Isolation and Characterization of Microsatellite Markers for Jasminum sambac (Oleaceae) Using Illumina Shotgun Sequencing

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Primer Note

Isolation and characterization of microsatellite markers for *Jasminum sambac* (Oleaceae) using Illumina shotgun sequencing[1]

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- *Premise of the study:* Microsatellite markers of *Jasminum sambac* (Oleaceae) were isolated to investigate wild germplasm resources and provide markers for breeding.
- *Methods and Results:* Illumina sequencing was used to isolate microsatellite markers from the transcriptome of *J. sambac.* A total of 1322 microsatellites were identified from 49,772 assembled unigenes. One hundred primer pairs were randomly selected to verify primer amplification efficiency. Out of these tested primer pairs, 31 were successfully amplified: 18 primer pairs yielded a single allele, seven exhibited fixed heterozygosity with two alleles, and only six displayed polymorphisms.
- *Conclusions:* This study obtained the first set of microsatellite markers for *J. sambac*, which will be helpful for the assessment of wild germplasm resources and the development of molecular marker–assisted breeding.

Key words: Illumina sequencing; *Jasminum sambac*; microsatellite markers; Oleaceae.

Jasminum sambac (L.) Aiton (Oleaceae) is an evergreen vine or shrub that is native to Pakistan and India; this species is cultivated as an ornamental plant worldwide because of its attractive and sweet fragrance (Ruan, 2014). Previous studies on this plant have mainly focused on its aromatic compounds (Edris et al., 2008), medicinal values (Sengar et al., 2015), cultivation physiology (He et al., 2010), and aromatic gene isolation (Ou, 2012; Sun et al., 2014). Only one study has reported the genetic diversity of *J. sambac* using inter-simple sequence repeat (ISSR) markers (Qiu et al., 2008). However, ISSR loci are dominant markers that are difficult to use in the calculation of heterozygosity and paternity analysis. As an important ornamental plant, it is necessary to develop a set of powerful markers for the assessment of wild germplasm resources and the development of molecular marker–assisted breeding.

Microsatellites or simple sequence repeats (SSRs) are powerful markers used in population genetics and molecular marker–assisted breeding because of their high level of polymorphism, ease of genotyping, and codominant inheritance (Li et al., 2002; Oliveira et al., 2006). Emerging high-throughput sequencing platforms make it possible to discover a large number of microsatellite markers in a short time (Suresh et al., 2013). In the present work, transcript-based microsatellite markers were developed for *J. sambac* by using Illumina sequencing.

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METHODS AND RESULTS

Because of the temporal and spatial specificity of gene expression, RNA was isolated from a flower from a single individual of *J. sambac* to find molecular markers associated with the most important ornamental organs. The extraction was performed using a Quick RNA isolation kit (BioTeke Corporation, Beijing, China) following the manufacturer's protocol. RNA concentration was measured using a NanoDrop ND1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA). The construction of cDNA libraries and RNA-Seq were performed by the Biomarker Biotechnology Corporation (Beijing, China). Sequencing was conducted using an Illumina HiSeq 2500 system (Illumina, San Diego, California, USA). The obtained raw reads were cleaned by removing adapter sequences and then assembled de novo using Trinity (Grabherr et al., 2011). Microsatellite searching was performed using MISA (Thiel et al., 2003), and searching parameters were set as di-, tri-, tetra-, penta-, and hexanucleotide motifs with a minimum of five repeats. Primer pairs were designed with Primer3 (Rozen and Skaletsky, 1999). The product size range was set at 100–400 bp, and the other primer design parameters were set at default values.

Fresh leaves of *J. sambac* were collected from 24 individuals from two cultivated populations in South China Botanical Garden (SCBG: 23°11′24″N, 113°21′40″E) and Kunming Botanical Garden (KMBG: 25°07′05″N, 102°44′15″E). The leaves were preserved in silica gel and used as the source of DNA. Vouchers were deposited in the herbarium of Henan Agricultural University (HEAC; SCBG population: voucher no. HHAU-3201-3213, KMBG population: voucher no. HHAU-3214-3224). The total genomic DNA (gDNA) of 24 individuals was extracted using a DNA extraction kit (Plant #DP305; Tiangen Biotech, Beijing, China) following the manufacturer's protocol. PCR was carried out using a 30-µL reaction mixture consisting of 30 ng of gDNA, 3 µL of 10× buffer, 6 mM of each dNTP, 9 µM of each primer, and 1 unit of *Taq* DNA polymerase (Tiangen Biotech). The PCR reaction consisted of an initial denaturation step at 95°C for 5 min; followed by 35 cycles at 94°C for 40 s, annealing at a specific temperature (see Table 1) for 45 s, and 72°C for 50 s; followed by a final extension at 72°C for 8 min. The amplified fragments were electrophoresed on an 8% native polyacrylamide gel and visualized through silver staining. PCR products were sized relative to a 50-bp DNA ladder (TaKaRa Biotechnology Co., Dalian, Liaoning, China). Number of alleles (A) and inbreeding coefficient (F_{IS}) were calculated using FSTAT 2.9.3.2 (Goudet, 1995). Observed heterozygosity (H_o), expected heterozygosity (H_e),

TABLE 1. Primer sequences and characterization of 31 microsatellite loci isolated from *Jasminum sambac*.

