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Applications in Plant Sciences

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Primer Note

Isolation and characterization of microsatellite loci in the invasive herb *Solidago altissima* (Asteraceae)[1]

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- *Premise of the study:* Microsatellite markers were developed in the perennial herb *Solidago altissima* from populations within its introduced range in Japan to assess its population structure and to facilitate tracking of invasion expansion.
- *Methods and Results:* Using 454 pyrosequencing, 16 microsatellite primer sets were developed for *S. altissima*. The primer sets were tested on 70 individuals sampled from three populations in Japan. The primers amplified di- and trinucleotide repeats with five to 25 alleles per locus, and the expected heterozygosity ranged from 0.46 to 0.92.
- *Conclusions:* These results indicate the utility of primers in *S. altissima* for future research on a wide range of applications, including tracking of invasion dynamics and investigating population genetics of the species.

Key words: 454 sequencing; Asteraceae; invasion; polyploidy; *Solidago altissima*.

Tall goldenrod (*Solidago altissima* L.; Asteraceae) is considered to be among the most invasive of introduced plants in Japan (Shimizu, 2003), China (Huang and Guo, 2004), and Taiwan (Li, 1978). It is a rhizomatous herbaceous perennial native to a variety of successional habitats such as prairies, woodland edges, and old fields throughout eastern North America. Diploid, tetraploid, and hexaploid cytotypes are known to occur at a continental scale and even on very fine spatial scales in some local populations in its native range (Halverson et al., 2008), while only hexaploids have been found in Japan (Sakata, unpublished data). After the species was introduced to Japan in the early 19th century as an ornamental plant, it has continuously expanded its range and has now invaded disturbed areas throughout Japan (Shimizu, 2003). Two closely related species, *S. canadensis* L. and *S. gigantea* Aiton, have also become invasive throughout Europe. *Solidago altissima* is also well studied as a model plant for insect–plant interactions, and effects of the variation among its genotypes on the diversity and composition of foliage-based arthropods have been studied (e.g., Crutsinger et al., 2008). However, these studies used amplified fragment length polymorphism (AFLP) analysis to identify individuals and calculate the genotypic diversity of *S. altissima*, and no microsatellite markers are available for population genetic studies. There is a great need for molecular studies that address pathways of introduction, genetic variability within and between populations, and the potential for rapid evolutionary changes in tall goldenrod since its introduction. Here we report 16 nuclear microsatellite loci for *S. altissima* developed using 454 next-generation sequencing, which will facilitate studies on the invasion dynamics and population genetics of the species.

METHODS AND RESULTS

A fresh leaf sample of *S. altissima* was collected from an individual grown at the greenhouse of the Center for Ecological Research, Kyoto University, Japan (34.97136°N, 135.957892°E). A voucher was deposited at KYO (accession number: 00020479). Genomic DNA was extracted using a DNeasy Plant Mini Kit (QIAGEN, Germantown, Maryland, USA). A DNA library for *S. altissima* was constructed using the GS Junior Titanium Series kit (Roche, Mannheim, Germany). A 500-ng aliquot of the genomic DNA was nebulized at 0.24 MPa for 1 min and purified using the MinElute PCR Purification Kit (QIAGEN). The purified DNA fragments were end-repaired and A-tailed using the Rapid Library Preparation Kit (Roche) and ligated to the Rapid Library Adapter (Roche) with RL Ligase (Roche). Suitably sized DNA fragments were selected by removing short fragments with AMPure XP beads (Beckmann Coulter, Brea, California, USA). Fragments were then mixed with capture beads and amplified by emulsion polymerase chain reaction (emPCR) from the DNA library with the GS Junior Titanium emPCR Kit (Roche). After emPCR, beads were collected, and beads capturing the DNA library were enriched before annealing with sequencing primers to selectively capture beads with sequenceable amounts of template DNA. Amplified fragments were sequenced using the GS Junior Benchtop system (Roche), obtaining 70,602 DNA sequences.

