Forest Systems
24(2), eRC04, 4 pages (2015)
eISSN: 2171-9845
http://dx.doi.org/10.5424/fs/2015242-07188
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)

RESOURCE COMMUNICATION

OPEN ACCESS

Characterization of functional SSR markers in *Prosopis alba* and their transferability across *Prosopis* species

María F. Pomponio[1*], Cintia Acuña[2], Vivien Pentreath[3], Diego L. Lauenstein[4], Susana M. Poltri[2] and Susana Torales[1]

[1] *Instituto de Recursos Biológicos (IRB), CIRN, Instituto Nacional de Tecnología Agropecuaria (INTA Castelar), Argentina.* [2] *Instituto de Biotecnología (IB), CICVyA, Instituto Nacional de Tecnología Agropecuaria (INTA Castelar), CC 25, Castelar B1712WAA, Argentina.* [3] *Universidad Nacional de Patagonia San Juan Bosco, Ciudad Universitaria Km 4 Comodoro Rivadavia-Chubut, Argentina.* [4] *Instituto de Fisiología y Recursos Genéticos Vegetales (IFRGV), Instituto Nacional de Tecnología Agropecuaria (INTA), km 5.5 (5119), Córdoba, Argentina*

Abstract

Aim of study: The aim of the study was to characterize functional microsatellite markers in *Prosopis alba* and examine the transferability to species from the *Prosopis* genus.

Area of the study: samples were obtained from natural populations of Argentina.

Material and Methods: Eleven SSR functional markers related to stress and metabolism were amplified in a sample of 152 genotypes from *P. alba*, *P. denudans*, *P. hassleri P. chilensis*, *P. flexuosa*, and interspecific hybrids.

Main results: In *P. alba*, the PIC average value was 0.36; and 6 out of the 11 primers showed high values of polymorphism ranging from 0.40 to 0.71. The cross-species transferability was high with high percentages of polymorphic loci.

Research highlights: The SSR markers developed in *P.alba* were easily transferred to other *Prosopis* species which did not have functional markers.

Keywords: genetic variation; functional markers; microsatellites; prosopis.

Abbreviations: PIC: Polymorphic Information Content; PCR: Polymerase Chain Reaction; SSR: Simple Sequence Repeat.

Citation: Pomponio, M.F., Acuña, C., Pentreath, V., Lauenstein, D.L., Poltri, S.M., Torales, S. (2015). Resource communication: Characterization of functional SSR markers in *Prosopis alba* and their transferability across *Prosopis* species. Forest Systems, Volume 24, Issue 2, eRC04, 4 pages. http://dx.doi.org/10.5424/fs/2015242-07188.

Received: 19 Dec 14. **Accepted**: 02 Mar 2015

Funding: This research was supported by the INTA PNBIO-1131044.

Competing interests: The authors have declared that no competing interests exist.

Correspondence should be addressed to María F. Pomponio: pomponio.florencia@inta.gob.ar

Introduction

Microsatellite markers have been extensively used because they are codominant, highly polymorphic and widespread across the genome. They are a very useful tool for studies on gene flow, demographic patterns and parental assignment. Microsatellites from transcribed regions have some advantages over genomics microsatellites. They have better allele resolution and high transferability among distantly related species because the primers are designed in highly conserved regions of the genome (Varshney *et al*., 2005).

The genus *Prosopis* (Fabaceae) comprises trees species and shrubs found in the Near East, North and Central Africa, North and South America, and the Caribbean. The main centre of diversity for Prosopis genus is located in Argentina with 27 species (Burkart, 1976). The species studied here are distributed in the phytogeographic provinces of the Chaco, Monte, Espinal, and Patagonia (Cabrera, 1976). These species are of economic interest because of their role as animal fodder, timber production, fuel wood and due to their ecological value for contributing to soil stabilization and nitrogen fixation (Pasiecznik *et al.* 2001).

In the *Prosopis* genus, SSR markers have been developed through the construction of enriched genomic microsatellite libraries (Mottura *et al.,* 2005; Alves *et al.,* 2014) and, through high generation sequencing techniques either from genomics (Bessega *et al.,* 2013) and transcriptomics (Torales *et al.,* 2013).

