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# Isolation and characterization of sixteen polymorphic microsatellite loci for *Scaevola taccada* (Goodeniaceae), a widespread coastal plant

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[Shi M.-M., Guo X.-M., Zhou L.-X. and Zhang D.-X. 2014 Isolation and characterization of sixteen polymorphic microsatellite loci for *Scaevola taccada* (Goodeniaceae), a widespread coastal plant. *J. Genet.* **93**, e66–e69. Online only: http://www.ias.ac.in/jgenet/OnlineResources/93/e66.pdf]

## Introduction

Since the era of Darwin, island systems have continually drawn attention from ecologists (Nakanishi 1988; Kueffer *et al.* 2010) and evolutionary biologists (Emerson 2002; Losos and Richlefs 2009). Insular species are characterized by geographic isolation, distinct population boundary and small population size, thus considered as good examples to study species differentiation and origins (Francisco-Ortega *et al.* 2001; Wei*et al.* 2008).

*Scaevola taccada* (Gaertn.) Roxb. (Goodeniaceae) is a large, bushy shrub or tree up to 7-m tall (Hong and Howarth 2011), distributed widely in the Pacific and Indian coastal strands, and has even radiated in parts of the South Pacific (Elmore 2008). It is salt-tolerant and occurs commonly along rocky and sandy or coralline shorelines, amongst the first pioneer plant colonizers (Liao 2008). In North America, *S. taccada* has become an invasive species, supplanting native coastal vegetation. For example, in some Florida areas it has begun to displace native beach plant *S. plumieri* (http://www.fleppc.org/). Overall, *S. taccada* is an important species with ecological significance. Research on this species has addressed its physiology (Alpha *et al.* 1996; Goldstein *et al.* 1996), phenology (Brooke *et al.* 1996), pollination biology and reproductive ecology (Elmore 2008; Liao 2008). However, nothing is known about its population genetic information such as genetic diversity, genetic structure and gene flow, although the information is critically important for conservation and management strategies (Wang *et al.* 2006). Microsatellite markers are well-characterized as powerful tools for population genetic investigations due to codominance, high polymorphisms and ease of scoring (Kalia *et al.* 2011). Here, we report the isolation and characterization of the first set of polymorphic microsatellite markers, which will provide a useful tool for population genetic studies for *S. taccada*.

## Materials and methods

Genomic DNA was extracted from silica gel-dried leaf tissue of one individual of *S. taccada* following the cetyltrimethylammonium bromide (CTAB) method (Doyle 1991). Microsatellite loci were developed following the protocol in Duan *et al.* (2012). About 250 ng of genomic DNA was digested with the enzyme *Mse*I (Promega, Madison, USA) and fragments of 200 to 800 bp were ligated with an *Mse*I adapter pair. The adapter-ligated fragments were amplified with *Mse*I-N primer (5′-GATGAGTCCTGAGTAAN-3′). PCR products were denatured and hybridized to 5′-biotinylated $(AC)_{15}$ probes. The probe-bound fragments were enriched by streptavidin-coated magnetic beads (Promega). After stringent washing, the captured DNA fragments were eluted with 50 $\mu$L of TE buffer and then amplified with *MseI*-N. PCR products were ligated into pMD19-T vector (Takara, Dalian, China) and transformed into *Escherichia coli* strain DH5$\alpha$ (TakaRa). Clones were picked randomly and tested by PCR using the M13+/M13− primers.

Finally, 440 clones were sequenced by an ABI3730 DNA Sequencer (Invitrogen, Guangzhou, China). After discarding sequences with few repeats or short-flanking sequences, 165 primer pairs were designed using Primer3web ver. 4.0.0 (http://primer3.ut.ee/). Of the 165 primer pairs, 90 produced clear bands of amplification products with expected sizes on 1% agarose gel. To test the effectiveness of these primers, we chose eight individuals for PCR from two insular populations, Baili Island (BL), Xiaowanshan Island (XW), and two

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**Keywords.** microsatellites; island biodiversity; genetic variation; *Scaevola taccada.*

from continental island Hainan Province in China, Changhua (CH) and Wanning (WN) (two individuals per population). Sixteen loci showed polymorphisms by 6% polyacrylamide gel electrophoresis, each of which forward primer was fluorescently labelled with HEX, FAM, ROX or TAMRA (Invitrogen) for further screening (table 1).

