Isolation and characterization of polymorphic microsatellite loci from the invasive plant Lantana camara L

ARTICLE *in* CONSERVATION GENETICS RESOURCES · MARCH 2011

Impact Factor: 1.17 · DOI: 10.1007/s12686-011-9501-9

Conservation Genet Resour
DOI 10.1007/s12686-011-9501-9

TECHNICAL NOTE

Isolation and characterization of polymorphic microsatellite loci from the invasive plant *Lantana camara* L.

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Received: 14 July 2011 / Accepted: 26 August 2011

Abstract *Lantana camara* is a highly invasive plant that has established itself in at least 60 different countries across the world. Here, we report development of ten microsatellite markers for this species. These microsatellite loci have 2–15 alleles per locus; with observed and expected heterozygosity of 0.022–0.833 and 0.336–0.848, respectively. These markers will be useful in addressing a variety of questions about *Lantana camara*, including those concerning breeding system, pollination and dispersal, genetic variation and population structure.

Keywords *Lantana camara* · Microsatellites · Invasive species · Ploidy

Lantana camara L. (Verbenaceae) is a highly invasive shrub that is now present in at least 60 countries across the world (Cronk and Fuller 1995). Strictly speaking a species complex, it is recognised as one of the hundred worst invasive species (Lowe et al. 2000). A native of tropical Central and South America it was taken to different parts of Europe in the early sixteenth century and was cultivated and bred as an ornamental (Stirton 1978). *Lantana camara* L. *sensu lato* spread from Europe to different parts of the world perhaps carried by British colonizers (Stirton 1978). In this paper, we report the development of ten microsatellite markers that could be used to address a wide diversity of questions involving its breeding system, genetic variation and structure, and population history and dynamics.

Leaves of *L. camara* were collected from different parts of India (32°21′N, 77°39′E and 10°35′N, 77°46′ E) and stored at −20°C until extraction. Genomic DNA was extracted from all the individuals using DNeasy Plant Mini Kit (Qiagen). The extracted DNA was purified and digested with RsaI and ligated to linker oligonucleotides SNX-F and SNX-R (5′-GTTT AAGGCCTAGCTAGCAGAATC-3′; 5′-GATTCTGCTAG CTAGGCCTTAAACAAAA-3′) using the rapid DNA ligation kit (Fermentas International). These DNA fragments were hybridized to biotinylated oligos where magnetic beads with biotin-labelled $(GT)_{13}$, $(CT)_{13}$, $(AAC)_8$, $(AAG)_8$, $(ACT)_8$ and $(ATC)_8$ oligonucleotides (as microsatellite probes) were used for the enrichment step and captured by using avidin beads (Qiagen). The beads and attached probes were separated magnetically from the supernatant. Following stringent washes, the bound DNA (pure gold) was recovered using Fermentas TA cloning kit. The pure gold DNA was incorporated into a pTZ57R plasmid (NEB) vector. *Escherichia coli* colonies were grown on agar plates prepared with IPTG and X-gal and the true transformants distinguished by their white colour were selected. Transformed colonies were screened by running PCRs with universal M13 primers plus the respective oligonucleotide repeat as a primer (Invitrogen) and positive clones were selected. Positive clones were sequenced in order to characterize the repeat arrays on a MegaBACE 1000. Out of the 289 clones screened, 106 contained microsatellite inserts. From these, we chose 14 unique microsatellites, and

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Published online: 07 September 2011

designed primers for them using the web-based software Primer3 (Rozen and Skaletsky 2000).

To study the polymorphism of these 14 loci, a total of 62 individuals of *L. camara*, sampled from different parts of India were tested. DNA was amplified by running a PCR on a 15 µL reaction consisting of 30 ng of DNA, 1 mM of each dNTP, 1× PCR reaction buffer (Sigma Aldrich, India) containing 1.5 mM $MgCl_2$, 1unit Taq polymerase (Sigma Aldrich, India) and 5 pico moles of primers. Initial PCR conditions (to check for polymorphism) were: 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, annealing temperature for 1 min and 72°C for 1 min. A final elongation step of 72°C for 10 min was added. The PCR products were separated by a 2.5% agarose gel and stained with ethidium bromide. The gels were run at 100 V for about 1.5–2 h, after which the number of alleles was scored. Out of 14 loci screened eight were polymorphic. We also screened 34 microsatellite primers of other two species (*Callicarpa subpubescens* and *Phyla canescens*) of Verbenaceae for cross-amplification (Mori et al. 2008; Fatemi and Gross 2008) in *L. camara*. Only five of these loci amplified well and two (both from *P. canescens*) were polymorphic.

