



# MICROSATELLITES REVEAL A HIGH GENETIC DIFFERENTIATION AMONG NATIVE *GEOFFROEA DECORTICANS* POPULATIONS IN CHILEAN ATACAMA DESERT

LOS MICROSATÉLITES REVELAN UNA ALTA DIFERENCIACIÓN GENÉTICA ENTRE POBLACIONES NATIVAS DE *GEOFFROEA DECORTICANS* DEL DESIERTO DE ATACAMA CHILENO

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## SUMMARY

**Background and aims:** The extreme conditions in the Chilean Atacama Desert are a major hurdle for the survival of any organism. Despite this, several legume populations of *Geoffroea decorticans* have thrived within this harsh, hostile environment for centuries. Here, we sought to determine the genetic variability in *G. decorticans* populations, given its wide distribution across the Atacama Desert, the driest and most ancient desert on Earth. The specific aims of the present study were to determine the level of genetic diversity and assess genetic structure in eight populations of *G. decorticans* from Chilean Atacama Desert.

**M&M:** Eighty-four *G. decorticans* individuals were selected for sampling across eight localities in Northern Chile. Five microsatellites were used to analyze genetic diversity, differentiation among populations, population structure and gene flow.

**Results:** The majority of the analyzed populations from the Atacama Desert displayed a high genetic diversity, with the exception of Pachica population. *G. decorticans* (chañar) populations also displayed a high genetic differentiation and a moderate gene flow given by the natural barrier imposed by the Atacama Desert. The eight chañar populations studied were separated in groups from Northern and Southern regions.

**Conclusions:** Microsatellites have provided valuable baseline information to understand the genetic diversity and structure of *G. decorticans* populations at the Atacama Desert.

## KEY WORDS

Gene flow, genetic structure, genetic variability, SSR markers.

## RESUMEN

**Introducción y objetivos:** Las condiciones extremas en el Desierto de Atacama de Chile son un obstáculo importante para la supervivencia de cualquier organismo. A pesar de esto, varias poblaciones de leguminosas de *Geoffroea decorticans* han prosperado en este entorno hostil y severo durante siglos. Aquí, tratamos de determinar la variabilidad genética en poblaciones de *G. decorticans*, dada su amplia distribución a través del Desierto de Atacama, el desierto más seco y antiguo de la Tierra. Los objetivos específicos del presente estudio fueron determinar el nivel de diversidad genética y evaluar la estructura genética en ocho poblaciones de *G. decorticans* del Desierto de Atacama chileno.

**M&M:** Ochenta y cuatro individuos *G. decorticans* fueron seleccionados para muestreo en ocho localidades en el norte de Chile. Se utilizaron cinco microsatélites para analizar la diversidad genética, la diferenciación entre poblaciones, la estructura de la población y el flujo de genes.

**Resultados:** La mayoría de las poblaciones analizadas del Desierto de Atacama mostraron una alta diversidad genética, con la excepción de población de Pachica. Las poblaciones de *G. decorticans* (chañar) también mostraron una alta diferenciación genética y un flujo genético moderado dado por la barrera natural impuesta por el Desierto de Atacama. Las ocho poblaciones de chañar estudiadas se separaron en grupos de las regiones norte y sur.

**Conclusiones:** Los microsatélites han proporcionado información valiosa de referencia para comprender la diversidad genética y la estructura de las poblaciones de *G. decorticans* en el Desierto de Atacama.

## PALABRAS CLAVE

Estructura genética, flujo de genes, marcadores SSR.

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## INTRODUCTION

The Atacama Desert in Northern Chile is probably the driest and the oldest desert on Earth. This is demonstrated by geological and soil mineralogical evidence (Hartley *et al.*, 2005; Clarke, 2006) and its extreme environmental conditions that include: extremely low relative humidity, high soil salt concentrations, low year average rainfall and high UV radiation (Azua-Bustos *et al.*, 2012). Despite these harsh conditions, seven Fabaceae species survive in this environment, namely *Prosopis tamarugo* Phil, *Prosopis chilensis* (Molina) Stuntz emend. Burkart, *Prosopis alba* Griseb, *Prosopis flexuosa* DC, *Prosopis burkartii* Muñoz, *Prosopis strombulifera* (Lam.) Benth and *Geoffroea decorticans* Burkart (Burkart, 1976; Calderon *et al.*, 2015; Carevic *et al.*, 2015; McRostie *et al.*, 2017; Garrido *et al.*, 2018; Contreras *et al.*, 2018). Indeed, *G. decorticans*, locally known as “chañar”, has a wide geographic distribution across the Atacama Desert. The chañar inhabit grouped in small groves and discontinuously along of the Atacama Desert, specifically in valleys, gulches, oasis and close to human populations; generally where there are subterranean water and fog (Contreras *et al.*, 2018). However, the dispersion of chañar seeds via avi-fauna species or by South American camelids (*Lama guanicoe*, *Vicugna vicugna*) has not been reported. Forest genetic resources in Chile’s arid zones are fragmented and reduced in extent as a result of indiscriminate extraction and unsustainable use (Gacitúa & Villalobos, 2012). This species can be found in northern Chile, from 18°29’S to 30°2’S of latitude, and in the 3-3,000 meters above sea levels (masl) range (Contreras *et al.*, 2018). Despite this, genetic studies on arid zones’ forests and resources including *G. decorticans* are scarce and scattered (Calderon *et al.*, 2015; Contreras *et al.*, 2018; Garrido *et al.*, 2018). Chañar reproduces by seeds or by gemiferous roots (Giménez *et al.*, 2013). The lateral buds, buds of basal stem and gemiferous roots have high capacity of regrowth when the tree suffering damages by cuts or burn (Giménez *et al.*, 2013). *Geoffroea decorticans* is facultatively xenogamous, therefore pollination occurs through the participation of pollinators or without them (Eynard & Galetto, 2002). A study by Squeo *et al.* (2008) indicates that Atacama’s *G. decorticans* has been classified as “Vulnerable” in terms of

