) Makino. For its long-term sustenance, it is necessary to establish adequate management plans for the conservation of this species with a good understanding of its genetic diversity, population structure, and genetic differentiation. Simple sequence repeats (SSRs) are significantly effective in species conservation and management because of their high polymorphism, abundance, codominance, and small length (Chen et al., 2011a). To date, no SSR markers have been reported in *B. tricuspis* (Hance) Makino. Therefore, development of SSR markers is imperative. In the present paper, we report 59 novel genomic SSR loci, developed through the specific length amplified fragment sequencing (SLAF) data of the genome.

MATERIAL AND METHODS

From the SLAF data library of *B. tricuspis* (Hance) Makino genome, 80 sequences, containing mono-, di-, tri-, tetra-, penta-, or hexanucleotide units repeated at least 15, 8, 5, 4, 3, or 3 times, respectively, were selected to evaluate the polymorphism. The SSR markers were designed with primer 5 software (Lalitha, 2000).

The major parameters used for primer design were according to Chen *et al.* (2011a). The primers were synthesized by BioAsia Biotech (Shanghai, China). Young leaves of 30 individuals were used for DNA extraction following the protocol of Tiangen DNA extraction kit (Beijing, China). Polymerase chain reactions were carried out in 10- μ L reaction volumes with 1X PCR buffer, 0.2 mM dNTP, 1U *Taq* DNA polymerase (Tiangen), 0.5 μ M each primer, and 0.5 μ L DNA under the following PCR conditions: 5 min at 94°C, followed by 30 cycles of 30 s at 95°C, 30 s at the primer-specific annealing temperature, 30 s at 72°C, and a final extension of 10 min at 72°C. The PCR products were separated on 8% polyacrylamide gels by electrophoresis, and stained using a silver dye according to Zhang et al. (2000). Observed heterozygosity (H_O), expected heterozygosity (H_E), and significant deviations from Hardy-Weinberg equilibrium (HWE) were estimated using Popgen1.32.

RESULTS AND DISCUSSION

Details of the developed microsatellite loci and their variability characteristics across 30 individuals of a natural population are summarized in Table 1. In total, 59 of 156 loci were successfully amplified and demonstrated to be polymorphic. The number of alleles per locus ranged from two to five and the observed and expected heterozygosities from 0.0000 to 1.0000 and from 0.0769 to 0.6751, respectively. Of the 59 loci, 25 displayed significant deviations from Hardy-Weinberg expectations ($P < 0.05$).

Table 1. Characterization of 59 microsatellite loci of *Boehmeria tricuspis* (Hance) Makino, described by locus name, repeat motif, forward (F) and reverse (R) primer sequences, size of allele (Size), optimal annealing temperatures (Ta), number of alleles (N_A), observed heterozygosity (H_O), expected heterozygosity (H_E), and significance of departure from Hardy-Weinberg equilibrium (HWE).

