Development of Microsatellite Markers for the Coastal Shrub Scaevola taccada (Goodeniaceae)

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PRIMER NOTE

DEVELOPMENT OF MICROSATELLITE MARKERS FOR THE COASTAL SHRUB *SCAEVOLA TACCADA* (GOODENIACEAE)[1]

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- *Premise of the study:* Microsatellite markers were developed for the coastal shrub species *Scaevola taccada* to estimate the population genetic structure, which may reflect different seed dispersal patterns.
- *Methods and Results:* Thirteen microsatellite primer sets were developed for *S. taccada* using 454 pyrosequencing. The primer sets were tested on 64 individuals sampled from two populations in Japan. Fragments were amplified using the primers, with one to 10 alleles per locus, and the expected heterozygosity ranged from 0.00 to 0.85.
- *Conclusions:* These results indicate the utility of markers in *S. taccada* for broad estimations of the population genetic structure of this species.

Key words: 454 sequencing; genetic structure; Goodeniaceae; *Scaevola taccada*; seed dispersal.

Scaevola taccada (Gaertn.) Roxb. (Goodeniaceae) is a coastal shrub species widely distributed along coastal areas of the Pacific and Indian oceans (Howarth et al., 2003). The white fleshy exocarp and the underlying corky layer of the fruit (Howarth et al., 2003) allow it to be transported in the avian gut (Kawakami et al., 2009; Emura et al., 2012) and by oceanic floating (Nakanishi, 1988). Due to its unique seed dispersal system, *S. taccada* has been found growing in various environments. Seashores (sandy shores and cliffs, consistent with dispersal by oceanic floating and birds) represent the primary growth environment of this species, but some populations exist in island interiors (dispersal by birds) (Satake et al., 1989; Emura et al., unpublished data). Following such a distribution pattern, some isolated populations might be in the process of speciation, which may be indicated by population genetic structure. To estimate genetic differentiation caused by the different seed dispersal patterns, highly variable genetic markers are required. Here, we report 13 nuclear microsatellite loci for *S. taccada* developed using 454 next-generation sequencing, which will be useful for estimating the population genetic structure of this species.

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METHODS AND RESULTS

A fresh leaf sample of *S. taccada* was collected from an individual growing in a coastal area of the Ogasawara Islands, Japan, and genomic DNA was extracted using the DNeasy Plant Mini Kit (QIAGEN, Germantown, Maryland, USA). The GS Junior Titanium Series Kit (Roche, Mannheim, Germany) and the SPRIworks Fragment Library System (Beckman Coulter, Brea, California, USA) were used for construction of a DNA library for *S. taccada*. A 500-ng aliquot of genomic DNA was nebulized at 0.24 MPa for 1 min, purified, end-repaired, and A-tailed using the SPRIworks Fragment Library Kit II (Beckman Coulter) and ligated to the Rapid Library Adapter (Roche) using RL Ligase (Roche). Suitably sized DNA fragments were selected by removing short fragments using SPRIworks Fragment Library Kit II (Beckman Coulter). Emulsion PCR (emPCR) was constructed for the desired fragments mixed with capture beads using the GS Junior Titanium emPCR Kit (Roche). After emPCR, the beads capturing the DNA library were enriched to selectively capture beads with sufficient amounts of template DNA for sequencing. The enriched beads were annealed with sequencing primers, and the amplified fragments were sequenced using the GS Junior Benchtop System (Roche). In the GS Junior sequencing, 30,497 DNA sequences were obtained. The sequences were screened to find potential microsatellite loci using the MSATCOMMANDER program (Faircloth, 2008). After screening, 423 repeat regions containing eight or more dinucleotide repeats were identified using Primer3 software (Rozen and Skaletsky, 2000) embedded in MSATCOMMANDER, and primers were successfully designed for a total of 72 repeats.

Twenty-three primer pairs were selected for amplification trials in eight *S. taccada* individuals, based on the repeat structure and avoiding sequences containing mononucleotide repeats. All of the forward primers designed from the selected loci were synthesized with a tag sequence (Boutin-Ganache et al., 2001) for fluorescent labeling. The sequence of each tag is indicated in the notes for Table 1. A modified protocol for the QIAGEN Multiplex PCR Kit (QIAGEN) was used for PCR amplification. The final volume of the PCR reaction mixture was 5 µL and contained the following: 16 ng extracted DNA, 2.5 µL Multiplex PCR Master Mix, 0.01 µM forward primer, 0.2 µM reverse primer, and 0.1 µM M13 (fluorescently labeled) primer. The PCR program was as follows: an initial denaturation at 95°C for 15 min; 30 cycles at

TABLE 1. Characteristics of 13 microsatellite primers developed for *Scaevola taccada*. All values are based on 64 samples from two populations on the Okinawa and Ogasawara islands in Japan.