conservation. Considered a valuable crop and well adapted to the dry conditions in Northern Chile *G. decorticans* is used for multiple applications. A Chañar fruit’s aqueous extract has demonstrated analgesic, antitussive and expectorant properties (Reynoso *et al.*, 2016), as well as antioxidant properties (bioactive polyphenols) against diseases associated with oxidative stress, inflammation and the metabolic syndrome (Costagama *et al.*, 2016; Jiménez-Aspee *et al.*, 2017).

Microsatellites, or simple sequence repeat (SSR) polymorphisms, are widely used molecular markers to study genetic diversity and structure. Previous studies have defined Fabaceae tree population’s genetics using microsatellite analyses (Andrianoelina *et al.*, 2009; Caetano *et al.*, 2012; Collevatti *et al.*, 2013), allowing precise level heterozygosity at a given locus. SSR is a powerful molecular tool due given its high reproducibility, a high level of polymorphism, codominance and random distribution across the genome (Zane *et al.*, 2002; Sahu *et al.*, 2012). A previous study by Naciri-Graven *et al.* (2005) described a total of eleven microsatellite markers in *Geoffroea spinosa* Jacq. *Geoffroea spinosa* is a species closely related to *G. decorticans* (Lamarque *et al.*, 2009). Hence, we used transferability of SSR markers from *G. spinosa* to *G. decorticans* to assess genetic variability. The specific aims of the present study are to determinate the level of genetic diversity and assess genetic structure in eight populations of *G. decorticans* from Chilean Atacama Desert. We expect that *G. decorticans* populations present high differentiation and low genetic diversity due to the influence by the Atacama Desert.

## MATERIAL AND METHODS

### *Plant material*

Eighty-four individuals of *Geoffroea decorticans* were selected for sampling across eight localities in Northern Chile: Azapa (AZA), Chaca (CHA), Pachica (PACH), San Pedro de Atacama (SP), Calama (CALA), Copiapó (COP), Alto del Carmen (AC) and Valle del Elqui (VIC). Sampling was conducted in March 2016 and young leaves from the season were collected. For different alogamous forest species or mixed mating system, Sebbenn (2002) suggests sampling between 30 to 50 trees

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per population for studies of genetic diversity and structure. However, our study reduced this number to 5-17 chañar individuals per site given their small numbers. Randomly selected trees were sampled at a ~30 meter distance in order to avoid kinship relationship between trees; according to studies performance by Gapare *et al.* (2005) in the *Picea sitchensis* (Bong) Carr. species. Fifty grams of young leaves were collected from each specimen and placed in polyethylene bags, and immediately transported at 4 °C and subsequently stored at -80 °C for analyses. GPS coordinates of each specimen collected are listed in Table 1.

### DNA extraction

Genomic DNA was extracted using a method described by Contreras *et al.* (2018) with modifications. For cell lysis, 100 mg of fine powder of young leaves, 14 µl of beta-mercaptoethanol, 14 µl of 10 mg/ml Proteinase K, 14 µl of 5% Sarkosyl, 0,045 g D-sorbitol (MW 182.17 g/mol) and 700 µl of CTAB preheated to 65°C for 15 min (4% p/v PVP-40, 100 mM Tris-HCl pH 8; 1,2 M NaCl; 20 mM EDTA, 2% CTAB) were added to each tube. They were stirred with a vortex and then incubated in a water bath for 60 min at 65°C, inverting the tubes every 15

