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Development of microsatellite markers for *Suriana maritima* (Surianaceae) using next-generation sequencing technology

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ABSTRACT. Our objective was to develop microsatellite markers for use in assessing genetic variation in the small shrub or tree species *Suriana maritima* (Surianaceae). In China, this species is found only as a few fragmented populations and individuals on the Paracel Islands. Using next-generation genome sequencing methodology, we developed 17 novel microsatellite markers for *S. maritima*. Fifty-four individuals from six populations of *S. maritima* were examined for polymorphisms; only one allele was detected for each of the markers. Microsatellite loci developed indicate a complete absence of genetic diversity for *S. maritima* on the Paracel Islands in China. These markers will be useful for examining genetic variation among *S. maritima* populations in other areas of the world.

Key words: *Suriana maritima*; Surianaceae; Microsatellite markers; Next-generation sequencing; Paracel islands

INTRODUCTION

Suriana maritima L., the sole species of *Suriana* (Surianaceae), grows as a perennial shrub or small tree on tropical Pacific coral islands, such as those of India, Indonesia, Philippines, and China. Usually, it grows in sandy areas or in crevices along the edges of such islands (Peng and Thomas, 2008). In China, *S. maritima* is restricted to the Paracel Islands, where only a few fragmented populations and individuals exist, because of the effects of climate change and human activity (Tong et al., 2013).

The taxonomic status of *S. maritima* among angiosperms has been controversial (Gutzwiller, 1961; Cronquist, 1981; Thome, 1983; Takhtajan, 1987; Fernando et al., 1992; Peng and Thomas, 2008; Bremer et al., 2009). At various points, *S. maritima* has been placed in the *Phytolaccaceae* (Gutzwiller, 1961), the *Simaroubaceae* (Cronquist 1981), and the Surianaceae (Thome, 1983; Fernando et al., 1992; Peng and Thomas, 2008). Takhtajan (1987) suggested placement of Surianaceae (including *S. maritima*) and *Simaroubaceae* in *Rutales*. Cronquist (1988) placed Surianaceae (including *S. maritima*) in *Rosales*. Bremer et al. (2009) suggested placement of Surianaceae (with five genera and eight species, including *S. maritima*), *Polygalaceae*, *Quillajaceae,* and *Leguminosae* in *Fabales*, and considered Surianaceae as sister to *Polygalaceae*. Therefore, *S. maritima* is an interesting species for the phylogenetic study of the Surianaceae and *Fabales* (including the Surianaceae, *Polygalaceae*, *Quillajaceae,* and *Leguminosae*) (Bremer et al., 2009).

Little is known about genetic variations among *S. maritima* populations and individuals in China, or in other areas of the world. In this study, we used next-generation sequencing to develop microsatellite markers, in order to assess genetic variation in *S. maritime*. Markers were used to analyze 54 individual *S. maritima* plants from six populations on the Paracel Islands.

MATERIAL AND METHODS

Leaf samples of *S. maritima* on the Paracel Islands, China, were collected from six populations: Chenhang (CH); Yongxing (YX); Guangjin (GJ); Dongdao (DD); Zhongjian (ZJ; all N = 10); and Jinyin (JY, N = 4; **Table S1**). Total genomic DNA was extracted from silica gel-dried leaf tissue, as previously described (Doyle and Doyle, 1987). One DNA sample from the ZJ population was subjected to restriction-site-associated DNA sequencing (RAD-seq), using a HiSeq2000 sequencer (Illumina, Inc., San Diego, California,USA). A total of 112,999 reads were obtained, and assembled using STACK version 1.24 (Catchen et al., 2011; 2013). A total of 473 microsatellites (di-, tri- and tetranucleotide; minimum of six repeats) were identified using MSATCOMMANDER software, version 0.8.2 (Faircloth, 2008); only 80 of these could be used for primer design, which was carried out using the program Primer3 (Rozen and Skaletsky, 2000) embedded in MSATCOMMANDER. Primers were tagged with the universal M13 sequence (GTAAAACGACGGCCAGT). Thirteen primers were discarded due to the complexity of their repeat motifs. Ultimately, 67 microsatellite loci were selected for subsequent testing with 12 individuals from the six populations (two for each population).

Amplifications by PCR were conducted in 10-μL total volume reactions that contained: 10 ng template DNA; 2 μL 10X buffer (Takara Biotechology (Dalian) Co., LTD., Dalian, China); 0.4 mM dNTPs; 10 mM $MgCl_2$; 0.5 U Taq DNA polymerase (Takara); 0.05 mM forward primer; 1.5 mM reverse primer; and 1.5 mM fluorescently labeled M13 primer. Amplifications were carried out in

a PTC-100 thermo cycler (MJ Research Inc., St. Bruno, Canada) under the following conditions: a melting step of 94ºC for 5 min; 35 cycles at 94ºC for 30 s, annealing temperatures (as per Table 1) for 60 s, and 72ºC for 30 s; and a final extension at 72ºC for 10 min. Amplified products were assessed on 2% agarose (w/v) gel. Amplification products were visualized using the genetic analyzer ABI3500 (Applied Biosystems, Carlsbad, California, USA), and the results were analyzed using GeneMapper 4.1 (Applied Biosystems).

