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Isolation and characterization of polymorphic microsatellite loci from the invasive plant *Solidago canadensis* (Asteraceae)

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ABSTRACT. *Solidago canadensis*, a clonal herb originally from North America (common name: Canada goldenrod), is an invasive species in many countries. We developed microsatellite primers for this species. Eleven polymorphic loci were generated and primers were designed. Polymorphism of these 11 loci was assessed in 35 plants from two populations (Wuhan and Shanghai) in China. The number of alleles per locus ranged from 3 to 14. The observed and expected heterozygosities varied from 0.0732 to 0.7391 and from 0.1177 to 0.8687, respectively. These microsatellite markers will be useful tools for studies of population genetics in the native and invasive range of this species.

Key words: Invasive plant; Microsatellite; Population genetics; *Solidago canadensis*

INTRODUCTION

Solidago canadensis (Asteraceae) is a perennial clonal shrub native to North America and has now been intentionally introduced in many countries, in most of which this species is an invasive plant (Li and Xie, 2002). This species is a polyploid weed with 2n = 18, 36 and 54 (Melville and Morton, 1982). *S. canadensis* poses a serious threat to native ecosystems and their biodiversity, and causes considerable economic loss to the regions they invade (Dong et al., 2006). Several biological control programs for *S. canadensis* have been conducted, but until now, there are only a few population genetic studies on this species, and all these studies involved samples in restricted areas based on dominant molecular markers, e.g., RAPD and ISSR (Ma, 2003; Huang and Guo, 2005; Dong et al., 2006). In order to better develop biological control strategies, knowledge of the population structure and genetic diversity of native and invasive populations and of the historical routes of invasion are necessary.

Microsatellites are widely applied in studies on plant population genetics because of the typically high levels of variability detected, the co-dominant inheritance in a Mendelian fashion, and reliable scorability (Beaumont and Bruford, 1999; Zhang and Hewitt, 2003). For the invasive species *S. canadensis*, there was no microsatellite marker available for a population genetics study. In this study, we characterized 11 microsatellite loci from *S. canadensis* using a developed isolation technique (Lian et al., 2006) for ongoing population genetic studies. The approach can substantially reduce the time in comparison with FIASCO (Fast Isolation by AFLP of Sequences Containing Repeats) protocol. Because a common fluorescent compound SSR primer can be used in polymorphism analyses for different loci and different species and because a fluorescent primer is rather expensive, the approach proposed here may save on investigation costs (Lian et al., 2006).

MATERIAL AND METHODS

Genomic DNA was extracted from silica-dried leaves using a modified CTAB procedure (Doyle and Doyle, 1990). The extracted DNA was dissolved in 30 µL TE buffer, and digested with blunt-end restriction enzyme *Eco*RV (Takara). The fragments were then ligated with a specific blunt adaptor (consisting of the 48-mer: 5'-GTAATACGACTCACTATAGGGCACGCGTGGT CGA-3' and 8-mer with the 3'-end capped with an amino residue: 5'-ACCAGCCC-3') using T4 DNA ligase (Takara). Fragments were amplified with $(AC)_6(TC)_5$ or $(TC)_6(AC)_5$, $(TC)_3(AC)_5$ and an adaptor primer, AP2 (5'-CTATAGGGCACGCGTGGT-3'). The products were purified using a DNA clean-up kit (Transgen Biotech) and transformed into cells (DH5α) after ligating with the pGEM-T vector (Promega). Single clones were PCR-amplified using SSR primers and AP2. The positive clones were sequenced on an ABI 3730 automated sequencer (Applied Biosystems). For the fragments amplified by the $(AC)_6(TC)_5$, $(TC)_6(AC)_5$ or $(TC)_3(AC)_5$ primer, a specific primer (IP1) was designed from the sequenced region flanking the microsatellite. The IP1 and the compound SSR primers were used as SSR markers. In total, 62 primer pairs were designed using the Primer Premier 5 program (Lalitha, 2000).