To characterize the genetic variability of *S. taccada* at 16 loci, we genotyped 64 individuals from the above mentioned four populations (table 2). Multiplex PCRs were performed in a total volume of 10 $\mu$L, which included 5 $\mu$L Master Mix (Zhuangmeng, Beijing, China), 0.2 $\mu$M of each primer pair and approximately 10 ng of genomic DNA. All loci were grouped into three multiplex PCR sets: i) Sse62, Sse116, Sse135, Sse145, Sse148, Sse171; ii) Sse61, Sse130, Sse155, Sse191, Sse408; and iii) Sse17, Sse40, Sse154, Sse172, except that Sse189 was amplified in a single PCR and then genotyped together with other loci in multiplex PCR set 1. Thermocycler protocol began with one cycle of 94°C for 4 min, followed by 35 cycles of 30 s at 94°C, 30 s at 58°C (except for Sse189, for which the annealing temperature was 52°C) and 30 s at 72°C, with a final extension of 10 min at 72°C. PCR products were separated by an ABI PRISM 3100 Genetic Analyser (Invitrogen) using an internal size standard GeneScan$^{TM}$ 500 LIZ. Allele binning and calling were done using GeneMarker ver. 2.4.0 (SoftGenetics LLC, State College, Pennsylvania, USA).

The number of alleles per locus for all individuals ($N_A$) and for each population ($A$) was calculated. Observed ($H_o$) and expected ($H_e$) heterozygosities per locus and population were estimated. Also, deviation from Hardy–Weinberg equilibrium (HWE) for each locus and population was tested. All above analyses were performed by GenALEx ver. 6.1 (Peakall and Smouse 2006). Micro-Checker ver. 2.2.3 (Van Oosterhout *et al.* 2004) was used for the test of null alleles.

## Results and discussion

Overall, 16 loci were found to be polymorphic among 64 individuals (table 2). Totally, 106 alleles were genotyped.

**Table 1.** Sequence and multiplex characteristics of 16 microsatellite loci isolated in *S. taccada*.

| Locus | Primer sequences (5′–3′) | Motif | Size (bp) | Multiplex | GenBank |
|---|---|---|---|---|---|
| Sse17 | F: <TAMRA> CTTGTGTGTCACGTGTGTGC<br>R: TATCCAGGTTGGCTTGGAAC | $(CT)_{13}(GA)_{14}$ | 188–204 | 3 | KF984511 |
| Sse40 | F: <FAM> AAGCCAAAACGTCATTATCCA<br>R: CAATCAAAAAGAAACGGATCG | $(AG)_{13}\ldots(TC)_{11}\ldots(TC)_{8}$ | 220–238 | 3 | KF984512 |
| Sse61 | F: <TAMRA> AGGCACAGGAGATGAAGTCG<br>R: GTTGCAGCTATGGAGGTCGT | $(AGA)_{20}$ | 173–183 | 2 | KF984513 |
| Sse62 | F: <ROX> GATGCAGGGAGACAGGAAGA<br>R: AGGAATTTTGACCACAGATGC | $(AG)_{21}$ | 146–158 | 1 | KF984514 |
| Sse116 | F: <TAMRA> ACCCAACACTTTCCTTTCCA<br>R: CGGATCATTCATCACATTGG | $(AC)_{12}$ | 168–180 | 1 | KF984515 |
| Sse130 | F: <HEX> GGCTTTTTCTGGGTCATCAA<br>R: GAGGAGAAGGGGAAGAAGGA | $(TTC)_{8}$ | 205–215 | 2 | KF984516 |
| Sse135 | F: <ROX> ACCAACAACCGAAGCAACAT<br>R: CATCTCAGGTATGCCAAATCC | $(AC)_{17}$ | 200–214 | 1 | KF984517 |
| Sse145 | F: <FAM> CAAGTTCCGTAGTGCATCACA<br>R: CCAATGTCAACACCATTTTCC | $(CT)_{12}$ | 210–220 | 1 | KF984518 |
| Sse148 | F: <FAM> TCATATCATCTATTTGGAATGAAGC<br>R: TGATCTCATCTGATATGATTCTGG | $(GA)_{23}\ldots(AG)_{5}(GT)_{7}$ | 278–294 | 1 | KF984519 |
| Sse154 | F: <ROX> GGTTAGATGTAACTGCTTGTGTGT<br>R: GCTCGGACTCAACTAAGTTTTGA | $(TG)_{15}\ldots(TG)_{7}\ldots(TG)_{7}\ldots(AG)_{6}$ | 189–211 | 3 | KF984520 |
| Sse155 | F: <ROX> CTTTGCCATTTCCACAATCA<br>R: GAAGCAACAACCAAGGGAGA | $(AG)_{23}$ | 210–230 | 2 | KF984521 |
| Sse171 | F: <FAM> GCTTTCCTTCTGCAAAACTCA<br>R: CCGCAGGGTACAGACTGTTT | $(GA)_{6}\ldots(AG)_{10}$ | 158–166 | 1 | KF984522 |
| Sse172 | F: <HEX> GGTTTCGGTGGAGAAAAACA<br>R: TTAGGGAGAGGGCCTTAGGA | $(CT)_{10}$ | 198–214 | 3 | KF984523 |
| Sse189 | F: <HEX> CGTTCTCATTTGGTCAAGCA<br>R: AAACTACAACAATGTGGTGGATAGA | $(TC)_{6}\ldots(CT)_{7}$ | 186–216 | 1* | KF984524 |
| Sse191 | F: <FAM> CAGATTTTGGGTGGTGAAGC<br>R: GGTCTTGCACTGATCTTCTCG | $(CT)_{17}$ | 181–203 | 2 | KF984525 |
| Sse408 | F: <ROX> GCTGGCTTCCTCAATCATTC<br>R: AAAAGCCCACACCATAGCTG | $(TC)_{9}$ | 179–185 | 2 | KF984526 |