In this study we report the characterization and transferability of 11 microsatellite markers that were

previously developed on *Prosopis alba* to four *Prosopis* species and hybrids. Polymorphism within them as among them is described.

Materials and methods

Genetic variation was characterized in four natural populations of *P. alba* and cross-species amplification was performed in 20 genotypes of four other *Prosopis* species and hybrids (Table 1). Total genomic DNA from leaves was extracted with Qiagen DNeasy Plant Mini Kit (Qiagen, Germany).

Eleven polymorphic SSRs located in functional genes related to stress and metabolism functions previously developed in *P. alba* (Torales *et al*., 2013) were used. The PCR amplifications were carried out as described in Torales *et al*. 2013 and the PCR products were genotyped with the ABI 3130 Genetic Analyzer (Applied Biosystems, USA) and analyzed by the GeneMapper Software (Applied Biosystems).

The orthology of the analyzed microsatellite loci was confirmed by sequencing analysis of amplicons. The PCR products were sequenced and then aligned with the MEGA software v5.2 (Tamura *et al*., 2011). Genetic diversity parameters and the probability of identity (PI) were estimated using GenAlEx 6.5 software (Peakall & Smouse, 2012). Polymorphic Information Content (PIC) was estimated with Microsatellite Toolkit (Park, 2001), and the frequencies of null alleles were estimated with the Gene Pop v. 4.2.2 software (Rousset, 2008).

Results and discussion

Eleven polymorphic loci were characterized in a sample of 52 individuals of *Prosopis alba*. The total number of alleles was 49 and the number of alleles per locus ranged from 2 to 10 with an average of 4.54. The PIC value ranged from 0.09 to 0.71 and the mean of Ho and He was 0.366 and 0.414 respectively. Eight out of 10 loci displayed very low null allele frequencies and 5 of them showed a high discrimination power (PI <0.5). The combined probability for 11 loci all together was 1.4E-05 (Table 2).

Our next step was to establish if the SSR markers could be applied across the *Prosopis* genus and to provide data on polymorphism among related species. For this purpose, we tested the 11 microsatellites in a sample of 20 individual per species. We found 100% of transferability of SSR from *Prosopis alba* to *P. denudans*, *P. hassleri, P. flexuosa, P. chilensis*, and the interspecific hybrids of the two last. Among the amplified loci, 6 loci (54.50%) were polymorphic in *P. denudans*, 7 loci (63.63%) were polymorphic in *P. flexuosa,* 8 loci (72.72%) were polymorphic in *P. chilensis* and hybrids and 10 loci (90.90%) were polymorphic in *P. hassleri*.

Among the species, the He per locus varied between 0.049 and 0.706 and the Ho between 0.050 and 0.722. The average PIC value was 0.44 in *P. denudans*; 0.31 in *P. flexuosa* and hybrids; 0.28 in *P. chilensis* and 0.29 in *P. hassleri* (Table 3).

To date, this is the first report on the transferability of 6 polymorphic SSRs to *P. denudans*. In addition, *P.*

Table 1. Geographic location from the *Prosopis* species

Species (series)	Number	Origin (Provenance)	Lat. (S)	Lon. (W)
P. alba (chilenses)	6	Campo Durán (Salta)	25° 06' 20''	61° 51' 52''
	32	La Unión (Salta)	23° 44' 10''	63° 11' 17''
	7	Isla Cuba (Formosa)	24° 18' 15''	61' 51' 52''
	7	Chañar Bajada (Santiago del Estero)	26° 15' 00''	63° 46' 14''
Total	52			
P. denudans (denudantes)	10	Diadema (Chubut)	45º46'30,7''	67º42'16.5''
	10	Cerro Dragón (Chubut)	45º 43' 35''	68º 23'15.3''
P. hassleri (ruscifoliae)	20	Posta Zalazar (Formosa)	25° 06' 20''	59° 06' 45''
P. flexuosa (chilenses)	20	Southern Chaco Árido	From 30° 30' to 32° 14'	From 64° 30' to 66° 15'
Hybrids	20	Southern Chaco Árido		
P. chilensis (chilenses)	20	Southern Chaco Árido		
Total	100			

