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PRIMER NOTE

MICROSATELLITE MARKERS FOR THE INVASIVE SPECIES *BIDENS ALBA* (ASTERACEAE)[1]

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- *Premise of the study:* Microsatellite markers were developed in the invasive species *Bidens alba* (Asteraceae) to assess its population structure and to facilitate tracking its expansion in China.
- *Methods and Results:* Using 454 pyrosequencing, 20 microsatellite primer sets were developed for *B. alba*. The markers were tested on one population of *B. alba* (30 individuals) and one population of the closely related *B. pilosa* (30 individuals) in China. For *B. alba*, all of the markers were polymorphic, and the number of alleles per locus ranged from three to 32. The expected heterozygosity values were from 0.3787 to 0.9284, and the Shannon–Wiener index was from 0.6796 to 2.8401.
- *Conclusions:* These markers will be useful for investigating the genetic structure, genetic diversity, and invasion dynamics of *B. alba* and will also be useful in studies of *B. pilosa*.

Key words: Asteraceae; *Bidens alba*; microsatellite marker; simple sequence repeat (SSR).

Bidens alba (L.) DC. (Asteraceae) is a cosmopolitan subtropical and tropical weed that is native to North and Central America (Ballard, 1986) and has recently become invasive in China. *Bidens alba* reproduces vigorously and has been rapidly spreading in southern China. It grows along roadsides and in abandoned farmland and orchards, resulting in a decline in soil fertility and crop production (Tian et al., 2010). *Bidens alba* is a tetraploid species ($2n = 48$) (Grombone-Guaratini et al., 2005; Knope et al., 2013). Currently, no microsatellite markers are available for population genetic studies of *B. alba*. In this study, we isolated and characterized 20 polymorphic microsatellites for *B. alba*, which can be used to assess its genetic variation within and among populations and track its invasion route in China.

METHODS AND RESULTS

Genomic DNA was extracted from silica gel–dried leaves using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). Genomic DNA from 20 individuals was mixed and sequenced using commercial services provided by Sangon Biotech (Shanghai, China) using 454 GS FLX Titanium (454 Life Sciences, a Roche Company, Branford, Connecticut, USA). A total of 149,204 reads with an average length of 423 bp were obtained, and a total of 11,049 reads contained microsatellite motifs.

One hundred and twenty-eight primer pairs from *B. alba* designed by Primer Premier 6.0 (Premier Biosoft International, Palo Alto, California, USA) were tested in 10 individuals as preparatory screening. Primers that produced reproducible and clearly defined bands were further tested for polymorphism in one *B. alba* population (30 individuals; 23.41505°N, 111.24734°E) and one population of the closely related *B. pilosa* L. (30 individuals; 25.26276°N, 111.32731°E). Voucher specimens (*S. Tang 20121001* for *B. alba* and *S. Tang 20120701* for *B. pilosa*) were deposited at the herbarium of Guangxi Normal University. PCRs were performed in 20-μL reaction volumes containing 1 unit of *Taq* polymerase (TaKaRa Biotechnology Co., Dalian, China), 2 μL of 10× PCR buffer, 0.4 μL of dNTPs (2.5 mM), 0.2 μL of each primer (50 μM), and 40 ng of genomic DNA. PCR amplification conditions were as follows: an initial denaturation at 94°C for 5 min, 30 cycles of 45 s at 94°C, 45 s at the optimized annealing temperature (Table 1), 45 s of extension at 70°C, ending with a 10-min extension at 72°C. PCR products were resolved on a 6% polyacrylamide denaturing gel using a 10-bp DNA ladder (Invitrogen, Carlsbad, California, USA) as the reference and visualized by silver staining.

In total, 20 highly polymorphic primer pairs were successfully amplified with expected sizes. These loci showed clearly defined banding patterns ranging from one to four alleles for each locus per individual. The expected heterozygosity (H_e) and the Shannon–Wiener index (H') were calculated with ATETRA version 1.2 a (Van Puyvelde et al., 2010), which includes all possible combinations of allele copy numbers in populations with partial heterozygotes.

As a result, all of the 20 microsatellite loci were polymorphic in *B. alba* and the number of alleles per locus varied from three to 32 alleles, with a mean of 13.4. H_e and H' were between 0.3787 and 0.9284 (mean = 0.7755) and 0.6796 to 2.8401 (mean = 1.8064), respectively. In *B. pilosa*, six loci were monomorphic. The number of alleles (A) per locus varied from one to 14, H_e varied from 0 to 0.8380, and H' ranged from 0 to 2.0937 (Table 2).

CONCLUSIONS

The 20 microsatellite loci developed for *B. alba* are useful for investigating the genetic structure, genetic diversity, and invasion dynamics of *B. alba*. Some of these loci will also be useful for *B. pilosa*.