Table 1. Characteristics of microsatellite markers for *Suriana maritima.*

Locus	Primer sequence (5´-3´)	Repeat motif	Size (bp)	T_a (ºC)	N	H_O	H_E	GenBank accession No.
S1	F: ACATTGGTGCATGCGTTGG							
	R: AGGCCTTGGTTTAGTACCC	$(AC)_9$	202	48	1	0.000	0.000	KP717426
S5	F: ACCAGCACAATGAAGTTGGC							
	R: AGAAGTTTGGTTATGCTGCG	$(AG)_{11}$	195	47	1	0.000	0.000	KP717427
S6	F: TTTAGTGCACAGCCACGTC							
	R: AACAAGGCCCTGAACGGAG	$(AG)_{10}$	318	56	1	0.000	0.000	KP717428
S7	F: GCCAGTTGTCGAATGCCTC							
	R: AGGAAGATCTCACTGAAACAAAGTC	$(CT)_6(CA)_{13}$	152	48	1	0.000	0.000	KP717429
S9	F: TAGCGATCGAGGCCTTTGG							
	R: AAATTCCAGGACAGGTGCG	$(AG)_{12}$	219	60	1	0.000	0.000	KP717430
S12	F: TGATGGTACAGCAAGTGGG							
	R: GCAACAAATAGCAAGCCCAAAG	$(GT)_{11}(GA)_7$	347	56	1	0.000	0.000	KP717431
S13	F: TCCAAACAATAAACAATGTCCATCC							
	R: ATGTGAGCACTTCCTGCCC	$(CT)_{10}$	228	56	1	0.000	0.000	KP717432
S21	F: TCCTTGGTTGGAGAATTGTTGC							
	R: ACAGAGTACACAAGGATCAAGTG	$(CT)_9$	207	47	1	0.000	0.000	KP717433
S22	F: AGTTGTTTAGTCATAGCAAACTGTC							
	R: CATTAATTGACCTTTGATGGGTTC	$(AG)_6$ TG $(AG)_9$	193	48	1	0.000	0.000	KP717434
S23	F: CCTTTGCAGGTACGTGAGC							
	R: TCACTAACACCCTATTTAAGCAC	$(ACT)_8$	178	60	1	0.000	0.000	KP717435
S27	F: AACAACATTGCAGCTTGGG							
	R: CATTGGCTGCGTAGTTGCC	$(AG)_{10}$	173	47	1	0.000	0.000	KP717436
S36	F: CAACTTCCTGAACACCGAGC							
	R: TGGTGGTAAAGGCCCTAGTC	$(GT)_{10}$	300	58	1	0.000	0.000	KP717437
S37	F: CCACCCTACGGTATTGAAGC							
	R: TCCGATCTTGAATATTGCTCGAC	$(CT)_{12}$	272	49	1	0.000	0.000	KP717438
S39	F: ACAGACCACATCGGAAGCC							
	R: AGGCGCCCATCCTTTGTC	$(AG)_9$	274	58	1	0.000	0.000	KP717439
S42	F: TCAAAGGCAATTGAGGCTGTC							
	R: GGTGACACTCCTCACCAAG	$(GT)_{10}$	363	58	1	0.000	0.000	KP717440
S43	F: ACCCGGGACATTTCTATTGG							
	R: TGTATGAATTGTTGAACCCTTGG	$(AG)_{13}$	208	47	1	0.000	0.000	KP717441
S51	F: CTACAAACCAATCCACCGC							
	R: TGTGTTCTTCGCAGACTTAGC	$(GT)_8(GA)_{10}$	196	58	1	0.000	0.000	KP717442

F = forward; R = reverse; T_a = PCR annealing temperature; N = number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity.

RESULTS AND DISCUSSION

Polymorphisms were assessed for the remaining 17 microsatellite loci using all 54 samples from the six populations. Among 67 microsatellite loci tested, 40 failed to amplify correctly in all samples, and 10 produced blurred bands. All loci were found to be monomorphic within and among populations, indicating a complete absence of genetic variation among *S. maritima* in China (Table 1). This lack of genetic variation may be the result of a recent founder effect or bottleneck, because of recent introduction and a single ancestor. Furthermore, repeated selfing and inbreeding would be also responsible further loss of genetic diversity of *S. maritima* in this study. These markers will be useful for assessing genetic variation among *S. maritima* populations in other parts of the world.

Conflicts of interest

The authors declare no conflict of interest.

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Supplementary material

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