Table 2. Microsatellite characterization in *P. alba*

Locus	Na	Amplicon Size	Ho	He	PIC	PI	Fa	Ar
I-P00930b	6	254-260	0.134	0.182	0.16	0.802	0.072	3.78
I-P00930c	2	234-237	0.096	0.092	0.09	0.978	0.950	2
I-P00930d	3	176-182	0.154	0.143	0.14	0.812	0.000	2
I-P03211	5	187-198	0.712	0.693	0.64	0.189	0.008	3.80
I-P03325a	5	270-277	0.442	0.443	0.40	0.428	0.054	3.79
I-P06286b	10	194-211	0.385	0.742	0.71	0.127	0.214	5.20
I-P06639	3	228-232	0.538	0.524	0.41	0.412	0.009	2
I-P07653	2	216-219	0.212	0.299	0.25	0.644	0.277	2
I-P10500	6	262-278	0.569	0.608	0.56	0.250	0.036	4.81
S-P1DKSFA	4	164-171	0.192	0.195	0.19	0.779	0.038	1.96
S-P1EPIV2	4	287-300	0.596	0.558	0.47	0.352	0.021	2.96
Average	4.54	–	0.366	0.414	0.36	1.4E-05*	–	

Na: Number of alleles; Amplicon Size: Allele Size Range in base pairs; Ho: Observed Heterozygosity; He; Expected Heterozygosity; Polymorphic Information Content (PIC); PI: Probability of genotypic identity (* which means combined probability of 11 markers); Fa: Frequencies of null alleles; Ar: allelic richness.

Table 3. Descriptive statistics of the analyzed markers in *Prosopis* species

Species	*P. alba*				*P. flexuosa*				Hybrids				*P. chilensis*				*P. denudans*				*P. hassleri*			
Locus	Na	Ho	He	PIC	Na	Ho	He	PIC	Na	Ho	He	PIC	Na	Ho	He	PIC	Na	Ho	He	PIC	Na	Ho	He	PIC
I-P00930b	6	0.134	0.182	0.16	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0
I-P00930c	2	0.096	0.092	0.09	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	3	0.100	0.096	0.09
I-P00930d	3	0.154	0.143	0.14	2	0.050	0.049	0.05	1	0	0	0	1	0	0	0	2	0.400	0.320	0.27	2	0.050	0.049	0.05
I-P03211	5	0.712	0.693	0.64	2	0.450	0.439	0.34	4	0.650	0.569	0.47	2	0.526	0.388	0.31	4	0.722	0.657	0.60	4	0.600	0.629	0.56
I-P03325a	5	0.442	0.443	0.40	6	0.450	0.553	0.51	5	0.550	0.595	0.54	5	0.500	0.544	0.51	1	0	0	0	6	0.250	0.468	0.43
I-P06286b	10	0.385	0.742	0.71	4	0.150	0.529	0.49	2	0.150	0.219	0.19	2	0.050	0.049	0.05	5	0.500	0.646	0.61	3	0.200	0.184	0.17
I-P06639	3	0.538	0.524	0.41	2	0.250	0.289	0.25	3	0.100	0.395	0.35	3	0.150	0.366	0.33	6	0.474	0.706	0.66	2	0.300	0.495	0.37
I-P07653	2	0.212	0.299	0.25	1	0	0	0	2	0.050	0.049	0.05	2	0.350	0.289	0.25	1	-	0	0	2	0.300	0.255	0.22
I-P10500	6	0.569	0.608	0.56	3	0.200	0.184	0.17	4	0.450	0.441	0.41	4	0.500	0.431	0.39	2	0.150	0.399	0.32	3	0.600	0.499	0.40
S-P1DKSFA	4	0.192	0.195	0.19	1	0	0	0	3	0.050	0.226	0.21	2	0.050	0.049	0.05	1	0	0	0	2	0.050	0.049	0.05
S-P1EPIV2	4	0.596	0.558	0.47	2	0.450	0.439	0.34	2	0	0.255	0.22	2	0.350	0.439	0.34	2	0.250	0.219	0.19	5	0.550	0.605	0.56
Average	4.54	0.366	0.414	0.36	2.27	0.286	0.354	0.31	2.55	0.286	0.344	0.31	2.27	0.310	0.319	0.28	2.36	0.357	0.421	0.44	3	0.300	0.333	0.29