The forward primers for all ten polymorphic loci (eight novel loci and two from other Verbenaceae) were labeled with fluorescent dyes (ABI) and the PCR products were genotyped on an ABI 3100 at the National Centre for Biological Sciences, Bangalore, India with LIZ-500 as size standard (Applied Biosystems) which includes the dyes NED, HEX, FAM and VIC. Output files were analysed with GENEMAPPER version 3.0 (Applied Biosystems). However, later we PCR multiplexed two sets of primers: (1) Lac4, Lac11, Lac12, Lac14; (2) Lac6, Lac13, phc 17 using a Qiagen PCR Multiplex Kit with reaction profile of 95°C for 15 min followed by 35 cycles of 94°C for 1 min, annealing temperature for 1 min, and 72°C for 1 min. Following the multiplex instruction manual, we used a final elongation step of 60°C for 30 min.

Interpreting allelic patterns in *L. camara* is complicated by the fact that ploidy is known to be variable in this species (Sen and Sahni 1955). Since the ploidy of our specimens was unknown, we used allelic patterns as well as a 'dosage effect' to infer ploidy (Landergott et al. 2006). If an individual showed three or more alleles for a locus we considered it tetraploid and genotypes were calculated by examining relative peak heights of the alleles. According to these rules, all individuals were found to be tetraploid. We calculated several measures for genetic diversity, allelic richness, and expected and observed heterozygosity using AUTOTET (Thrall and Young 2000). Here, expected heterozygosity is calculated for three partial (e.g. AAAB, AABB, AABC) and one full heterozygote (ABCD) and observed heterozygosities are calculated considering both

Table 1 Locus, repeat motif, primer sequences, expected product size range, annealing temperature (T_a), allele number (A), expected heterozygosity (H_e) and observed heterozygosity (H_o) for ten microsatellites in *Lantana camara*

Locus	Repeat motif	Primer sequence (5′–3′)		T_a (°C)	A	Allele size range	H_e		H_o	Genbank Accession number
		Left sequence (5′–3′)	Right sequence (5′–3′)				He(Ce)	He(Cd)		
Lac2	(CT)8	CAGAACAGGCGCATCATTTA	TTGAAATGGGTGGGAGAGAG	50	15	84–126	0.805	0.752	0.352	JN246080
Lac4	(AAC)5	CCTACAGCCGAACGACCTAC	TGCTTCCGCCTTTCTGTTAC	58	2	267–270	0.381	0.356	0.5	JN043466
Lac5	(CTT)8	GCTTCGGACACGTATCCACT	GGTTGAGGATTCGGTGAATG	65	2	335–344	0.375	0.350	0.5	JN043467
Lac6	(GA)7	AGGACCAATCATTCCCATCA	AGATCTTGGGCAGGACCTTT	60	6	344–380	0.578	0.54	0.46	JN043468
Lac11	(AG)8	CGGCTAAGTTCCAGCTTTGT	CGTTGTGTGGCCCTATTTTC	58	4	324–369	0.648	0.605	0.642	JN043472
Lac12	(GA)7	TCTCAACTTCGACGATGCAC	GATACCCGCCCATTTTCTTT	58	5	266–345	0.360	0.336	0.022	JN043469
Lac13	(CT)11	TCTTTTCGCCTGAACTTGCT	CAAGCACCCACGTAATGATG	60	12	300–330	0.848	0.792	0.798	JN043470
Lac14	(GT)7	CGCCCCTGTTTTTCTTTAGGT	CCTACCAACTGCCCAAAGCAC	58	12	328–352	0.801	0.748	0.833	JN043471
Phc17	(TC)14	GGAGGCCGTTTCTTTGTTTT	CCAAAGAATATGCTGATCAAAGAG	60	11	208–240	0.587	0.548	0.559	EU286562
Phc18	(ATG)6	TGGTCCTTGATGGCATTTTT	CCCAGAGCGAGCTTAATCAG	54	4	222–232	0.623	0.581	0.046	EU286561

random chromosome (RceS) and chromatid segregation (RcdS) (Bever and Felber 1992) as $H_E(Ce)$ and $H_E(Cd)$, respectively. Over all the ten loci, the number of alleles per locus ranged from 2 to 15, with an average of 7 alleles per locus. Observed heterozygosity over all the loci ranged between 0.022 and 0.833 with a mean of 0.48; expected heterozygosity was 0.336–0.848 with an overall mean of 0.593–0.635 (Table 1).

Invasive species like *L. camara* can be used to address several issues in evolution and ecology including answering questions about the rate of spread across new landscapes; pollination and dispersal distances; and population structure and local adaptation. Answers to many of these questions are also useful in the management and control of invasives. The mircosatellite markers that we have developed will help researchers in addressing these issues.

Acknowledgments We thank the National Centre for Biological Sciences, Bangalore, for financial support; and Uma Ramakrishnan and Samrat Mondal for their advice on the project and comments on this manuscript. AR is supported by a post-doctoral fellowship from the Department of Biotechnology, Government of India.

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