**Table 1.** Geographical data of the 84 *Geoffroea decorticans* individuals studied.

N°	Name	Latitude	Longitude	N°	Name	Latitude	Longitude
1	AZA1	18°29'34.9"S	70°16'42.7"W	43	SP7	22°57'13.7"S	68°13'52.2"W
2	AZA2	18°29'34.7"S	70°16'43.2"W	44	SP8	22°57'15.3"S	68°13'48"W
3	AZA3	18°29'34.7"S	70°16'43.3"W	45	SP9	22°57'17.6"S	68°13'50.8"W
4	AZA4	18°31'1.4"S	70°10'53.9"W	46	SP10	22°57'15.1"S	68°13'47.9"W
5	AZA5	18°30'54.2"S	70°11'22"W	47	CALA1	22°27'51.4"S	68°54'37.4"W
6	AZA6	18°30'7.9"S	70°14'56.1"W	48	CALA2	22°27'51.3"S	68°54'37.9"W
7	AZA7	18°30'54.2"S	70°11'22.1"W	49	CALA3	22°27'52.6"S	68°54'38"W
8	AZA8	18°30'54"S	70°11'22.4"W	50	CALA4	22°27'51.2"S	68°54'37.5"W
9	AZA9	18°30'20.6"S	70°12'59.9"W	51	CALA5	22°27'51.1"S	68°54'37"W
10	AZA10	18°30'20.9"S	70°12'58.6"W	52	COP1	27°22'47.7"S	70°19'5.2"W
11	AZA11	18°30'3.1"S	70°15'5.9"W	53	COP2	27°21'20.1"S	70°21'10.1"W
12	AZA12	18°30'2.3"S	70°15'5.7"W	54	COP3	27°20'57.1"S	70°21'22.9"W
13	AZA13	18°29'52.9"S	70°15'51.6"W	55	COP4	27°20'13.4"S	70°35'47.2"W
14	CHA1	18°48'9.7"S	70°10'13.2"W	56	COP5	27°20'13"S	70°35'48.2"W
15	CHA2	18°48'9.8"S	70°10'13.2"W	57	COP6	27°20'12.5"S	70°35'46.8"W
16	CHA3	18°48'9.3"S	70°10'13.8"W	58	COP7	27°20'12.3"S	70°35'46.6"W
17	CHA4	18°48'10.1"S	70°10'13.1"W	59	COP8	27°21'49"S	70°19'42.7"W
18	CHA5	18°48'10.1"S	70°10'13.1"W	60	COP9	27°20'12.2"S	70°35'47.2"W
19	CHA6	18°48'8.3"S	70°10'14.6"W	61	COP10	27°52'44.6"S	70°2'38.3"W
20	PACH1	19°51'51.7"S	69°24'28.7"W	62	COP11	27°20'46.8"S	70°21'36.3"W
21	PACH2	19°51'50.8"S	69°24'35.7"W	63	COP12	27°26'38.9"S	70°16'1.1"W
22	PACH3	19°51'50.2"S	69°24'36.7"W	64	COP13	27°24'11.4"S	70°17'50.1"W
23	PACH4	19°51'47.8"S	69°24'38.9"W	65	COP14	27°26'40.8"S	70°16'0.9"W
24	PACH5	19°51'46.6"S	69°24'38.8"W	66	COP15	27°25'21.3"S	70°16'14"W
25	PACH6	19°51'46.3"S	69°24'38.3"W	67	COP16	27°27'45.1"S	70°16'0.7"W
26	PACH7	19°51'51.7"S	69°24'28.3"W	68	COP17	27°20'39.3"S	70°21'46"W

Table 1. Continuation.

N°	Name	Latitude	Longitude	N°	Name	Latitude	Longitude
27	PACH8	19°51'51.4"S	69°24'29.4"W	69	AC1	28°46'38"S	70°28'21.2"W
28	PACH9	19°51'50"S	69°24'31"W	70	AC2	28°46'38.3"S	70°28'22.4"W
29	PACH10	19°51'49.1"S	69°24'31.7"W	71	AC3	28°46'40.7"S	70°28'21.2"W
30	PACH11	19°51'49"S	69°24'32"W	72	AC4	28°46'41.5"S	70°28'21.3"W
31	PACH12	19°51'48.8"S	69°24'38.5"W	73	AC5	28°46'42.5"S	70°8'17.5"W
32	PACH13	19°51'47.8"S	69°24'38.8"W	74	AC6	28°46'39.2"S	70°28'22.1"W
33	PACH14	19°51'47.7"S	69°24'39"W	75	VIC1	29°58'31.9"S	70°58'33.1"W
34	PACH15	19°51'46.6"S	69°24'39"W	76	VIC2	29°58'31.8"S	70°58'33"W
35	PACH16	19°51'46.3"S	69°24'38.8"W	77	VIC3	29°58'31.7"S	70°58'32.9"W
36	PACH17	19°51'6.3"S	69°24'37.9"W	78	VIC4	30°2'22.5"S	70°41'52"W
37	SP1	22°57'17.9"S	68°13'49.6"W	79	VIC5	30°2'38.5"S	70°42'52.6"W
38	SP2	22°57'17.5"S	68°13'50.8"W	80	VIC6	30°2'38.5"S	70°42'55.4"W
39	SP3	22°57'15.5"S	68°13'47.9"W	81	VIC7	30°2'38.7"S	70°42'52.9"W
40	SP4	22°57'15.1"S	68°13'46.9"W	82	VIC8	30°2'29.9"S	70°43'21.5"W
41	SP5	22°57'14.8"S	68°13'46.9"W	83	VIC9	30°2'22.9"S	70°41'53"W
42	SP6	22°57'14.8"S	68°13'47.1"W	84	VIC10	30°2'29.6"S	70°43'21.2"W

