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Resource Communication. Identification of single nucleotide polymorphisms (SNPs) at candidate genes involved in abiotic stress in two *Prosopis* species and hybrids

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Abstract

Aim of the study: Identify and compare SNPs on candidate genes related to abiotic stress in *Prosopis chilensis*, *Prosopis flexuosa* and interspecific hybrids

Area of the study: Chaco árido, Argentina.

Material and Methods: Fragments from 6 candidate genes were sequenced in 60 genotypes. DNA polymorphisms were analyzed.

Main Results: The analysis revealed that the hybrids had the highest rate of polymorphism, followed by *P. flexuosa* and *P. chilensis*, the values found are comparable to other forest tree species.

Research highlights: This approach will help to study genetic diversity variation on natural populations for assessing the effects of environmental changes.

Key words: SNPs; abiotic stress; interspecific variation; molecular markers.

Introduction

Single Nucleotide Polymorphism (SNP) is the substitution of only one nucleobase in DNA fragments homologous. In recent years, the discovery SNP candidate-gene resequencing has been a major focus in tree genomics. The SNPs frequency in trees is high, generally in the range of 1 SNP per 100 bp among the species that have been surveyed (Neale & Kremer, 2011).

Prosopis chilensis and *Prosopis flexuosa* are two hardwood arboreal species which grow in sympatry in the Argentinean arid Chaco region. Genetic diversity was studied in the *Prosopis* species using isozyme markers (eg. Saidman *et al*., 1998); Random Amplified Polymorphic DNAs (RAPDs) (*eg.* Elmeer & Almalki, 2011) and Simple Sequence Repeats (SSRs) (*eg.* Mottura, 2006). Frequency of SNPs markers based on ESTs has been reported in *P. juliflora* (*eg.* Sablok & Shekhawat, 2008), but there have not been carried out studies of genetic diversity in *Prosopis* species with these markers yet.

The objective of the present study was to identify and to compare levels of nucleotide polymorphism of SNPs based on five drought and salinity adaptation related candidate genes in two *Prosopis* species and their hybrids.

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This work has one Supplementary Table that does not appear in the printed article but that accompany the paper online

Abbreviations used: NCBI: National Center for Biotechnology Information; PCR: Polymerase chain reaction; SNPs: Single Nucleotide Polymorphisms; RAPDs: Random Amplified Polymorphic DNAs; SSR: Simple Sequence Repeats.

Table 1. Candidate genes selected, PCR primers and length of regions analyzed

Gene name	Accession number of *P. juliflora* sequences for primer design/Primers from bibliography	Primer sequence (5'-3')	Size of fragment (bp)	Exon/intron distribution (bp)	Percent of coding regions (%)
Early responsive to dehydration (*ERD 15*)	DW360105	F:CTGGTTTGAACCCAAATGCT R:ATGCCCAGATCCAACATCTC	214	Exon: 214	100%
Hak3p	DW360037	F:CCACATGTCAGAGCTGAGGA R:ATGCTGAAGTCCCCAACAAC	240	Exon: 240	100%
sHSP	DW359918	F:CTGCCGATAAGTCCGTCAAT R:GTCCTCCACCTGAACCTTGA	160	Exon: 160	100%
PHD finger	DW359867	F:GACGTGAAATGGAGGCTTCT R:CTCTTTTCTCCGCACAATCC	600	Exon :81, Intron: 519	13.5%
Rab 7 GTP	EF591762	F:CGGCGAATGTTATTGAAGGT R:TCCCAAATCTGCAATGTGAA	256	Intron: 155, Exon: 101	39%
Alcohol dehydrogenase (ADH I)	Sang *et al*., 1997	F: CCTCGCATATTTGGTCACGAAG R: GGGCACACCAACAAGTACTG	260	Intron: 158, Exon:102	39%
Alcohol dehydrogenase (*ADH II*)	Fukuda *et al*., 2005	F:ATATTTGGTCAYGAAGCTGG R: CCCTTRAGMGTCCTCTCATTC	377	Intron: 41, Exon: 336	89%
Total			2,107	Intron: 873, Exon: 1,234	58%

Materials and methods

Genomic DNA from young leaves from a total of 60 genotypes, 20 from each species (*P. chilensis* and *P. flexuosa*) and 20 hybrids from the southern area of arid Chaco region of Argentina, was extracted using the DNeasyPlant Mini Kit (QIAGEN).

All candidate genes have been chosen based on literature and also on information from ESTs sequences of *Prosopis juliflora* (Suja *et al*., 2007) available in the National Center for Biotechnology Information (NCBI) database.

We analyzed fragments of *ADH* gene, a glycolytic enzyme (Fukuda *et al*., 2005) and fragments of candidate genes induced in response to dehydration: *ERD 15*, a transcription factor which is a negative regulator of the abscisic acid (ABA) and whose overexpression in *Arabidopsis thaliana* reduces ABA sensitivity and decrease the drought tolerance (Kariola *et al*., 2006); *PHD finger*, a transcription factor associated with chromatin remodelling complex that improve stress tolerance in transgenic *Arabidopsis* plants (Wei *et al*., 2009) and a cytosolic class II low molecular weight heat shock protein (*sHSP*) that shows a tissue-specific expression of sunflower heat shock proteins in response to water stress (Almoguerra *et al*., 1993). Also we analyzed two fragments of candidate genes involved in salt stress tolerance: *Rab 7 GTP*, a small GTP-binding protein whose overexpression on transgenic tobacco confers salt stress tolerance (George & Parida, 2011) and *Hak3p*, a potassium transporter whose expression is regulated in response to salinity stress in common ice plants (Su *et al*., 2002).