Locus	Repeat	Primer sequences (5'-3')	Size (bp)	Ta (°C)	N_A	H_O	H_E	HWE P value
N1	(T) ₁₉	F: TGATTCCGTGTGTGTGTTTC R: CCCGAAATTATGCCAACTGT	130	59	2	0.1875	0.1754	0.74
N2	(T) ₁₈	F: TGCATGAGCTCTGATCTTCCT R: TTGGTCTGTTAAGACTCAGAACTGT	117	60	2	0.3215	0.3992	0.30
N3	(A) ₁₅	F: TGAGCAAGGTTTTTGACGTTTC R: TGCAACTCCGAGATGAAAA	139	59	2	0.0000	0.5116	0.00
N4	(AG) ₁₀	F: AGACCTCCGTTTCAGAGGT R: CACCGCAGCAAACTCTACAC	106	60	3	0.1796	0.2282	0.43
N5	(AG) ₉	F: TCTCACCAGCCAAACAAAGA R: GATTCTCTGCTCGCTCATC	148	59	2	0.0000	0.4249	0.00
N6	(TC) ₁₀	F: TTCTCACCCTTAATCTCGGC R: AATCTCTGGACTAATATCTGTGATTC	113	57	3	0.3333	0.4723	0.01
N7	(GA) ₈	F: TCACACAGTAAGACAAAGAGAAAGG R: CGTAGCCAAATGTCTTCAA	137	59	2	0.0000	0.5085	0.00
N8	(AG) ₉	F: ATGGCTTTGATGGGATTGAA R: CTGCTCTCACACGCACACC	113	60	2	0.0769	0.0769	1.00
N9	(TAA) ₅	F: TGGGAAAATGAATTTCAAAGAA R: TTAACATTTGCTCGGTGTAATCA	149	57	2	0.0000	0.4994	0.00
N10	(TGT) ₁₂	F: CACCTCTCTGCGGTTGTTT R: TGTCAGGAGGACAAAGGCTC	118	60	3	0.3077	0.4819	0.06
N11	(ATT) ₅	F: AACAAAGGTTGCCTTCCCAT R: TAAGACAAGCGTGTGGCAAG	117	60	2	0.0000	0.1079	0.00
N12	(AGA) ₅	F: CTCCTCAATCATGCCAAATC R: TATCGCTTCTTGGGCTTCT	110	59	2	0.3600	0.4700	0.30
N13	(TATT) ₅	F: CGAATCCATTGACATCTCTG R: CGAATGTGCAATCTTAAAGCA	148	58	2	0.3889	0.4746	0.43
N14	(AAAC) ₆	F: AAGCTGCTCTTCAGTCGCAT R: TTCTCTATTCTTCTCTCTCGC	145	59	3	0.7070	0.6435	0.16
N15	(TAAT) ₅	F: AAATCAAATCATCTTGCAAAAGC R: AAACAAAACGGATTGCTTTCT	140	57	2	0.1700	0.1760	0.60
N16	(ATGT) ₉	F: AATAACACAATCAGTTTATGACAATCA R: TTGATTGTGTTAAATAGTGTGAAGAGA	113	57	3	0.6522	0.6570	0.23
N17	(AAAG) ₆	F: GCAAAATAGGGGCTGTCAAAC R: TGAAGTTTCCAAAGCAACGA	106	59	2	0.6000	0.4271	0.06
N18	(AAAT) ₆	F: CAGCAATTCAGTTACCCGA R: CCAATTAGCTATTAGCGGTTCTTTT	170	59	2	0.9615	0.5090	0.00
N19	(AGAA) ₅	F: CTGAGAGCTGTGACACTGATG R: TGATTTTATGTGTGCTGGTTG	116	57	2	0.3610	0.4720	0.30
N20	(TAAAA) ₅	F: TCAATATGATAAATTTGACTCTGATGC R: CCCAAAGTTTCGTCCTATGAT	120	58	2	0.0000	0.3638	0.00
N21	(AAACA) ₅	F: TCATTTTCTTTTCACGTTCCC R: TCAAGCTTCTTCTTACTATTGCTC	114	59	3	0.5526	0.4423	0.27
N22	(AAAAT) ₄	F: TCGACTTCGTACACGACAT R: GAGAAAGAAAGTGGCGATGA	115	60	2	0.1176	0.2995	0.02
N23	(AAAAC) ₆	F: TCTCCCAGATCTGGTTTAC R: TTCAACTTCTCATCGTTCTTGA	136	59	2	0.0417	0.3112	0.00
N24	(AAAATC) ₅	F: GGATTGGAAGGACTGGTGAG R: TTTTGCTATACCCCGCAAAG	158	59	2	0.1250	0.5250	0.02
N25	(CATCAC) ₅	F: CCATAAGAAGACATGTTTGCCA R: CGACGAAGACGATAACGACA	101	59	5	0.9333	0.6418	0.06
N26	(GATACC) ₅	F: TGTCTGACTCATCAGCAAGG R: AATAGTCGGACACCCGATCC	115	59	2	0.0833	0.2283	0.02
N27	(GAGAAG) ₃	F: CCAATCAGAAAACGAGAATTGA R: CAAAATTCGGGAGGCATTTA	170	59	2	0.0000	0.3339	0.00
N28	(AG) ₉ (GA) ₅	F: ATAGGGTTACCAATGCAGC R: TTTTTCCTGCTTTTACACC	144	59	2	0.2778	0.5000	0.06
N29	(A) ₁₆	F: GGGTCTCAATCGAATCCTAAAA R: TTTGCTGACCCAAATTTCA	108	59	3	0.6364	0.6480	0.31
N30	(T) ₁₇	F: CTTAGGTGAAGTTCGGCGAC R: TGAAGAATCTTGTGTGATTTTCTT	126	58	3	0.0000	0.6621	0.00
N31	(T) ₁₆	F: CAACAAAATTTAATCAACAAACCA R: AGCAAGTCTTCTTGCACGG	108	59	2	0.3846	0.3167	0.25
N32	(A) ₁₅	F: AACCAAAACCTCAATCCCA R: CTTGCAAAACAGTCCGAGA	125	59	3	0.7407	0.6744	0.15
N33	(A) ₁₇	F: GTCGCAAGAAGGGCAGATAG R: TCCAAAACAGAAGGCCAGG	142	59	3	0.0000	0.6079	0.00

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Table 1. Continued.