**Note:** All DNA samples are deposited in the CRIDESAT laboratory of the Universidad de Atacama.

min. The tubes were then centrifuged at 14,000 rpm for 15 min at 4°C, and the top aqueous phase was taken for further processing (~700 µl) in a new tube. Subsequently, 800 µl of phenol/chloroform/ UltraPure™ isoamyl alcohol (25:24:1) solution were added to each tube and mixed for 10 min at 120 rpm in a shaker-incubator at 20°C. These tubes were then centrifuged at 14,000 rpm for 15 min at 4°C and the top phase was recovered for further processing (~550 µl), trying not to perturb the lower fraction of the tube. This supernatant was transferred to a new tube. Next, 8 µL of 10 m/ml RNase were added to every tube, followed by incubation at 55°C for 8 min. Two thirds of isopropanol at -20°C were added to the tubes (367 µL of isopropanol for 550 µl of solution in this case), which were then inverted 30 times at room temperature to promote mixing. The liquid was then transferred to a Hi-Bind DNA mini column (Omega Bio-tek) attached to a 2 ml collection tube and left incubating for 2 min at room temperature. The mini columns were centrifuged at 14000 rpm for 2 min and all the precipitate was discarded. They were then washed once with 700 µL of 90%

ethanol at room temperature, centrifuged at 14000 rpm for 2 min and the precipitate was discarded. This was followed by addition of 700 µl of 70% ethanol with 10 mM NH<sub>4</sub>OAc at room temperature, centrifugation at 14,000 rpm for 2 min and discard of the precipitate. The empty mini columns were centrifuged at 14,000 rpm for 2 min to remove the remaining ethanol and the collection tube was replaced by a new 1.5 ml tube. Then, 60 µl of TE preheated to 65°C were added to each mini column, followed by incubation at 65°C for 5 min. Finally, the tubes were centrifuged at 14,000 rpm for 2 min, the mini column was discarded and the 1.5 ml tube with the extract was stored at -20°C for further use. Quality and total DNA concentration were verified by Colibri Microvolume Spectrophotometer (Titertek Berthold, Germany) at 260, 280, and 230 nm. Genomic DNA integrity was checked in a 0.7% agarose gel.

#### SSR Amplification

Amplification of SSRs were performed using the primer pairs listed in Table 2 as previously described by Naciri-Graven *et al.* (2005). In order

Table 2. Characteristics of SSR primer pairs and PCR amplification studied in *Geoffroea decorticans* individuals.

Locus (*) (accession)	DNA sequence (5' - 3') and fluorophore (FAM, HEX)	Motif	Ta (°C)	PCR amplification in <i>G. decorticans</i> DNA	Design of new primer pairs: DNA sequence (5' - 3') and fluorophore (FAM, HEX)	Ta (°C)	PCR amplification in <i>G. decorticans</i> DNA
Gsp.G226 AY644746	F: 5'AATCCAAATGTTGGTCTCG3' R: 5'CTGACTAATCCTTCACAACC3'	(TC) <sub>22</sub>	60	NO	F:5'-GCTGCCCTTGATTTTCGATGCAAAATGCT-3' R:5'-GCCCTTCCCTAAACCTCACTGCC-3'	54	Yes
Gsp.F119 AY644745	F:5'-GGCTAAATCTGGCTCACCTTG-3' R:5'-GATGATGAATTTGGGTCTTCC-3'	(CA) <sub>10</sub>	55	Yes	-	-	-
Gsp.A149 AY644737	F:5'-GAGTGGAGTCACAGAAAAACAG-3' R:5'-CAAATCTTGAAACTCGGAACC-3'	(GA) <sub>15</sub>	55	Yes	-	-	-
Gsp.B284 AY644740	F: 5'AGCCCATCTTGGGGATGAG3' R: 5'TCGTTCCTCAAGGCTCTGATACTG3'	(TC) <sub>8</sub> (TATG) <sub>2</sub>	55	NO	F:5'-AGCCCATCTTGGGGATGAGCCT-3' R: 5'-TGCATCCCTCTCTCTGGCGTC-3'	53	Yes
Gsp.A021 AY644738	F: 5'CAACCAGTAGGATGTTTGTG3' R: 5'CATTTGGTCAAACTAATTTTGC3'	(TA) <sub>4</sub> (TG) <sub>14</sub>	56	NO	F:5'-CACACCAGTAGGATGTTTGTCA-3' R:5'-TCCATCTTATCATCTTTCTCTCCT-3'	51	Yes
Gsp.I168 AY644748	F:5'-ATGTGCAATGCCCACTAAC-3' R: 5'-AAGAAGAACCCTAATGATGG-3'	(CT) <sub>34</sub>	54	NO	with three different primer pairs	-	NO
Gsp.B264 AY644733	F:5'-AACACTCAGTTGGCGTGC-3' R:5'-AGCAGCCACTAATACACAAG-3'	(CT) <sub>14</sub> (AT) <sub>2</sub>	53	Yes	-	58	-
Gsp.B291 AY644741	F:5'-GTTGACTGTTTATTACCCTTCTC-3' R:5'-TCAGCGTAACAATTCAGAATG-3'	(CT) <sub>37</sub> (CA) <sub>10</sub> CTCA(CT) <sub>3</sub>	50	NO	with three different primer pairs	-	NO
Gsp.A104 AY644736	F:5'-AAGCAGGTTGCCAACATGG-3' R:5'-GCGAACATTTTCAAGAGATCG-3'	(GT) <sub>11</sub> and (GA) <sub>6</sub>	55	Yes	-	-	-
Gsp.B458 AY644742	F: 5'-TCACTGATTTTGTAGTGG-3' R: 5'-GTTCTACGGTCCCTTTGTAAGAG-3'	(CA) <sub>10</sub> (TA) <sub>6</sub>	55	NO	F:5'-TCACTGATTTTGTAGTGG-3' R:5'-TAACGGTCTACGGTCCCTTTGT-3'	53	Yes
Gsp.B331 AY644734	F:5'-TACATTGATTTTGTATGGAC-3' R:5'-GTTTGAAGTATTGAAGTCTTA-3'	(CA) <sub>14</sub> TG(CA) <sub>4</sub>	56	NO	with three different primer pairs	-	NO