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TABLE 1. Characteristics of 20 polymorphic microsatellite markers in *Bidens alba.*

Locus	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)	T_a (°C)	GenBank accession no.
Ba1	F: TTCAGAAATAGTCAAAGGGTT	$(AAT)_7$	184–230	53	KF872208
	R: TAGTAATAGCAAGCAAAGCA				
Ba2	F: CTATTCTTTCGGGATAGAGG	$(ATG)_{12}$	144–236	52	KF872209
	R: GCATTAAGTATTAACGATTGACT				
Ba3	F: TCATATTTCTAGTCCTGCTGC	$(CAT)_7$	144–194	56	KF872210
	R: GCTGTCTACATCTTACCCTCC				
Ba4	F: TTGTGAACATACATACGTGGGA	$(CAT)_{18}$	166–230	54	KF872211
	R: TGGTTTGATGAAGCAAGCAG				
Ba5	F: GGAGACTACCACCATAGATTG	$(ATA)_7$	202–224	54	KF872212
	R: GATAATGACATCAGATGAGCC				
Ba6	F: ACGACGATCTTTGACTTTCC	$(TTA)_7$	170–208	54	KF872213
	R: CCGATTTCACTGGACCTATT				
Ba7	F: TGTCACATGGTCCCGATAAG	$(TTA)_9$	288–316	55	KF872214
	R: ATGGGTACATCACGGTCTTC				
Ba8	F: ATCAGCACGTTGTTCCTAGT	$(AAT)_7$	240–290	54	KF872215
	R: GTCAGTTTCAGCAACGAATG				
Ba9	F: TTGGAATGGAGGGAGTGAAT	$(AAT)_{10}$	176–290	58	KF872216
	R: AGGTAAGGTCGGGTTGAGAA				
Ba10	F: ATTTAGGTGCGGGATGGACT	$(GAT)_8$	200–270	58	KF872217
	R: ACGGCTGATAACCGAACGAG				
Ba11	F: ACATGATCGTCAAGACCCAA	$(ATT)_{10}$	160–260	56	KF872218
	R: ACAGACCCATTTCCAACCTC				
Ba12	F: TCTGCTCGTGCTCGTTCATA	$(TAA)_7$	202–318	58	KF872219
	R: GCCGTCCTAATGGTTCACTC				
Ba13	F: GTTGGAGTACGGAAACGGCTAA	$(TAT)_{10}$	186–282	60	KF872220
	R: GCATCGCTGCTTCTGGACAA				
Ba14	F: GGAAGAACGTCGCTGAAGGC	$(AAT)_{11}$	238–340	60	KF872221
	R: ACCCGAACCACTCCACCATA				
Ba15	F: TTAAAGGTCATCGTGATGGCGTAA	$(TCT)_7$	224–250	59	KF872222
	R: AAGGCGAGGGCGGAGATAGA				
Ba16	F: TTCTGAAGCTCCATCCATTC	$(TTG)_{10}$	280–352	56	KF872223
	R: GATTCTGACCTCGTACTCGTAG				
Ba17	F: GGGTTTGAATATGAGCAATG	$(AAT)_7$	192–204	54	KF872224
	R: GAAAGAGCCTCTAAAGCAGA				
Ba18	F: ATCGCATCAGATCCATCGTC	$(TAA)_5(TAT)_5$	170–222	60	KF872225
	R: GAAACCTCACCAAATCCTCC				
Ba19	F: AACGGTGGTCAAACTCTTGG	$(ATT)_{33}$	176–254	56	KF872226
	R: CCACCTGGCAGCTATAATCC				
Ba20	F: AATAGGCGGAGGAAGACGTT	$(TGA)_{28}$	158–186	53	KF872227
	R: TCAATTCATTCATTGACCTAATTCT				

Note: T_a = annealing temperature.

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TABLE 2. Results of marker screening in *Bidens alba* and *B. pilosa.*

	B. alba (*N* = 30)			*B. pilosa* (*N* = 30)		
Locus	*A*	H_e	*H′*	*A*	H_e	*H′*
Ba1	18	0.8159	1.9620	1	0.0000	0.0000
Ba2	7	0.7422	1.4501	1	0.0000	0.0000
Ba3	14	0.7904	1.9171	1	0.0000	0.0000
Ba4	32	0.9284	2.8401	8	0.7200	1.4553
Ba5	10	0.7621	1.6140	3	0.5460	0.8604
Ba6	15	0.8475	2.0272	3	0.1772	0.3771
Ba7	16	0.8696	2.1827	4	0.5558	0.9162
Ba8	8	0.7610	1.6188	6	0.6829	1.2395
Ba9	14	0.7654	1.8645	14	0.8380	2.0937
Ba10	18	0.8580	2.1811	6	0.6329	1.1316
Ba11	7	0.7017	1.3557	6	0.7132	1.3045
Ba12	14	0.7463	1.8020	1	0.0000	0.0000
Ba13	12	0.7141	1.5434	3	0.5870	0.9601
Ba14	18	0.7882	1.8878	10	0.8082	1.8309
Ba15	12	0.8207	1.9177	4	0.6036	1.0675
Ba16	11	0.7819	1.6952	7	0.7518	1.5176
Ba17	5	0.7821	1.6957	1	0.0000	0.0000
Ba18	15	0.8021	1.7816	8	0.6326	1.2040
Ba19	18	0.8544	2.1114	9	0.7389	1.5192
Ba20	3	0.3787	0.6796	1	0.0000	0.0000

Note: *A* = number of alleles; H_e = expected heterozygosity; *H′* = Shannon–Wiener diversity index.