BioOne COMPLETE

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

Authors: Li, Yong, and Zhang, Weirui

Source: Applications in Plant Sciences, 3(10)

Published By: Botanical Society of America

URL: https://doi.org/10.3732/apps.1500063

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://bioone.org/journals/Applications-in-Plant-Sciences on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Applications in Plant Sciences

Applications in Plant Sciences 2015 3(10): 1500063

Primer Note

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

Yong Li[2,4,5] and Weirui Zhang[3,4]

[2]College of Forestry, Henan Agricultural University, Zhengzhou 450002, People's Republic of China; and [3]Institute of Chinese Materia Medica, Henan University, Kaifeng 475004, People's Republic of China

- *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.

[1]Manuscript received 3 June 2015; revision accepted 6 July 2015.

Funding for this project was provided by the National Natural Science Foundation of China (31100272).

[4]These authors contributed equally to this work.

[5]Author for correspondence: liyongrui1@126.com

doi:10.3732/apps.1500063

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),

 © 2015 Li and Zhang. Published by the Botanical Society of America. This work is licensed under a Creative Commons Attribution License (CC-BY-NC-SA).

Downloaded From: https://bioone.org/journals/Applications-in-Plant-Sciences on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

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.

Downloaded From: https://bioone.org/journals/Applications-in-Plant-Sciences on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

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.

LITERATURE CITED

EDRIS, A. E., R. CHIZZOLA, AND C. FRANZ. 2008. Isolation and characterization of the volatile aroma compounds from the concrete headspace and the absolute of *Jasminum sambac* (L.) Ait. (Oleaceae) flowers grown in Egypt. *European Food Research and Technology* 226: 621–626.

GOUDET, J. 1995. FSTAT (version 1.2): A computer program to calculate *F*-statistics. *Journal of Heredity* 86: 485–486.

GRABHERR, M. G., B. J. HAAS, M. YASSOUR, J. Z. LEVIN, D. A. THOMPSON, I. AMIT, X. ADICONIS, ET AL. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology* 29: 644–652.

HE, L. S., B. XIA, X. J. MENG, C. Y. WANG, F. PENG, AND R. WANG. 2010. Physiological and biochemical responses of *Jasminum sambac* L. under natural temperature reduction. *Journal of Nanjing Agricultural University* 33: 28–32.

LI, Y. C., A. B. KOROL, T. FAHIMA, A. BEILES, AND E. NEVO. 2002. Microsatellites: Genomic distribution, putative functions and mutational mechanisms: A review. *Molecular Ecology* 11: 2453–2465.

OLIVEIRA, E. J., J. G. PÁDUA, M. I. ZUCCHI, R. VENCOVSKY, AND M. L. C. VIEIRA. 2006. Origin, evolution and genome distribution of microsatellites. *Genetics and Molecular Biology* 29: 294–307.

OU, X. F. 2012. Cloning and analysis on HPL and GDS of *Jasminum sambac*. Master's thesis, Fujian Agriculture and Forestry University, Fuzhou, China.

QIU, C. Y., G. Q. GAO, B. L. CHEN, R. Y. ZHOU, AND J. Q. ZHANG. 2008. ISSR analysis on genetic diversity of *Jasminum sambac. Hubei Agricultural Sciences* 47: 744–747.

ROUSSET, F. 2008. GENEPOP'007: A complete reimplementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* 8: 103–106.

ROZEN, S., AND H. J. SKALETSKY. 1999. Primer3 on the WWW for general users and for biologist programmers. *In* S. Misener and S. A. Krawetz [eds.], Methods in molecular biology, vol. 132: Bioinformatics methods and protocols, 365–386. Humana Press, Totowa, New Jersey, USA.

RUAN, Y. J. 2014. Research on the mutation effects of *Jasminum sambac* (Linn.) Aiton in vitro after colchicine treatment. Master's thesis, Northwest University, Xi'an, China.

SENGAR, N., A. JOSHI, S. K. PRASAD, AND S. HEMALATHA. 2015. Anti-inflammatory, analgesic and anti-pyretic activities of standardized root extract of *Jasminum sambac. Journal of Ethnopharmacology* 160: 140–148.

SUN, J., G. X. CHEN, N. X. YE, S. H. LÜ, Z. Q. LIU, W. HUANG, AND Z. D. LIN. 2014. Cloning and expression analysis of deoxyoxylulose-5-phosphate synthase gene related to aroma from *Jasminum sambac* and isolation of its promoter. *Acta Horticulturae Sinica* 41: 1236–1244.

SURESH, S., J. H. PARK, G. T. CHO, H. S. LEE, H. J. BAEK, S. Y. LEE, AND J. W. CHUNG. 2013. Development and molecular characterization of 55 novel polymorphic cDNA-SSR markers in faba bean (*Vicia faba* L.) using 454 pyrosequencing. *Molecules (Basel, Switzerland)* 18: 1844–1856.

THIEL, T., W. MICHALEK, R. K. VARSHNEY, AND A. GRANER. 2003. Exploiting EST database for the development and characterization of gene-derived SSR markers in barley (*Hordeum vulgare* L.). *Theoretical and Applied Genetics* 106: 411–422.

Downloaded From: https://bioone.org/journals/Applications-in-Plant-Sciences on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use