(\*) Naciri-Graven *et al.* (2005)

to evaluate the expected allele range size in *G. decorticans* individuals, PCR reactions with each set of primers were prepared, and new primer pairs from accession were designed when PCR amplification was not obtained with Naciri-Graven's primers (Table 2). PCR reactions were prepared in 24 µl that contained: 12 µl Master Mix SapphireAmp Fast PCR 2X (Takara-Clontech), 2 µl of genomic DNA (5 ng/µl), 1 µl of each primer (forward and reverse) at 5 µM concentration, and 8 µl nuclease-free water. The amplifications were performed in a Labnet MultiGene OptiMax Thermal Cycler under the following conditions: an initial step of 3 minutes at 94°C, 45 cycles of 25 seconds at 94°C, 25 seconds at annealing temperature (Ta) (see Table 2), and 25 seconds at 72°C, followed by a final extension step of 3 minutes at 72°C. PCR products were detected in an ABI3730XL Genetic Analyser (Applied Biosystems). For capillary electrophoresis detection, forward SSR primers were labelled with 5'-fluorescence dyes 6-FAM and HEX, and the size standard used in the sequencer was Gene Scan™ 400 HDTM Rox (Applied Biosystems). Allele sizes were determined using the Peak Scanner Software (Applied Biosystems, version 1.0).

#### Data analysis

Null alleles from each locus were detected in a MICRO-CHECKER v.2.2.3 (van Oosterhout *et al.*, 2004). For every population and locus, Hardy-Weinberg Equilibrium (HWE) with permutation tests and Linkage disequilibrium (LD) between all pairs of loci tests were assessed using the Arlequin v. 3.1 (Excoffier *et al.*, 2005) and FSTAT v. 2. 9. 3 (Goudet, 2001) software. LD test was carried out on 10,000 permutations. The total number of alleles per locus ( $N_a$ ), effective number of alleles ( $N_e$ ), observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), mean number of alleles ( $MNA$ ), allelic richness ( $A_R$ ), private allele ( $A_p$ ), Shannon's information index ( $I$ ) and Wright's F statics parameters ( $F_{IS}$ ,  $F_{IT}$  and  $F_{ST}$ ) were also calculated using FSTAT v. 2. 9. 3 (Goudet, 2001) and Arlequin v. 3.1 (Excoffier *et al.*, 2005). Population pairwise Fst values were performed on 1,000 permutations. The polymorphic information content (PIC) for each SSR locus was estimated using the formula:

$PIC = 1 - \sum p_i^2$ , where  $p_i$  is the frequencies of the different alleles detected in the locus. The average gene flow ( $N_m$ ) among paired populations was indirectly estimated by a traditional genetic differentiation method based on  $F_{ST}$  value ( $N_m = (1-F_{ST})/4F_{ST}$ ) (Slatkin & Barton, 1989).

To estimate genetic variability within and among populations, the nonparametric test analysis of molecular variance (AMOVA) was calculated using GenAlex v. 6.5 (Peakall & Smouse, 2012) with 1,000 permutations.

The establishment of genetic relationship using Nei's genetic distances from 84 individuals and eight populations was calculated by Phylip 3.6 software (Felsenstein, 1989) based on Neighbour joining (NJ) and UPGMA (unweighted pair group method with arithmetic mean) clustering methods; bootstraps of 1,000 replicates were performed to test the robustness of the clusters. Dendrograms were constructed by FigTree 1.4.0 (Rambaut, 2012). A multivariate analysis was carried out by metric multidimensional scaling (MDS) as the grouping technique using PAST program (Hammer *et al.*, 2001).

We assess isolation-by-distance by testing the relationship between genetic and geographic distances using software GenAlex v. 6.5 (Peakall & Smouse, 2012) and significances was carried out on 1000 permutations. Both, geographical coordinate and genetic distances were calculated for GenAlex in a single analysis.

The genetic structure of the 84 individuals of *G. decorticans* was determined by a Bayesian cluster analysis, using the STRUCTURE v.2.3 software (Pritchard *et al.*, 2000). For the analysis, an admixture model with correlated allele frequencies was used without the LocPrior option. The optimum number of subpopulations ( $K$ ) was identified after five independent runs for each value of  $K$  ranging from 1 to 8, with a burn-in period of 100,000 iterations followed by Markov Chain Monte Carlo (MCMC) repetitions of 10,000 iterations. We examined two criteria with mean log-likelihood of  $K$  ( $Ln(K)$ ) and delta  $K$  ( $\Delta K$ ) values to find the appropriate values of  $K$  for the population structure (Evanno *et al.*, 2005). The results from STRUCTURE were processed using the web program STRUCTURE HARVESTER (Earl & Vonholdt, 2012) to identify the optimal groups ( $K$ ).