Locus	Repeat	Primer sequences (5'-3')	Size (bp)	Ta (°C)	N _A	H _O	H _E	HWE P value
N34	(T) ₁₇	F: GCTAACCTAGTCAGGGCAGG R: TAGCCGGAATCGAACTCTTG	113	58	2	0.2174	0.1981	0.60
N35	(A) ₁₈	F: TGGAGAAGAAATGATGCACAA R: GGATTGAATCTCATTATATTTGTTTT	133	57	3	0.5238	0.4797	0.64
N36	(GA) ₁₃	F: AAGGGGAAACTCGGCAG R: CAAATGCCCAATAACCCAAG	134	60	3	0.0000	0.5243	0.00
N37	(TC) ₈	F: TCAGATCCAACGGCTGTAAA R: GTTCTCCGATTTTGGGGATT	128	59	3	0.5833	0.5771	0.86
N38	(GA) ₁₁	F: CTTACCGGCTACGAGCTACC R: TTAATGGTCGCTGATACTGAGTT	109	57	3	0.4815	0.6730	0.12
N39	(GA) ₁₂	F: AGCCTAATATATAGGGCGAAAAGTG R: CCAAGGAATTGTGTCGTG	100	60	3	0.0667	0.4994	0.00
N40	(CT) ₁₁	F: CAACCAATTTTCATTACCAAGACA R: ATTTTGGAGCCGAATAGCA	149	59	3	1.0000	0.6215	0.00
N41	(AAT) ₅	F: TTTTGTGAATAGCAAGCAAGC R: ATAAGGCCCTGGTCTACGGA	153	58	2	0.0000	0.5085	0.00
N42	(ATA) ₅	F: TGGCACCACATTCTCTCTTC R: AAGCCTGCTTCAACCGTAAA	110	59	3	0.1852	0.2956	0.06
N43	(ATA) ₇	F: CCATCTGAACTCCTTCTCAGC R: TAATGGTTGGGGTCATTGGT	117	59	2	0.0000	0.4987	0.00
N44	(TTA) ₅	F: AACTCCAACGCTAGCGACAC R: TCAACGAATTAGAGAAAATGAATCC	111	59	3	0.0000	0.5672	0.00
N45	(TCT) ₅	F: GTCAATGGAAAGTTGGAAGGG R: ATTACTGCGACAGGAGGTCG	114	59	4	0.4091	0.4165	0.51
N46	(TCC) ₆	F: TCGCAGATCTCCTTCTATCG R: CTGTTCCCTTTCATCACGGT	131	59	2	0.4138	0.3339	0.08
N47	(TCTA) ₅	F: CCATTACCCCTCTCATGGT R: CCTTTCAGAAAGAGGAAGGTTG	146	58	4	0.5833	0.6480	0.20
N48	(TATG) ₄	F: ACCAATGATAACGCCGCTA R: GCTTATTCCGACTTTTAAACGGT	140	59	4	0.5333	0.6751	0.15
N49	(CTGAT) ₃	F: ACATGTTTCCACCCAAGG R: GAGAGGGGTGGTCTACGGT	104	59	3	0.1333	0.5898	0.00
N50	(AAAAG) ₄	F: AAAAACAACGGAGTTGACCG R: TCTTCCTCGCCATTATCGAC	122	60	4	0.5000	0.4653	0.87
N51	(ATAAA) ₃	F: TGGTTCAATGTAATTTGGGGGA R: ATACGGTTGTCAACACTGCG	158	59	3	0.4483	0.5124	0.27
N52	(GAAACA) ₃	F: CCACCACCATCGGAGTAAAG R: CGCCAACATATAGGCATTAGGA	142	59	3	0.3000	0.2695	0.84
N53	(ATCAAC) ₃	F: GACGAGGAGAGCCTTCCAT R: CCGTTCACCTAATGCACAAA	148	59	3	1.0000	0.6153	0.00
N54	(TTGCC) ₅	F: TCGCCAGAAGACAAAATTGA R: TCCGAGGATGAAAAAGGATG	154	59	3	0.0667	0.3712	0.00
N55	(CAGAAA) ₃	F: GAAACTCTCTGAGCCATGC R: TGCTCCCACTTCGTCTCTG	152	59	2	0.2105	0.1935	0.66
N56	(AAAAAT) ₃	F: CCTCCAGAATCCTGATAGTICA R: CATTGTGTTGAATGGTTTACTGT	162	59	3	0.3333	0.4983	0.24
N57	(CAAAAA) ₃	F: AGGAGCCCCCAAGTATCAAT R: TGAGGACGTCATGTTCTTAGC	141	59	4	0.5517	0.6679	0.17
N58	(TTTTTA) ₃	F: AAATTGCAATTCTGGTTGTTG R: TTTCACCTCAGCTCGTATCAACA	113	59	3	0.0000	0.4428	0.00
N59	(GTGTGG) ₄	F: TGTGGTGCTTGATACACGGT R: ATATGACGCCCCACAGATTG	156	60	2	0.2222	0.2032	0.65

Polymorphism analyses were performed on data from 30 individuals.

To the best of our knowledge, this is the first report about SSR markers of *B. tricuspis* (Hance) Makino, and it should be useful for genetic analyses and resource conservation in *B. tricuspis* (Hance) Makino.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research financially supported by the National Natural Science Foundation of China (Grant

#31471547 and #30900913), the Hunan Provincial Natural Science Foundation of China (Grant #13JJ4116), the National Modern Agro-industry Technology Research System (Grant #CARS-19-E04), and the National Key Technology Support Program (Grant #2013BAD01B03-13).

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