The sequences were screened for potential microsatellite loci by MSATCOMMANDER (Faircloth, 2008). We screened sequences for ≥8 dinucleotide repeats and ≥6 trinucleotide repeats using the Primer3 software (Rozen and Skaletsky, 2000) embedded in MSATCOMMANDER. A total of 2629 repeats were found, consisting of 2082 dinucleotide and 547 trinucleotide repeats. Primers were successfully designed for a total of 370 repeats, including 156 dinucleotide and 214 trinucleotide repeats. Based on the repeat structure and

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Table 1. Characteristics of 16 microsatellite primers developed in *Solidago altissima*. All values are based on 70 samples representing Japanese populations located in Hokkaido, Hyogo, and Kagoshima (N = 22–24 for each).

Locus	Primer sequences (5′–3′)	Fluorescent label[a]	Repeat motif	T_a (°C)	Allele size (bp)	GenBank accession no.
Salt1	F: ACTACTGGCGGCGTAACAG R: CCGCCGGTTTCAATGTTGG	NED	$(ATT)_{15}$	57	180–250	AB724096
Salt2	F: TGCATGGTTATTGGTTTCAAATGG R: CACCCTCACTAGGAGAAACTTG	VIC	$(AAT)_6$	57	185–200	AB724097
Salt3	F: GAAGAAGAGCTTCGGATGTTGT R: CGCCTAAATTGACTTGACACAG	FAM	$(ATT)_{14}$	57	150–230	AB724098
Salt4	F: CAGAAGAAACCGCCGGAAG R: GAGCAATGCAGTGGCAGAC	VIC	$(AAC)_6$	57	275–305	AB724099
Salt5	F: GGCGAAGAAGGGTCGTTTG R: CCGGAAAGCGTTTGATGGG	FAM	$(TGA)_8$	57	280–320	AB724100
Salt6	F: ACATCTTACCTCCCGGCTC R: CAGCTGACCAGATCAATCACC	NED	$(GTT)_7$	57	305–340	AB724101
Salt7	F: GGGACAAGGTCCATCAAATCC R: ACTCCTCTTCTTCTTTGATAACCG	FAM	$(GAT)_6$	57	365–380	AB724102
Salt8	F: ATAGAAGTGGGTCGGCTCC R: CCCGACTAAGCCCATTTCC	VIC	$(GGT)_6$	57	400–415	AB724103
Salt9	F: TCTTGCTTGTCTTCAGTGGG R: GCGTAACTTCAGTCGCCTC	NED	$(AAT)_8$	57	400–500	AB724104
Salt13	F: TGAAATTCCAACGTGGCAG R: GCCAAATGACACCCAACCG	FAM	$(AAT)_8$	57	270–300	AB724105
Salt14	F: CTGGTCACACTCCTAAAGCC R: ATCGGAAGTCGGGACAAGC	VIC	$(AAT)_8$	57	260–280	AB724106
Salt16	F: CAACGACAGCACATTCGGG R: CGACACGTCATCAATCGCC	VIC	$(AAT)_6$	57	315–325	AB724107
Salt17	F: GCACCTTCTCCAAATGCCG R: TGTCCGCTGATACCAGTCC	FAM	$(ACC)_8$	57	370–390	AB724108
Salt18	F: CCTCCACAAACCTTCCGTC R: AATCTGGGAAACACGTCGC	VIC	$(ATC)_6$	57	315–355	AB724109
Salt19	F: GTACCATTCCACCAAGCCG R: GGCCGACTTGAACCCATTC	NED	$(TTG)_8$	57	270–340	AB724110
Salt21	F: TCCTTCCTTGTCGAATCGGG R: AAACAAGGCTCCCAGTTGC	FAM	$(AT)_8$	57	190–200	AB724111

Note: T_a = annealing temperature.
[a]Sequence of the fluorescent labels: FAM = CACGACGTTGTAAAACGAC; NED = CTATAGGGCACGCGTGGT; VIC = TGTGGAATTGTGAGCGG.

avoiding sequences containing mononucleotide repeats, 84 primer pairs (23 dinucleotide and 61 trinucleotide loci) were selected for amplification trials in eight individuals from throughout the invasive range of *S. altissima*. For all the loci, the forward primer was synthesized with a tag sequence (5′-CACGACGTTGTAAAACGAC-3′) (Boutin-Ganache et al., 2001). PCR amplifications were performed according to a modified protocol using the QIAGEN Multiplex PCR Kit (QIAGEN). The PCR reaction mixture had a final volume of 5 µL, which included 16 ng of extracted DNA, 2.5 µL of Multiplex PCR Master Mix, 0.01 µM of forward primer, 0.2 µM of reverse primer, and 0.1 µM of M13 (fluorescently labeled) primer. The amplification profiles

Table 2. Results for primer screening of all samples of 16 microsatellite loci from three populations of *Solidago altissima*.