## RESULTS

Our study evaluated the genetic diversity in 84 *G. decorticans* individuals using SSR markers. Five out of eleven SSR primer pairs tested amplified PCR products of the expected size. We designed new primer pairs that flanked SSRs on the seven remaining DNA sequences that gave negative (accessions). This way, eight primer pairs amplified PCR products, however three of them (Gsp.G226, Gsp.F119 and Gsp.B458) were excluded due to their low polymorphism and poor amplification. Overall, five polymorphic SSR loci were used in our study and the characteristics of these SSRs are summarized in Table 2. A linkage disequilibrium (LD) test for each population detected significant equilibrium deviation in 17 out of 80 loci combinations, with a 5% significance level. Loci pairs with significant linkage disequilibrium were corrected by Bonferroni's test and, after correcting multiple tests with all loci were performed. The analysis with MICRO-CHECKER program not showed null alleles in all loci. A total of 40 alleles were detected among Chañar individuals. The number of alleles generated by each SSR marker ranged from 5 to 12 with an average of 8 alleles per locus (Table 3). The highest number of alleles was observed in the Gsp.A104 locus (12 alleles) and the lowest number of alleles was observed in the Gsp.A021 and Gsp.A264 loci (5 alleles) (Table 3). The effective number of alleles for each locus ranged from 1.91 to 3.28, with an average of 2.54. The size of the amplified allele fragments ranged from 168 bp (Gsp.B264) to 343 bp (Gsp.B284). The PIC value was in the range 0.640 (Gsp.A021) to

0.832 (Gsp.A104) with a mean PIC of 0.755 for all loci (Table 3). The expected heterozygosity ranged from 0.669 in the Gsp.B284 locus to 0.464 in the Gsp.A264 locus, averaging 0.550 for all loci. The observed heterozygosity ranged from 0.819 in the Gsp.B284 locus to 0.510 in the Gsp.A021 locus, averaging 0.649 for all loci. The range of allele numbers among all loci was the lowest in the PACH population and the highest in VIC, with values of 9 and 27, respectively. The average of allelic richness ( $A_r$ ), ranged between 1.78 in the Pachica population and 4.35 in the Vicuña population (Table 4). Private alleles, exclusive to the population were observed in VIC ( $A_r = 7$ ); in AZA, SP and AC ( $A_r = 2$ ); COP ( $A_r = 1$ ); and private alleles from the rest of the populations were not found. The average number of alleles varied between  $1.8 \pm 0.200$  in the PACH population and  $5.4 \pm 0.748$  in the VIC population (Table 4). The Shannon index ( $I$ ) varied between 0.509 in the PACH population and 1.422 in the VIC population. The highest value of  $H_o = 0.833 \pm 0.129$  was observed in AC, while the lowest value was found in the populations of SP and CALA,  $H_o = 0.520 \pm 0.080$  and  $H_o = 0.520 \pm 0.136$ , respectively. The highest  $H_e$  value estimated within the populations was identified in VIC ( $0.706 \pm 0.041$ ), while the lowest value was estimated in PACH ( $0.357 \pm 0.095$ ). For most analyzed populations the average observed heterozygosity was higher than the expected (AZA, CHA, PACH, CALA, COP and AC), being deviated from the HWE (non-significant).

The inbreeding level ( $F_{IS}$ ) for each population varied between -0.701 and 0.103, indicating an excess of heterozygotes in six populations and

**Table 3.** Diversity statistics of five polymorphic SSR loci used in 84 *Geoffroea decorticans* individuals.

Locus	$N_a$	$N_e$	PIC	$H_o$	$H_e$
Gsp.A149	9	2.320 ( $\pm 0.271$ )	0.781	0.552 ( $\pm 0.128$ )	0.512 ( $\pm 0.083$ )
Gsp.B284	9	3.170 ( $\pm 0.383$ )	0.822	0.819 ( $\pm 0.098$ )	0.669 ( $\pm 0.045$ )
Gsp.A104	12	3.280 ( $\pm 0.593$ )	0.830	0.808 ( $\pm 0.096$ )	0.636 ( $\pm 0.064$ )
Gsp.A021	5	2.020 ( $\pm 0.315$ )	0.640	0.510 ( $\pm 0.128$ )	0.488 ( $\pm 0.079$ )
Gsp.B264	5	1.910 ( $\pm 0.192$ )	0.703	0.558 ( $\pm 0.068$ )	0.464 ( $\pm 0.041$ )
Mean	8 ( $\pm 2.683$ )	2.540 ( $\pm 0.180$ )	0.755 ( $\pm 0.081$ )	0.649 ( $\pm 0.150$ )	0.550 ( $\pm 0.092$ )

**Note:** total number of alleles per locus ( $N_a$ ), effective number of alleles ( $N_e$ ), polymorphic information content (PIC), observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ). Values in parentheses are standard deviation ( $\pm$ )