We designed all primers using Primer 3 software (Rozen and Skaletsky, 2000), except for *ADH* that were obtained from Sang *et al*. (1997) and Fukuda *et al*. (2005). Information on genes, primers and accession numbers is summarized in Table 1.

Polymerase chain reactions (PCR) were performed in a final volume of 20 µl containing 20 ng of template DNA, 0.2 mM each dNTP, 0.5 µM of each primer, 1 × PCR buffer, 1.5 mM MgCl2, and 0.25 U Platinum *Taq*DNA polymerase (Invitrogen), under the following conditions: 94°C for 2 min, 33 cycles of 92°C for 50 s, 55°C for 50 s and 72°C for 1 min, with a final extension of 15 min at 72°C. Amplified products were examined by 1.0% agarose gel electrophoresis and then purified using a Macherey-Nagel "PCR clean-up Gel extraction" (NucleoSpin Extract II) Kit.

Purified samples were sequenced from both ends (F and R) on an ABI PRISM 3100 genetic analyzer. The DNA sequence chromatogram was edited with FinchTV

Table 2. SNPs, Nucleotide and haplotype diversity

Gene	*P. chilensis*							*P. Flexuosa*							*Hybrids*						
	N	SNPs			H	Hd	π	N	SNPs			H	Hd	π	N	SNPs			H	Hd	π
		Tot (Av.)	E	I					Tot (Av.)	E	I					Tot (Av.)	E	I			
sHSP	40	1	1	—	2	0.512	0.0039	38	1	1	—	2	0.102	0.0006	40	1	1	—	2	0.097	0.0009
ERD 15	36	4	4	—	6	0.617	0.0052	32	5	5	—	6	0.697	0.0097	34	6	6	—	10	0.743	0.011
Hak3P	40	3	3	—	4	0.529	0.0025	36	4	4	—	5	0.346	0.0018	38	5	5	—	5	0.724	0.0046
PHD finger	36	4	0	4	4	0.424	0.0009	28	7	—	7	7	0.807	0.0026	32	11	—	11	7	0.825	0.0046
Rab 7 GTP	28	7	1	6	7	0.825	0.0124	32	12	4	8	7	0.694	0.015	38	10	6	4	6	0.637	0.0124
ADH I	34	—	—	—	0	—	—	38	8	1	7	7	0.842	0.0102	40	9	—	9	4	0.585	0.0085
ADH II	30	6	5	1	2	0.497	0.0079	30	4	2	2	7	0.8	0.0039	28	9	7	2	5	0.762	0.0096
Total	244	25 (84.28)	14 (88.14)	11 (79.36)	25	0.486	0.0046	234	41 (51.39)	17 (72.58)	24 (36.37)	41	0.612	0.0062	250	51 (41.31)	25 (49.36)	26 (33.57)	39	0.624	0.0073

References: N = number of samples, H = Number of haplotypes, Hd = haplotype diversity, Av = average frequency, E = exon regions, I = intron regions, π = nucleotide diversity.

software (version 1.4.0) and aligned with BioEdit software.

Haplotype reconstruction and the estimation of polymorphism was performed using DnaSP software v 5.0 (Librado & Rozas, 2009). The identity of the sequences was confirmed through BLASTN homology searches and the assignment of coding and non-coding regions were defined by aligning genomic sequences against a reference mRNA sequence.

Results and discussion

We amplified 2,107 bp from partial sequences of 6 candidate genes on P. *chilensis*, *P. flexuosa* and hybrids, consisting 1,234 bp of coding and 873 bp of non coding regions (Table 2).

The average SNP frequency was one per 84 bp in *P. chilensis*, one per 51bp in *P. flexuosa* and one per 41bp in (Table 2). In comparison with *Prosopis juliflora,* the SNP frequency from ESTs library 1.60 SNPs / 100 bp (Sablok & Shekhawat, 2008), result intermediate between *P. flexuosa* and hybrids. Similar to previous studies, the SNP frequency found in *Prosopis* species, was lower in coding regions than in non-coding regions, being mostly synonymous rather than non-synonymous (see supplementary material).

The difference in the abundance of SNPs between species and hybrids was also reflected by different average values of π and Watterson´s θw, being the average over all five gene higher in hybrids (0.0073), and *P. flexuosa* (0.0062), and lower in *P. chilensis* (0.0046) (Table 2).

In the same way, the mean nucleotide diversity is comparable to the nucleotide diversity found in similar studies in forest trees species ie. *Populus* (Ismail *et al*., 2012), *Pinus* (González Martinez *et al*., 2006; Wachowiak *et al*., 2011) and *Quercus* (Derory *et al.,* 2012).

The number of haplotypes ranged from two to seven across all the loci.

Pairwise Fst revealed the highest differentiation occurs between *P. flexuosa* and *P. chilensis* (Fst = 0.461), than between species and hybrids, whereas hybrids seem to be more similar to *Prosopis chilensis* (Fst = 0.180) than *P. Flexuosa* (Fst = 0.221).These results agree with an earlier work of Mottura (2006) with SSR markers.

These SNP markers identified in candidate genes can be use to estimate genetic diversity related to adaptive variation of natural populations, as complement to other neutral molecular markers, or future investigation for association mapping for identification of genomic regions that underlying phenotypic traits like drought stress response.

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