	Hokkaido (N = 22)			Hyogo (N = 24)			Kagoshima (N = 24)			
Locus	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	P_{ID}
Salt1	8	0.91	0.84	17	0.99	0.90	12	0.97	0.89	0.008
Salt2	4	0.91	0.74	7	0.99	0.77	4	0.70	0.66	0.13
Salt3	12	0.99	0.90	22	0.96	0.95	14	0.80	0.92	0.006
Salt4	7	0.96	0.83	8	1.00	0.75	8	0.88	0.77	0.09
Salt5	11	0.95	0.86	13	0.92	0.88	10	1.00	0.88	0.19
Salt6	8	0.71	0.77	11	0.96	0.87	7	1.00	0.80	0.05
Salt7	1	0.00	0.00	5	0.88	0.69	4	0.91	0.67	0.27
Salt8	8	1.00	0.84	7	0.96	0.77	5	0.96	0.72	0.05
Salt9	15	1.00	0.90	17	1.00	0.88	9	0.91	0.86	0.01
Salt13	6	1.00	0.73	10	0.96	0.81	10	0.96	0.81	0.09
Salt14	4	0.77	0.56	6	0.98	0.77	5	0.86	0.70	0.09
Salt16	5	0.32	0.52	3	0.42	0.50	3	0.21	0.31	0.48
Salt17	7	0.93	0.78	10	0.96	0.81	10	0.90	0.81	0.05
Salt18	11	1.00	0.89	9	0.96	0.84	7	1.00	0.80	0.08
Salt19	8	0.90	0.82	10	0.92	0.87	7	0.95	0.81	0.08
Salt21	3	0.94	0.64	7	1.00	0.76	6	1.00	0.79	0.05
Average	7.38	0.83	0.73	10.13	0.93	0.80	7.56	0.88	0.76	0.09

Note: A = number of alleles; H_e = expected heterozygosity; H_o = gametic heterozygosity; N = sample size; P_{ID} = probability of identity.

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included an initial denaturation at 95°C for 15 min, followed by 30 cycles of 30 s at 94°C, 1.5 min at 57°C, and 1 min at 72°C, and a final extension for 30 min at 60°C. PCR product size was measured using the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, USA) and Genotyper version 4 (Applied Biosystems). Of these, 15 trinucleotide primers and one dinucleotide primer amplified well and were easy to score unambiguously (Table 1). The 68 primer pairs remaining failed to produce the expected product size or were not consistently amplifiable. The 16 successful primer pairs were amplified in 70 individuals sampled from three populations located in northern, central, and southern Japan (Hokkaido: 43.057372°N, 141.508487°E; Hyogo: 34.41131°N, 135.16072°E; Kagoshima: 31.613611°N, 130.420487°E) with the same protocol used in the amplification test described above. One sample from each population has been vouchered at KYO (accession numbers: 00020488–00020490).

Number of alleles per locus (A), gametic heterozygosity (H_o) (Moody et al., 1993), and expected heterozygosity (H_e) were calculated by the program GenoDive version 2.0b19 (Meirmans and Van Tienderen, 2004) for each polymorphic locus over each population. Probability of identity (P_{ID}) (Waits et al., 2001) was obtained to indicate if these loci are useful for fingerprinting analysis and analyzing clonal structure of *S. altissima* after calculating the allele frequency of 70 samples by POLYSAT (Clark and Jasieniuk, 2011) using the function "simpleFreq." For all samples in all populations, the number of alleles per polymorphic locus ranged from five to 25, H_o from 0.31 to 0.99, H_e from 0.46 to 0.92, and P_{ID} from 0.006 to 0.48. The values of A, H_o, H_e, and P_{ID} for each polymorphic locus over each population are given in Table 2.

CONCLUSIONS

We have characterized 16 microsatellite loci for the invasive herb *S. altissima*. These microsatellite loci will be useful for investigating the genetic structure, contemporary gene flow, fingerprinting, and invasion dynamics of *S. altissima*.

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