**Table 4.** Diversity statistics in *Geoffroea decorticans* populations.

Population	$A_R$	$A_P$	MNA	$I$	$H_o$	$H_e$	$F_{ST}$	$F_{IS}$	$N_m$
AZA	2.680 (± 0.790)	2	2.800 (± 0.374)	0.875 (± 0.161)	0.661 (± 0.061)	0.523 (± 0.083)	0.196 (± 0.061)	-0.118 ns	1,023
CHA	2.390 (± 1.130)		2.400 (± 0.510)	0.723 (± 0.222)	0.667 (± 0.183)	0.444 (± 0.123)	0.254 (± 0.074)	-0.505 ns	0.730
PACH	1.780 (± 0.440)		1.800 (± 0.200)	0.509 (± 0.132)	0.624 (± 0.190)	0.357 (± 0.095)	0.263 (± 0.091)	-0.701 ns	0.699
SP	3.600 (± 1.360)	2	4.200 (± 0.860)	1.145 (± 0.225)	0.520 (± 0.080)	0.597 (± 0.091)	0.160 (± 0.031)	0.090 *	1.310
CALA	2.600 (± 0.890)		2.600 (± 0.400)	0.755 (± 0.165)	0.520 (± 0.136)	0.468 (± 0.079)	0.207 (± 0.084)	-0,130 ns	0.957
COP	3.550 (± 1.000)	1	4.400 (± 0.748)	1.198 (± 0.175)	0.729 (± 0.101)	0.631 (± 0.066)	0.172 (± 0.059)	-0.148 ns	1.202
AC	2.660 (± 0.370)	2	2.800 (± 0.200)	0.831 (± 0.054)	0.833 (± 0.129)	0.519 (± 0.033)	0.177 (± 0.051)	-0,562 ns	1.160
VIC	4.350 (± 1.050)	7	5.400 (± 0.748)	1.422 (± 0.138)	0.640 (± 0.129)	0.706 (± 0.041)	0.144 (± 0.058)	0.103 ns	1.485

**Note:** (AZA) Azapa, (CHA) Chaca, (PACH) Pachica, (SP) San Pedro de Atacama, (CALA) Calama, (COP) Copiapó, (AC) Alto del Carmen, (VIC) Valle del Elqui. Allelic richness ( $A_R$ ), number of private alleles ( $A_P$ ), mean number of alleles (MNA), Shannon’s Information Index ( $I$ ), observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity, mean genetic differentiation ( $F_{ST}$ ), local inbreeding coefficient ( $F_{IS} = 1 - H_o/H_e$ ) and gene flow ( $Nm$ ). Significance level: ns: not significance; \* $p < 0.05$ . Values in parentheses are standard deviation (±)

suggesting a HWE in SP and VIC populations, however, HWE was significant only for SP ( $P < 0.05$ ). A significant genetic differentiation ( $F_{ST}$ ) was observed between the eight populations ( $P < 0.001$ ) and within populations ( $P < 0.001$ ). The  $F_{ST}$  value ranged from 0.144 to 0.254 in VIC and CHA, respectively (Table 4). Genetic differentiation values were calculated between pairs of populations with a level of significance between  $P < 0.01$  and  $P < 0.001$  (Table 5). The gene flow ( $Nm$ ) was calculated according to the values of genetic differentiation, which varied between 0.699 in the population of PACH to 1485 in VIC (Table 4). The pairwise Nei’s genetic distance values between populations ranged in 0.319 among PACH to AZA populations, and 2.006 among PACH and CHA populations.

The AMOVA analysis indicated that the genetic differentiation among populations was 29% ( $P=0.0001$ ) of the total variance. The highest variation (71%;  $P=0.0001$ ) was found among individuals within populations (Table 6). The mean  $F_{ST}$  (0.2975;  $P < 0.0001$ ) and  $F_{IT}$  (0.1733;  $P < 0.0001$ ) were significant, while the mean  $F_{IS}$  (-0.1768) was not significant.

To illustrate the genetic relationships between individuals and populations, a NJ and UPGMA dendrograms were constructed based on Nei’s genetic distance, respectively. The results indicated that the 84 analyzed individuals could be clustered in three groups: Cluster I composed mostly by AZA individuals, Cluster II composed by PACH individuals and Cluster III composed by the rest of

**Table 5.** Pairwise genetic differentiation  $F_{ST}$  values (below the diagonal) and Nei’s genetic distance (above the diagonal) among 8 *Geoffroea decorticans* populations.

	AZA	CHA	PACH	SP	CALA	COP	AC	VIC
AZA	-	1.351	0.319	0.698	1.211	1.201	0.632	0.691
CHA	0.400**	-	2.006	0.797	0.951	0.779	0.856	1.025
PACH	0.242**	0.555**	-	0.561	1.296	1.143	0.757	1.144
SP	0.252**	0.283**	0.316**	-	0.517	0.716	0.593	0.600
CALA	0.371**	0.365*	0.500**	0.198**	-	0.547	0.447	0.449
COP	0.313**	0.265**	0.397**	0.211**	0.198**	-	0.660	0.407
AC	0.257**	0.326**	0.399**	0.206**	0.195*	0.214**	-	0.480
VIC	0.212**	0.267**	0.380**	0.154**	0.154*	0.110**	0.139*	-

Significance level: \*  $p < 0.01$ ; \*\* $p < 0.001$ .