Locus	Primer sequences (5′–3′)	Repeat motif	Fluorescent label[a]	T_a (°C)	Allele size range (bp)	GenBank accession no.
Stac05	F: TGGTGGTTAACAAGTGGCAAG	$(AG)_8$	FAM	57	175–179	AB872256
	R: GTTACTCAAGTCTCTTATTAGCAGTTC					
Stac06	F: GGCACCAGCTTCTGTAACC	$(AG)_9$	FAM	57	270–274	AB872257
	R: GCAGTGGTGGATGCATTCTG					
Stac09	F: CATGAGCCAAGGAAACGTCC	$(AG)_9$	VIC	57	325–335	AB872258
	R: GCTTCGGCTCTTCACAAGG					
Stac10	F: CAGCAGGAATGCACTAAGACC	$(AT)_{11}$	VIC	57	223–253	AB872259
	R: GGAGGGATGGAAAGGTCCG					
Stac11	F: TTTCGCAAGATCCCGGC	$(AT)_8$	NED	57	254	AB872260
	R: TGGAAGTTGGTAAATGGGTCAG					
Stac13	F: TCTTAACGACTCCTGTTCACC	$(AC)_9$	FAM	57	209	AB872261
	R: CCGTCTCCATCCCTTCGTG					
Stac15	F: ACTCATCAGACAAGGTAACGG	$(AC)_{10}$	VIC	57	368–372	AB872262
	R: AGCAGTCTACTTCCCACGC					
Stac17	F: CTGCAACGGTCATTCGTCC	$(AT)_9$	NED	57	289–303	AB872263
	R: TGCAACCTATATGCTATGTGCTC					
Stac18	F: GAGGGAAGGTCAGAGGTGC	$(AG)_9$	FAM	57	189–193	AB872264
	R: TCAGACAAGTGTAATAGAAGGTCATC					
Stac19	F: AAAGTCGGGCAGTAGGTGC	$(AG)_8$	FAM	57	250	AB872265
	R: GGAGTTGATGAATGCTCGGC					
Stac21	F: CCCTCAAGGCTACTGTCGG	$(CT)_{13}$	FAM	57	297–304	AB872266
	R: TTGCGTTCTTCCGCAATCC					
Stac24	F: ATTGATTAGTAGGTGAGAATCGTG	$(AT)_{10}$	NED	57	191–193	AB872267
	R: TCGGTATTGTCCTAATCTTCCG					
Stac27	F: ATACATCATCGTACCCAAATTCC	$(AT)_8$	NED	57	199–203	AB872268
	R: GAGCTCCTTGAAATGTCGCC					

Note: T_a = annealing temperature.

[a] Sequence of the fluorescent labels: FAM = 5′-CACGACGTTGTAAAACGAC-3′, NED = 5′-CTATAGGGCACGCGTGGT-3′, VIC = 5′-TGTGGAATTGTGAGCGG-3′.

94°C for 30 s, 57°C for 1.5 min, and 72°C for 1 min; and a final extension at 60°C for 30 min. The PCR product size was determined using the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, USA) and GeneMapper software (Applied Biosystems). Thirteen primer pairs amplified well and could be scored easily and unambiguously (Table 1). These primer pairs were used for amplification in 64 individuals sampled from two populations located on the Ogasawara Islands (26.61184°N, 142.17543°E) and the Okinawa Islands (26.13975°N, 127.79644°E), using the PCR program used for amplification described above. One specimen from each population was collected and deposited at the Kyoto University Museum herbarium (accession numbers: KYO 00037380 and KYO 00037381).

The number of alleles for a given locus ranged from one to 10 (mean: 2.5). The observed and expected heterozygosities were 0.00–0.91 (mean: 0.23) and 0.00–0.85 (mean: 0.27), respectively (Table 2). Deviation from Hardy–Weinberg equilibrium (HWE) and the linkage disequilibrium (LD) between loci were tested using FSTAT version 2.9.3 (Goudet, 1995). Two loci (Stac21 and Stac24) exhibited a significant deviation from HWE ($P < 0.05$) in the Ogasawara population. There was no evidence of LD for any loci pairs. Fixation index was calculated using FSTAT and its values were –0.18–1.00.

TABLE 2. Results for primer screening of all samples for 13 microsatellite loci in two populations of *Scaevola taccada*.

	Okinawa ($N = 32$)				Ogasawara ($N = 32$)			
Locus	A	H_o[a]	H_e	F_{IS}	A	H_o	H_e	F_{IS}
Stac05	2	0.22	0.28	0.25	3	0.53	0.60	0.13
Stac06	3	0.44	0.46	0.07	2	0.41	0.36	–0.12
Stac09	4	0.22	0.20	–0.07	1	0.00	0.00	NA
Stac10	10	0.91	0.85	–0.05	4	0.44	0.48	0.11
Stac11	1	0.00	0.00	NA	1	0.00	0.00	NA
Stac13	1	0.00	0.00	NA	1	0.00	0.00	NA
Stac15	3	0.47	0.52	0.11	3	0.47	0.53	0.13
Stac17	2	0.03	0.03	0.00	5	0.53	0.65	0.20
Stac18	3	0.53	0.60	0.13	3	0.50	0.42	–0.18
Stac19	1	0.00	0.00	NA	1	0.00	0.00	NA
Stac21	3	**0.08**	0.47	0.84	3	0.06	0.15	0.58
Stac24	2	**0.00**	0.12	1.00	1	0.00	0.00	NA
Stac27	2	0.25	0.38	0.35	2	0.03	0.03	0.00

Note: A = number of alleles; F_{IS} = fixation index; H_e = expected heterozygosity; H_o = observed heterozygosity; N = sample size; NA = not applicable.

[a] Numbers in boldface show deviations from Hardy–Weinberg equilibrium.

CONCLUSIONS

We have characterized 13 microsatellite loci for *S. taccada*. These microsatellite loci will be useful for estimating population genetic structure possibly resulting from the various seed dispersal patterns of *S. taccada*.

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