Locus	Primer sequences (5′–3′)	Repeat motif	T_a (°C)	Allele size (bp)	*A*	GenBank accession no.
Js004	F: CCAAATTGTCATTGGGCTCT	$(CTT)_5$	48	214	1	KR339145
	R: GCTAGCTTTGATGGGTTGGA					
Js010	F: TGCGAAGACTCTCAGCAGAA	$(AG)_6$	48	189	1	KR339151
	R: AACAGCTTCACGCTCTCCTC					
Js011	F: AACATCCAAACAGGCCAAAA	$(CA)_9$	48	206	1	KR339152
	R: CAGAAGGAATCCACCCTTCA					
Js012	F: GACGGTCGGTCCTCACTTTA	$(GAC)_5$	48	228	1	KR339153
	R: ACTTGAATGGATCAAACGGC					
Js016	F: CCATTGGCTGGAGAGATGAT	$(TAC)_6$	48	251	1	KR339157
	R: CCCACTGCCAAGTCCTTTTA					
Js020	F: ACATGAATCGAGGAAAACCG	$(TTC)_5$	48	269	1	KR339161
	R: GAATGGCGAAGGAAAATGAA					
Js021	F: GAAGAGGGAACTACCCGGTC	$(GT)_6$	48	275–285	2	KR339162
	R: ATGAGAGCAAAGAGGGGACA					
Js029	F: TTCTCTCACATCGGTGGTTG	$(ATGT)_5$	48	278	1	KR339170
	R: TGACAAGAACCAAACCAATCC					
Js030	F: AACTCCGTGTACTCCGCAAG	$(GA)_7$	48	245–251	2	KR339171
	R: GCGAAAATCAAACTGCCATC					
Js033	F: GAAATCTGATGCTGCAACCA	$(AT)_7$	48	256–262	2	KR339174
	R: AAAGAGTTCATCCATTCGGG					
Js035	F: GACTTTGCGAGGGAAAACAG	$(TGG)_6$	48	252–254	2	KR339176
	R: CCAACCCTTCGACTCCTACA					
Js040	F: GTAGATTCGGCGTTACTCGG	$(AAG)_6$	48	204–213	2	KR339181
	R: CTTTTCTTCATAGCCCGACG					
Js041	F: GGAATTGTGGATGGCTCACT	$(CAAA)_5$	48	136–142	3	KR339182
	R: TGAGAGTTGGATGGGCTTTT					
Js042	F: AGAAATTTTTCCGGCTACGG	$(CCG)_5$	48	212–218	3	KR339183
	R: CCCATGACTAACCCGGTAGA					
Js050	F: TCCAAGAAAATGAACGGGAT	$(TAC)_5$	48	268	1	KR339191
	R: TGAATTGGCCTATCCTTTGG					
Js055	F: TGTTGCTCCTTCACATCAGC	$(CTC)_6$	48	171–180	2	KR339196
	R: GCCCCATCGTAGGGTAAAAT					
Js057	F: CTGATCTCTGCCACGTTCAA	$(CCG)_5$	48	278	1	KR339198
	R: AAAATAACAAAAATCCTCCGCA					
Js061	F: TCTTGGGTTGGCTTCAGAAC	$(AGC)_5$	48	242	1	KR339202
	R: CAGCGAAGTGAGTCTGGTCA					
Js062	F: TGAAACTACCGGGTCTTTGG	$(AGA)_5$	48	230	1	KR339203
	R: ATTAATCGGTCCTGAAGGGG					
Js063	F: CCCATCTCACCCTAACCTCA	$(TA)_6$	50	188–194	2	KR339204
	R: GAAAAATTCTTGGATCTTCTTGC					
Js064	F: TCAACGCCTTAAATTGCTTG	$(CT)_6$	48	275–281	2	KR339205
	R: CCACAAACCTTCGAGGAGAC					
Js068	F: ATAAAGCACAACATCCCGCT	$(AG)_6$	48	123	1	KR339209
	R: TTTCTCACTCCGGCACTTCT					
Js073	F: GCCTCGAAATGTTGGAATGT	$(GAA)_6$	48	213	1	KR339214
	R: CCAAACTACGAAGGGGAAAA					
Js075	F: ACGCAATCTACCCCATTGAG	$(GAT)_5$	48	266	1	KR339216
	R: TCCTTCAGCAACATTGCATC					
Js076	F: TGTAACGCGAAACGGAATTT	$(TGG)_6$	48	163–172	2	KR339217
	R: ACCAACCACGGTGTTTCTTC					
Js077	F: TTTGCCATCAATGTCTCCCT	$(GT)_9$	48	143	1	KR339218
	R: CCTGTTCCTGTTTCCATCAGA					
Js079	F: CAAGAAAATTGACCCCATCG	$(TA)_7$	48	157–163	2	KR339220
	R: GACTTGGTCGCCATTGTTTT					
Js084	F: TCATAACCCCTCGCTTTTTG	$(CCA)_5$	48	212	1	KR339225
	R: AAGCTTGGGGGAGGAAGTTA					
Js085	F: CCATGGACAAACATTGTGGA	$(AAC)_5(AGC)_5$	48	204–207	2	KR339226
	R: TTTCAAAGAGCGGAACCAGT					
Js086	F: TCCACCTCTCGGGTTATTTG	$(GAA)_6$	48	150	1	KR339227
	R: AAAGTCTGCAAAAGGGAGCA					
Js100	F: AAGCATCGAGAATCGAGCATA	$(AG)_6$	48	230	1	KR339241
	R: AACTCATCCTCCCCCACG					

