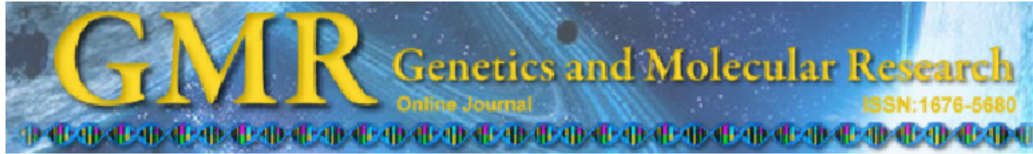

Research Article

Isolation and characterization of polymorphic microsatellite loci from aerial yam (*Dioscorea bulbifera* L.)

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ABSTRACT. *Dioscorea bulbifera* L. is widely distributed in pantropical regions along the equator. The taxonomic treatment of this species is ambiguous due to its extreme polymorphic morphological characters. In order to provide tools to facilitate the study of genetic diversity, population structure, patterns of gene flow, and the mating system of this species, and to assess intraspecific variability and relationships in *D. bulbifera*, 14 novel polymorphic microsatellite loci were developed using the dual-suppression PCR technique. The number of alleles per locus ranged from 4 to 17, with an average of 9.93. The mean observed heterozygosities were 0.7327 and 0.7223, and the mean Shannon-Wiener indices were 1.6431 and 1.811 in the Nanjing and Nanchong populations, respectively. All novel microsatellite loci showed high levels of polymorphism, indicating that these markers offer great potential significance and profound influence for future studies of this species.

Key words: *Dioscorea bulbifera* L.; Genetic diversity; SSRs; Microsatellite loci; Taxonomic delimitation

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INTRODUCTION

The aerial yam, *Dioscorea bulbifera* L. (family Dioscoreaceae) is widely distributed in pantropical regions along the equator (Ting et al., 1985; Ting and Michael, 2000). In China, it is considered to have medicinal properties, is widely used in traditional Chinese medicine (Ting et al., 1978), and is cultured as foodstuffs in some areas because of its edible bulbils (Lebot, 2009), whereas in North America, it is treated as an invasive weed (Croxton et al., 2011).

D. bulbifera is polyploid, with n = 9 seen in Africa and in the Americas and n = 10 in Asia (Martin and Ortiz, 1963). The taxonomic treatment of this species is ambiguous due to its extreme polymorphic morphological characteristics (Ramser et al., 1996). Hence, in this study, we developed and characterized a set of microsatellite loci (simple sequence repeats, SSRs) for *D. bulbifera*, to serve as tools for studying the genetic diversity, population structure, patterns of gene flow, and mating system of this species and to assess intraspecific variability and relationships in *D. bulbifera*.

MATERIAL AND METHODS

D. bulbifera plants were collected from 2 tetraploid populations; the Nanjing population in Jiangsu province and the Nanchong population in Sichuan province. Voucher specimens for the sampled populations have been deposited in the Herbarium of the Institute of Botany, Jiangsu Province, and the Chinese Academy of Sciences (NAS). Genomic DNA isolation was carried out using a modification of the hexadecyltrimethylammonium bromide (CTAB) method (Doyle, 1991). One individual from the Nanjing population was selected for digestion with the *Eco*RV restriction enzyme (Takara, Dalian, China) in order to construct a DNA library for *D. bulbifera*. After digestion, the fragments were ligated with a specific blunt adaptor (consisting of the upper strand 5'-GTA ATA CGA CTC ACT ATA GGG CAC GCG TGG TCG ACG GCC CGG GCT GGT-3' and the lower strand, with the 3'-end capped with an amino residue: 5'-ACC AGC CC-3') by T4 DNA ligase (Takara). Subsequently, the fragments were PCR-amplified from the *Eco*RV DNA library using the compound SSR primer $(AC)_6(AG)_5$ or $(TC)_6(AC)_5$ and an AP_2 adaptor (5'-CTA TAG GGC ACG CGT GGT-3'). Each 50-µL PCR reaction contained 25-50 ng genomic DNA, 1X PCR buffer with $MgCl_2$, 0.2 mM each dNTP, 0.5 U Ex Taq polymerase (Takara), and 0.5 mM each compound SSR primer and AP_2. The PCR amplification conditions were as follows: 1 cycle of 9 min at 94°C, 30 s at 62°C, and 1 min at 72°C; 5 cycles each of 30 s at 94°C, 30 s at 62°C, and 1 min at 72°C; 35 cycles each of 30 s at 94°C, 30 s at 60°C, and 1 min at 72°C; ending with a final cycle of 30 s at 94°C, 30 s at 60°C, and 9 min at 72°C. The products were purified using a DNA clean-up kit (Axygen, Union, CA, USA) and transformed into competent DH5α cells (Takara) after ligation with the pMD 19-T vector (Takara). A single clone was checked using the M13-47 (5'-CGC CAG GGT TTT CCC AGT CAC GAC-3')/RV-M (5'-GAG CGG ATA ACA ATT TCA CAC AGG-3') universal primers. Positive clones were obtained and sequenced on an ABI Prism 3730 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). Specific primers were designed based on sequences flanking the compound SSR primers using Primer Premier 5.0 (Clarke and Gorley, 2001).