Each forward primer was 5′ fluorescently labelled with HEX, FAM, ROX or TAMRA. Multiplex indicates multiplex PCR reaction sets: loci with the same number in this column were amplified with annealing temperature at 58°C and genotyped together, except Sse189 which were individually amplified at 52°C, and then genotyped together with others (*).

**Table 2.** Locus-specific measures of genetic diversity in four populations of *S. taccada*.

| | | BL ($n = 19$) | | | | XW ($n = 8$) | | | | CH ($n = 10$) | | | | WN ($n = 27$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locus | $N_A$ | $A$ | $H_o$ | $H_e$ | $P$ | $A$ | $H_o$ | $H_e$ | $P$ | $A$ | $H_o$ | $H_e$ | $P$ | $A$ | $H_o$ | $H_e$ | $P$ |
| Sse17 | 7 | 4 | 0.056 | 0.640 | ***$^N$ | 5 | 0.250 | 0.600 | **$^N$ | 4 | 0.556 | 0.575 | ns | 4 | 0.261 | 0.622 | ***$^N$ |
| Sse40 | 6 | 3 | 0.278 | 0.252 | ns | 4 | 0.500 | 0.592 | ns | 4 | 0.778 | 0.739 | ns | 5 | 0.760 | 0.707 | ns |
| Sse61 | 10 | 4 | 0.765 | 0.636 | ns | 5 | 0.625 | 0.800 | ns | 6 | 0.900 | 0.758 | ns | 8 | 0.615 | 0.827 | *$^N$ |
| Sse62 | 5 | 4 | 0.684 | 0.559 | * | 5 | 0.750 | 0.842 | ns | 3 | 0.700 | 0.584 | ns | 5 | 0.615 | 0.735 | ns |
| Sse116 | 5 | 2 | 0.579 | 0.462 | ns | 3 | 0.750 | 0.542 | ns | 3 | 0.600 | 0.568 | ns | 4 | 0.593 | 0.627 | ns |
| Sse130 | 2 | 2 | 0.556 | 0.508 | ns | 2 | 0.250 | 0.233 | ns | 1 | 0.000 | 0.000 | nd | 2 | 0.692 | 0.507 | * |
| Sse135 | 9 | 5 | 1.000 | 0.749 | * | 4 | 0.200 | 0.778 | ns | 5 | 0.500 | 0.725 | Ns | 5 | 0.292 | 0.793 | ***$^N$ |
| Sse145 | 3 | 3 | 0.263 | 0.656 | ** | 3 | 0.125 | 0.508 | *$^N$ | 3 | 0.500 | 0.626 | ns | 3 | 0.280 | 0.533 | *$^N$ |
| Sse148 | 10 | 3 | 0.278 | 0.452 | ns | 5 | 1.000 | 0.725 | ns | 4 | 0.800 | 0.705 | ns | 8 | 0.560 | 0.747 | ns |
| Sse154 | 8 | 4 | 0.833 | 0.646 | ns | 7 | 0.875 | 0.833 | ns | 5 | 0.700 | 0.753 | ns | 5 | 0.769 | 0.756 | ns |
| Sse155 | 8 | 4 | 0.833 | 0.702 | ns | 4 | 0.500 | 0.675 | ns | 5 | 0.900 | 0.732 | ns | 7 | 0.667 | 0.619 | ns |
| Sse171 | 4 | 3 | 0.316 | 0.428 | ns | 3 | 0.500 | 0.633 | ns | 2 | 0.800 | 0.505 | * | 3 | 0.077 | 0.298 | ***$^N$ |
| Sse172 | 6 | 3 | 0.333 | 0.294 | ns | 4 | 0.500 | 0.700 | ** | 4 | 0.800 | 0.679 | ns | 5 | 0.720 | 0.722 | ns |
| Sse189 | 8 | 5 | 0.167 | 0.744 | ***$^N$ | 4 | 0.250 | 0.592 | ns | 4 | 0.222 | 0.471 | ns | 5 | 0.038 | 0.708 | ***$^N$ |
| Sse191 | 7 | 4 | 0.500 | 0.532 | ns | 4 | 0.875 | 0.692 | ns | 6 | 0.800 | 0.805 | ns | 6 | 0.692 | 0.705 | ** |