Note: *A* = number of alleles; T_a = PCR annealing temperature.

linkage disequilibrium (LD), and Hardy–Weinberg equilibrium (HWE) were calculated using GENEPOP 4.2 (Rousset, 2008).

A total of 42.35 million reads were obtained from the RNA-Seq data. The assembly of reads resulted in 49,772 unigenes, with a mean size of 846 bp. Out of these unigenes, 1322 microsatellites contained sufficient flanking sequences for primer design and were deposited in GenBank (KR339142–KR340463). A total of 100 primer pairs were randomly selected for further PCR characterization. Among these, 69 primer sets were discarded due to nonspecific amplification.

TABLE 2. Genetic diversity parameters for six polymorphic microsatellite loci from two cultivated populations of *Jasminum sambac*.[a]

	SCBG				KMBG			
Locus	*A*	H_e	H_o	F_{IS}	*A*	H_e	H_o	F_{IS}
Js033	2	0.473	0.769	−0.600	2	0.500	1.000*	−1.000
Js035	1	0.000	0.000	—	2	0.397	0.545	−0.333
Js041	3	0.462	0.615	−0.297	1	0.000	0.000	—
Js042	3	0.349	0.308	−0.103	2	0.091	0.091	0.000
Js055	2	0.426	0.615	−0.412	2	0.463	0.727	−0.539
Js063	2	0.497	0.923*	−0.846	2	0.500	1.000*	−1.000

Note: *A* = number of alleles; F_{IS} = inbreeding coefficient; H_e = expected heterozygosity; H_o = observed heterozygosity.

[a]Locality and voucher information: SCBG (South China Botanical Garden: 23°11′24″N, 113°21′40″E), voucher no. HHAU-3201-3213; KMBG (Kunming Botanical Garden: 25°07′05″N, 102°44′15″E), voucher no. HHAU-3214-3224.

* Significant deviation from Hardy–Weinberg equilibrium.

The remaining 31 primer pairs were used for polymorphism verification. Eighteen primer pairs yielded a single allele, seven exhibited fixed heterozygosity with two alleles, and only six displayed polymorphisms (Table 1). For these polymorphic primer pairs, the *A*, H_o, H_e, and F_{IS} of each population ranged from one to three, 0.000 to 1.000, 0.000 to 0.500, and −1.000 to 0.000, respectively (Table 2). The six primer pairs exhibited low polymorphism. The most likely reason for this phenomenon was the narrow genetic basis of the cultivated populations. The 31 microsatellite sequences were searched in the nonredundant protein database using BLAST (Appendix S1). Nine loci matched significantly with coding regions in the known genes. Loci Js033 and Js063 significantly deviated from HWE ($P < 0.05$) due to excessive heterozygosity. No significant pairwise LD was observed among these loci. The microsatellite primers reported in this study will be helpful for the assessment of wild germplasm resources and the development of molecular marker–assisted breeding of *J. sambac*.

CONCLUSIONS

In this study, 1322 microsatellites were isolated from *J. sambac*. A total of 100 primer pairs were randomly selected to verify primer amplification efficiency. Out of these tested primer pairs, 18 primer pairs yielded a single allele, seven exhibited fixed heterozygosity with two alleles, and six displayed polymorphisms. This is the first set of microsatellite markers developed for *J. sambac*, which will be helpful for the assessment of wild germplasm resources and the development of molecular marker–assisted breeding.

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