FITOGENÉTICOS

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GENETIC DIVERSITY OF ALGARROBO (*Prosopis chilensis* Mol. Stuntz) IN COQUIMBO'S REGION, CHILE.

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ABSTRACT

Prosopis (Mimosoideae, Leguminosae) genus constitutes an important genetic resource for arid and semiarid environments. In Chile, *Prosopis chilensis* (Mol) Stuntz, (algarrobo) is the species with the widest distribution range, displaying a high phenotypic variability. Moreover, in Chile the species conforms a periferical metapopulation biogeographically isolated caused by the Andes. The aim of this study was to analyze its genetic diversity in Coquimbo's Region using six nuclear and eight chloroplastic microsatellite markers. A dendrogram of genetic diversity with nuclear loci displayed 143 genotypes. For chloroplastic analysis, one locus showed five haplotypes. Results suggest no difference between Elqui and Limari and slight but not significant difference with Choapa. This should be corroborated with further studies to the south of its distribution range.

INTRODUCTION

The genus *Prosopis* (Mimosoideae, Leguminosae) constitutes an important genetic resource for arid and semiarid environments. This genus is composed by 44 species of shrubs and trees described by Burkart (1976) whose native distribution range includes three continents, with 40 *Prosopis* species found solely In America (Pasiecznik *et al*., 2001). Trees are principally harvested for pods, fuel or timber and considered useful both for production and for environmental conservation initiatives. *Prosopis* species are included in continuous propagation planning by national forestry departments and international organizations (Pasiecznik *et al*., 2001). In Chile, six native species of the genus are found, being *Prosopis chilensis* (Mol) Stuntz, (algarrobo) the species with the widest distribution range, displaying a high phenotypic variability. Moreover, in Chile the species conforms a periferical metapopulation biogeographically isolated caused by the presence of the Andes.

Coquimbo´s Region (29°-31°S), displays three transversal valleys presenting *P.chilensis* populations which can be considered geographically isolated and with an increasing pluviometry to the south. These characteristics could facilitate genetic divergence of the species in the Region. The aim of this study was to analyze the genetic diversity of *P. chilensis* in this Region using nuclear and chloroplastic microsatellite markers

MATERIAL AND METHOD

Study sites of populations of *P.chilensis* were selected from each valley of Coquimbo's Region: Elqui, Limarí and Choapa. At least 30 individuals were collected from two subpopulations on each valley plus some others individuals, conforming an initial set of 192 samples. Outgroups of *P. flexuosa*, *P. alba* and *P. tamarugo* were also included. DNA was extracted from young expanding leaves according to Lodhi *et al*. (1994).

Nuclear microsatellite analysis was based on six loci, Mo05, Mo07, Mo08, Mo09, Mo13 and Mo16 described for *P. chilensis* by Mottura *et al*. (2005). PCR reactions were performed according to Mottura *et al*. (2005). Initial known allele's size were established using four external *P. chilensis* samples analyzed by capillary electrophoresis with an ABI-310 automated sequencer equipped with GeneMapper ID v3.2 software (Applied Biosystems). The alleles genotyped by capillary electrophoresis were used as standar alleles. The samples of the study were principally analyzed by denaturing 6% polyacrylamide (PAA) sequencing gel electrophoresis revealed by silver staining after PCR amplifications performed without fluorophores labeled primers. At least two independent SSR reactions were performed for each individual and recurrent capillary electrophoresis were performed with samples displaying unknown alleles.

Chloroplastic microsatellite analysis comprised eight cloroplastic microsatellite loci, six of them described as polymorphic for Tobbaco: ccmp2, ccmp3 and ccmp5 (Weising and Gardner, 1999) and ccSSR9, ccSSR10 and ccSSR21 (Chung and Staub, 2003) plus two polymorphic loci described for the leguminous soybean, RP19 and SOYCP (Powell *et al.*, 1995). PCR reactions were performed as described by the respective authors. Each locus was analyzed by denaturing 6% polyacrylamide sequencing gel electrophoresis revealed by silver staining. In the case of polymorphism detection, samples were grouped and representative samples of each haplotype were analyzed by capillary electroforesis on an ABI-310 sequencer as described, using an HEX fluorophore labeled primer in the PCR reaction.

Population genetics calculations were done with Genetix program (www.univ-montp2.fr/~genetix/genetix/genetix.htm). Genetic diversity between the individuals was analyzed using DICE coefficient implemented by the SimQual procedure of NTSYSpc V. 2.0 program (Rohlf, 1997). A dendrogram was constructed from a binary matrix 0/1 (absence/presence of the allele) using unweighted pair group method average (UPGMA) clustering (Sneath and Sokal, 1973).

RESULT AND DISCUSSION

The final analysis was done with 184 samples. In the case of nuclear loci, Mo07 and Mo16 were discarded. Between the 4 remaining loci, 28 alleles were found and their heterocigosity indexes were calculated. A dendrogram of the genetic diversity displayed 143 genotypes and scarce identity groups, with no significant relation between the genetic diversity and the origin of the samples, what will be confirmed by AMOVA analysis. For the eight chloroplastic loci assayed, only locus ccSSR10 presented polymorphic bands, displaying five haplotypes between the populations. These results indicate that there would be no difference in the genetic diversity between samples from Elqui and Limari and slight but not significant difference with Choapa samples, what should be corroborated with a genetic diversity analysis with samples collected to the south of its distribution range, Aconcagua and Maipo valley.

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