| Sse408 | 8 | 4 | 0.611 | 0.490 | ns | 3 | 0.500 | 0.692 | ns | 6 | 1.000 | 0.821 | ns | 6 | 0.538 | 0.673 | ns |
| Mean | 6.6 | 3.6 | 0.503 | 0.547 | | 4.1 | 0.528 | 0.652 | | 4.1 | 0.660 | 0.628 | | 5.1 | 0.511 | 0.661 | |

BL Baili island, Guangdong province; XW Xiaowanshan island, Guangdong province; CH: Changhua, Hainan province; WN: Wanning, Hainan province; $N_A$; total number of alleles; $A$, number of alleles; $H_o$, observed heterozygosity; $H_e$, expected heterozygosity; $P$, probability of deviation from HWE. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant; nd, not determined due to monomorphy; $^N$ presence of null alleles.

The number of alleles per locus ranged from 2 to 10 with a mean of 6.6. Except for Sse130 in CH being monomorphic, all loci were polymorphic in each population. Observed heterozygosity varied from 0.056 to 1.000 with a mean of 0.503 in BL, from 0.125 to 1.000 with a mean of 0.528 in XW, from 0.000 to 1.000 with a mean of 0.660 in CH and from 0.038 to 0.769 with a mean of 0.511 in WN, respectively, while expected heterozygosity ranged from 0.252 to 0.749 with a mean of 0.547 in BL, from 0.233 to 0.842 with a mean of 0.652 in XW, from 0.000 to 0.821 with a mean of 0.628 in CH and from 0.298 to 0.827 with a mean of 0.611 in WN (table 2). No significant deviation from HWE was detected for Sse40, Sse116, Sse148, Sse154, Sse155 and Sse408 in all populations. However, the other loci showed significant deviations ($P < 0.05$) in either one or more populations, probably due to the presence of null alleles, as indicated by Micro-Checker analysis (table 2), or limited number of samples.

These results confirm that the microsatellite markers reported here are suitable for population genetic studies of *S. taccada*. As the first set of microsatellite markers in *Scaevola*, they could be used in investigation of population genetic variation, genetic structure and gene flow in *S. taccada* and in related species in general, and comparative levels of genetic variation in continental versus insular populations in particular. Further, we expect that these markers will provide a foundation for the management of germplasms of *S. taccada*.

**Acknowledgements**

This work was supported by Ministry of Science and Technology of China (grant number: 2013FY111200) and the foundation of Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences.

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Received 8 January 2014, in revised form 27 February 2014; accepted 3 March 2014
Published online: 8 August 2014