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

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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.

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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 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
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 altogether 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 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* and hybrids, 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.

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).

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)                 | 45°46’30,7”   | 67°42’16.5”   |
|                          | 10     | Cerro Dragón (Chubut)            | 45°43’ 35”    | 68°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             | From 30° 30’  to 32° 14’ | From 64° 30’ to 66° 15’ |
| *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-P067653  | 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**|    |               | 0.366| 0.414| 0.36 | 1.4E-05* |     |

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

| Species | *P. alba* | *P. flexuosa* | *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 |
| I-P00930b | 6  | 0.134 | 0.382 | 0.16 | 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  | 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 |
| I-P03211 | 5  | 0.712 | 0.691 | 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 |
| I-P03325a | 5  | 0.442 | 0.443 | 0.40 | 6  | 0.450 | 0.535 | 0.51 | 5  | 0.550 | 0.595 | 0.54 | 5  | 0.500 | 0.544 | 0.51 | 4  | 0.526 | 0.388 | 0.31 |
| I-P06286b | 10 | 0.385 | 0.742 | 0.71 | 4  | 0.150 | 0.259 | 0.49 | 2  | 0.150 | 0.219 | 0.19 | 2  | 0.050 | 0.049 | 0.05 | 5  | 0.500 | 0.646 | 0.61 |
| I-P06639  | 3  | 0.538 | 0.524 | 0.41 | 2  | 0.250 | 0.289 | 0.25 | 3  | 0.100 | 0.395 | 0.35 | 3  | 0.100 | 0.366 | 0.33 | 6  | 0.474 | 0.706 | 0.66 |
| 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  |
| 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 |
| 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  |
| S-P1EPIV2 | 4  | 0.596 | 0.558 | 0.47 | 2  | 0.450 | 0.439 | 0.34 | 2  | 0.250 | 0.255 | 0.22 | 2  | 0.350 | 0.439 | 0.34 | 2  | 0.250 | 0.219 | 0.19 |
| **Average** |    | 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 |

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