Na: number of alleles; Ho: Observed heterozygosity; He: Expected heterozygosity; PIC: Polymorphic Information Content.

hassleri increased to 15 the SSR available for the analysis of this species (5 of them were previously described in Mottura *et al.,* 2005) and increased also in *P. alba*, *P. chilensis* and *P. flexuosa.*

To confirm the presence of microsatellite regions and their orthology with those regions in *P. alba*, we sequenced and compared the obtained amplicons. The observed polymorphism mainly resulted from variations in repeat number of SSR motif (data not shown), which confirms the conserved nature of coding regions.

As a result, all the markers were transferred to four *Prosopis* species, with few or no available microsatellite markers. This set complements previous studies on development of SSR markers in *Prosopis* spp, and were proposed for conservation genetic analysis, evolutionary relationships and association studies of adaptive traits.

References

Alves FM, Zucci MI, Azevedo-Tozzi AM, Sartori ALB, SOUZA AP, 2014. Characterization of microsatellite markers developed from *Prosopis rubriflora* and *Prosopis ruscifolia* (Leguminosae-Mimosoideae), legume species that are used as models for genetic diversity studies in Chaquenian areas under anthropization in South America. BMC Research Notes 7: 375. http://dx.doi.org/10.1186/1756-0500-7-375

Bessega CF, Pometti CL, Miller JT, Watts R, Saidman BO, Vilardi JC, 2013. New Microsatellite loci for *Prosopis alba* and *P. chilensis* (Fabaceae). Applications in Plant Sciences 5: 1200324. http://dx.doi.org/10.3732/apps.1200324

Burkart A, 1976. A monograph of the genus *Prosopis* (Leguminosae subfam. Mimosoideae). J Arnold Arbor. 57: 219-249.

Cabrera AL, 1976. Regiones Fitogeográficas Argentinas. In Enciclopedia Argentina de Agricultura y Jardinería. Ed. W. F. Kugler. Editorial ACME, Buenos Aires. 85 pp.

Mottura M, Finkeldey R, Verga A, Gailing O, 2005. Development and characterization of microsatellite markers for Prosopis chilensis and P*rosopis flexuosa* and cross-species amplification. Mol Ecol Notes 5: 487–489. http://dx.doi.org/10.1111/j.1471-8286.2005.00965.x

Park SDE, 2001. Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection. Ph.D. thesis. University of Dublin, Ireland. Exel Microsatellite Toolkit.

Pasiecznik N M, Felker P, Harris P, Harsh LN, Cruz G, Tewari JC, Cadoret K, Maldonado L, 2001. The *Prosopis juliflora–Prosopis pallida* complex: a monograph. HDRA, Coventry, UK. 172 pp.

Peakall R, Smouse PE, 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28: 2537-2539. http://dx.doi.org/10.1093/bioinformatics/bts460

Rousset F, 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol. Ecol. Resources 8: 103-106. http://dx.doi.org/10.1111/j.1471-8286.2007.01931.x

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S, 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol Biol Evol 28: 2731-2739. http://dx.doi.org/10.1093/molbev/msr121

Torales S, Rivarola ML, Pomponio MF, González S, Acuña CV, Fernández P, Lauenstein D L, Verga A, Hopp EH, Paniego NB, Marcucci Poltri SN, 2013. De novo assembly and characterization of leaf transcriptome for the development of functional molecular markers of the extremophile multipurpose tree species *Prosopis alba*. BMC Genomics 14: 705. http://dx.doi.org/10.1186/1471-2164-14-705

Varshney RK, Gramer A, Sorrells ME, 2005. Genic microsatellite markers in plants: features and applications. Trends Biotechnol 23: 48-55. http://dx.doi.org/10.1016/j.tibtech.2004.11.005