The designed primers were used to amplify microsatellite repeats from 47 *D. bulbifera* individuals from the 2 sampled populations. In order to evaluate their levels of polymorphism, the compound SSR primers were labeled with fluorescent dyes (6-FAM or HEX). The amplified products were analyzed by fluorescence capillary electrophoresis on an ABI Prism 3730 automated DNA sequencer (Applied Biosystems). The data were compiled and scored using GeneMaker 1.95

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(Soft-Genetics, State College, PA, USA). The number of alleles (N_A), observed heterozygosities (H_O), and Shannon-Wiener index (*H*') were determined using ATETRA (Puyvelde et al., 2010).

RESULTS

In total, 14 microsatellite loci were identified (Table 1). The number of alleles per locus ranged from 4 to 17, with an average of 9.93. The H_O and *H*' values ranged from 0.3046 to 0.8353 and 0.5667 to 2.1287, respectively, in the Nanjing population, and from 0.3914 to 0.9041 and 0.7802 to 2.3365, respectively, in the Nanchong population (Table 2).

Table 1. Characterization of fourteen microsatellite loci in *Dioscorea bulbifera*.

Locus	Primer sequence (5'-3')	Repeat motif	Ta (°C)	Size (bp)	N_A	Accession No.
DBSSR1	F: ACACACACACACAGAGAGAGAG R: AGAAGTTTGTTGCCCGTC	$(AC)_6(AG)_{20}…(GGA)_3$	54	215	9	JX908765
DBSSR2	F: ACACACACACACAGAGAGAGAG R: AACGCATCCCACCACTTC	$(AC)_6(AG)_{13}$	54	199	13	JX908766
DBSSR3	F: ACACACACACACAGAGAGAGAG R: CACGATGGAGGAACACTT	$(AC)_6(AG)_{19}$	54	265	12	JX908767
DBSSR4	F: ACACACACACACAGAGAGAGAG R: GAAAAGGAGAAGCCGAAT	$(AC)_6(AG)_9$	54	206	4	JX908768
DBSSR5	F: ACACACACACACAGAGAGAGAG R: TTGATTGAAAAGGAGGCT	$(AC)_6(AG)_9$	54	235	12	JX908769
DBSSR6	F: ACACACACACACAGAGAGAGAG R: GAACAATGCAAATCAGTAAA	$(AC)_6(AG)_{11}$	54	136	12	JX908770
DBSSR7	F: ACACACACACACAGAGAGAGAG R: GCAAATCGTGCGAAATAC	$(AC)_6(AG)_7(AC)_8…(AG)_7$	52	369	14	JX908771
DBSSR8	F: TCTCTCTCTCTCACACACACAC R: TGACGGAGAACTACAAGC	$(TC)_6(AC)_{13}$	48	279	17	JX908772
DBSSR9	F: ACACACACACACAGAGAGAGAG R: TCCTTGGTCCTTGAACTT	$(AC)_6(AG)_{15}$	48	85	8	JX908773
DBSSR10	F: ACACACACACACAGAGAGAGAG R: AGAAGTTTGTTGCCCGTC	$(AC)_6(AG)_{22}$	54	219	8	JX908774
DBSSR11	F: ACACACACACACAGAGAGAGAG R: TGATTCAGATAAGCCAAAC	$(AC)_6(AG)_{19}$	48	121	9	JX908775
DBSSR12	F: ACACACACACACAGAGAGAGAG R: TTGCTTTACCGACATCCA	$(AC)_6(AG)_{10}$	53	241	6	JX908776
DBSSR13	F: TCTCTCTCTCTCACACACACAC R: AACAGCCCACCGACTAAA	$(TC)_6(AC)_6$	54	189	11	JX908777
DBSSR14	F: TCTCTCTCTCTCACACACACAC R: TCTAAGGAGCAGCCGAAT	$(TC)_6(AC)_6$	50	214	4	JX908778