In order to analyze the level of polymorphism within the isolated SSR loci, 35 individuals of *S. canadensis* from the Wuhan population in Hubei Province and Shanghai population in Shanghai, China, were genotyped. PCR carried out in 20-µL reaction mixtures containing 2.5-5 ng template DNA, 0.2 mM of each dNTP, 1X PCR buffer, 2.5 mM $MgCl_2$, 0.5 U Easy *Taq* polymerase (Transgen Biotech) and 0.5 mM of each specific primer designed in

the present study and fluorescent SSR primer. Touchdown PCR was performed for SC47: an initial denaturation at 94°C for 5 min; 10 cycles of 45 s at 94°C, 45 s at a locus-specific start temperature reduced by 0.5°C each subsequent cycle, and 45 s at 72°C; 25 cycles of 45 s at 94°C, 45 s at locus-specific annealing-end temperature, and 45 s at 72°C, and a final extension at 72°C for 30 min. For other primers, the PCR protocol consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of 45 s at 94°C, a specific temperature for 45 s (Table 1), and 72°C for 45 s, followed by a final extension at 72°C for 30 min. The reaction products were detected using an ABI 3730 sequencer and read using the Genemaker Apply Software. The test for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) between pairs of loci were performed using POPGENE (Yeh et al., 1999). The number of observed alleles per locus (N_A), and the observed (H_O) and expected (H_E) heterozygosities for the test populations were also calculated using POPGENE (Yeh et al., 1999).

RESULTS AND DISCUSSION

As a result, 11 of 62 primer pairs were polymorphic, and the remaining 51 primers were not used due to a low level of genetic variation or the occurrence of unexpected PCR bands. The characteristics of these 11 microsatellite loci are presented in Table 1. The number of alleles per locus ranged from 3 to 14, with an average of 6.6. H_O and H_E varied from 0.0732 to 0.7391 and from 0.1177 to 0.8687, respectively (Table 1). Eight of 11 loci showed significant deviation from HWE ($P < 0.001$) (Table 1), and two pairs of loci showed a significantly different LD value ($P < 0.01$). These microsatellite markers will be useful tools in population genetic studies of this significant invasive plant species.

Table 1. Characterization of 11 polymorphic microsatellite loci in *Solidago canadensis*.

Locus	Repeat motif	Primer sequence (5'-3')	T_a (°C) start-end	Size range (bp)	N_A	H_O	H_E	Accession No.
SC10***	$(AC)_6(TC)_5$	F: ACACACACACACTCTCTCTCTC R: CGGTCGGTTGGTTGCTAA	62	109-115	3	0.0732	0.1177	HM584915
SC18***	$(AC)_6(TC)_{11}$	F: ACACACACACACTCTCTCTCTC R: AGATGGTTTCTGCCGTCGTG	65	171-259	6	0.4634	0.4932	HM584917
SC23 n.s.	$(TC)_6(AC)_9$	F: TCTCTCTCTCTCACACACACAC R: CGGAGGACCTTACGATGT	56	117-139	8	0.6341	0.5769	HM584918
SC25 ***	$(TC)_6(AC)_5$	F: TCTCTCTCTCTCACACACACAC R: TAACCGAAGGTGGACATA	60	100-154	7	0.3125	0.5263	HM584919
SC39***	$(AC)_6(TC)_5$	F: ACACACACACACTCTCTCTCTC R: TGAGGCGGCAGTAGTGAA	55	168-188	7	0.3913	0.3082	HM584920
SC40 n.s.	$(AC)_6(TC)_5$	F: ACACACACACACTCTCTCTCTC R: TTCAACAAATGGCTACAC	55	300-314	5	0.5217	0.5585	HM584921
SC45 n.s.	$(TC)_6(AC)_7$	F: TCTCTCTCTCTCACACACACAC R: GCTTTGGAGTTCTATTATTTGAG	55	120-146	4	0.7391	0.6357	HM584922
SC46***	$(TC)_7(AC)_7$ $(ATAC)_2$	F: TCTCTCTCTCTCACACACACAC R: GTGAGGTTTCCCGTGAGGTCGTC	55	185-205	5	0.0870	0.3208	HM584923
SC47***	$(TC)_6(AC)_5$ $(TC)_{22}$	F: TCTCTCTCTCTCACACACACAC R: TTCGTCGTCTTATCTCGTCTATT	63-53	106-144	8	0.5854	0.6131	HM584924
SC51***	$(TC)_3(AC)_{22}$	F: TCTCTCACACACACAC R: GGGACGAGGTGCAGGTGAGTTTG	59	102-136	14	0.6829	0.8687	HM584926
SC54***	$(TC)_6(AC)_6$ $(AAC)_4$	F: TCTCTCTCTCTCACACACACAC R: CTCTTTGCGAATACTTTGGTTGC	62	160-176	6	0.6341	0.7040	HM584927

F = forward primer; R = reverse primer; T_a = locus specific annealing temperature (touchdown program: start temperature-end temperature); N_A = the number of alleles detected; H_O = observed heterozygosity; H_E = expected heterozygosity. ***Significant departures from Hardy-Weinberg equilibrium at $P < 0.001$. n.s. = not significant.

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