Ta = annealing temperature.

Table 2. Results of initial primer screening in two populations of *Dioscorea bulbifera*.

Locus	NanJing (N = 26) 32.03°N, 118.46°E			NanChong (N = 21) 30.49°N, 106.04°E		
	N_A	H_O	*H*'	N_A	H_O	*H*'
DBSSR1	7	0.8191	1.8736	7	0.7683	1.6215
DBSSR2	11	0.8243	1.9419	9	0.8256	1.9148
DBSSR3	6	0.7811	1.6523	11	0.8765	2.2269
DBSSR4	4	0.4953	0.9397	3	0.5540	0.8898
DBSSR5	8	0.7722	1.7585	12	0.8912	2.3365
DBSSR6	9	0.7370	1.6618	12	0.8900	2.3148
DBSSR7	13	0.8350	2.1287	14	0.9041	2.4631
DBSSR8	11	0.7663	1.8548	13	0.8786	2.3510
DBSSR9	7	0.7035	1.3949	8	0.6292	1.3389
DBSSR10	8	0.7931	1.7747	7	0.7523	1.6010
DBSSR11	8	0.8353	1.8954	8	0.8330	1.8813
DBSSR12	6	0.7728	1.6152	6	0.7748	1.6124
DBSSR13	10	0.8236	1.9447	10	0.8408	2.0278
DBSSR14	3	0.3046	0.5667	4	0.3914	0.7802
Mean	7.30	0.7327	1.6431	8.86	0.7223	1.8114

N = sample size for each population.

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DISCUSSION

All novel microsatellite loci isolated from *D. bulbifera* in the present study showed high levels of polymorphism, indicating that these markers will be of great potential significance and profound influence in future research related to the genetic diversity, population structure, patterns of gene flow, and mating system of this species. Furthermore, the microsatellites developed herein will contribute toward better taxonomic delimitation for the aerial yam.

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REFERENCES

Clarke KR and Gorley RN (2001). Primer v5: User Manual/Tutorial. Primer-E Ltd., Plymouth.

Croxton MD, Andreu MA, Williams DA, Overholt WA, et al. (2011). Geographic origins and genetic diversity of air-potato (*Dioscorea bulbifera*) in Florida. *Invasive Plant Sci. Manag.* 1: 22-30.

Doyle JJ (1991). DNA Protocols for Plants. In: Molecular Techniques in Taxonomy (Hewitt GM and Johnston A, eds.). Springer-Verlag, Berlin.

Lebot V (2009). Tropical Root and Tuber Crops Cassava, Sweet Potato, Yams and Aroids. Crop Production Science in Horticulture Series.

Martin FW and Ortiz S (1963). Chromosome number behaviour in some species of *Dioscorea*. *Cytologia* 28: 96-101.

Puyvelde VK, Geert VA and Triest L (2010). ATETRA, a new software program to analyse tetraploid microsatellite data: comparison with TETRA and TETRASAT. *Mol. Ecol. Resour.* 10: 331-334.

Ramser J, Weising K, Kahl G, Lopez-Peralta C, et al. (1996). Genomic variation and relationships in aerial yam (*Dioscorea bulbifera* L.) detected by random amplified polymorphic DNA. *Genome* 39: 17-25.

Ting CT and Michael GG (2000). Dioscorea Linnaeus. In: Flora of China (Wu ZY and Raven PH, eds.). St. Louis, Missouri Botanical Garden Press, Science Press Beijing.

Ting CT, Chang MC and Ling PP (1985). Flora of China. Science Press, Beijing.

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