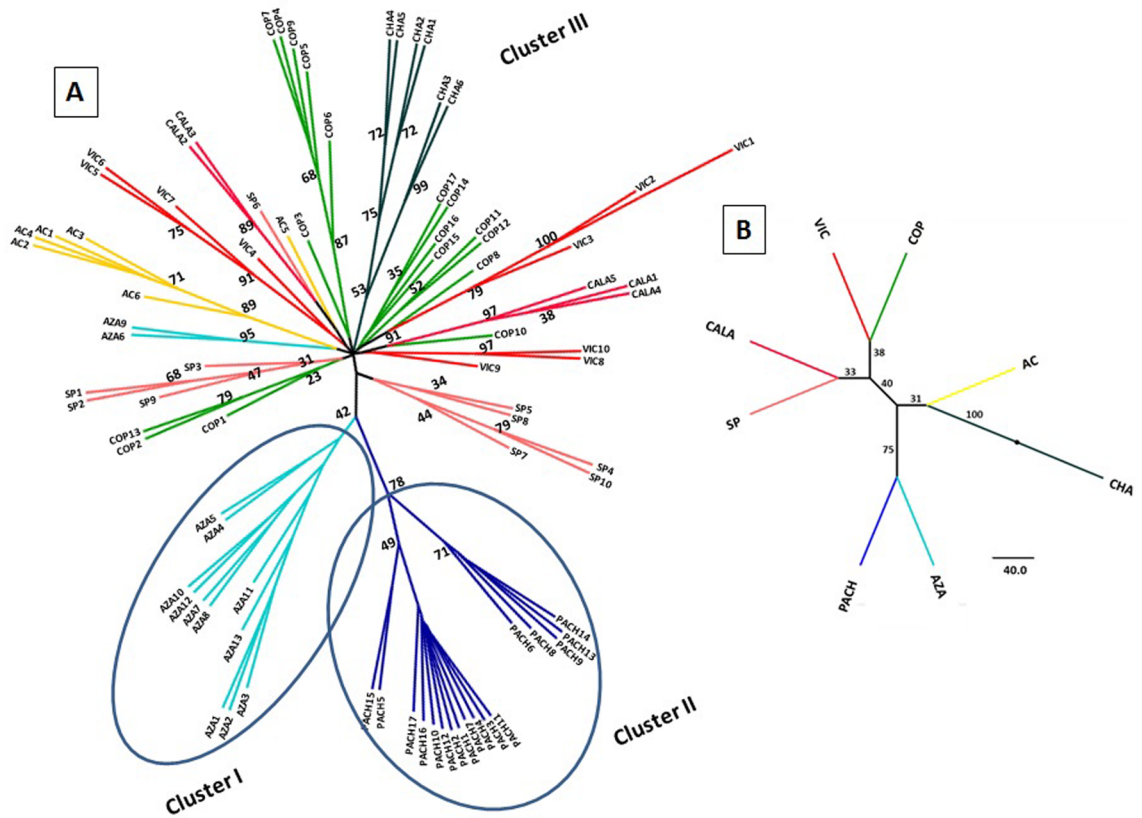
**Table 6.** Analysis of molecular variance (AMOVA) based on 5 SSR loci in 8 populations of *Geoffroea decorticans*.

Source of variation	DF	Sum of squares	Variance components	Percentage of variation	P value(*)
Among populations	7	92,024	0.575	29	0.001
Within populations	160	223,708	1.398	71	0.001
Total	167	315,732	1.973	100	

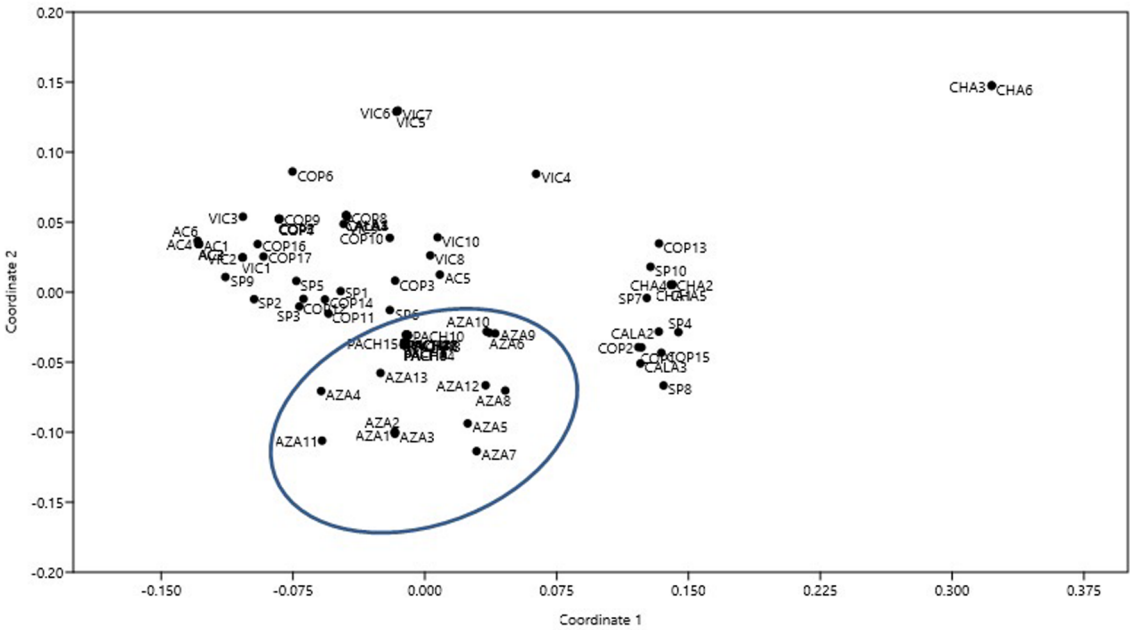
(\*) significance test by 1000 permutations.

the individuals (Fig. 1A). Cluster I and II grouped the AZA and PACH individuals, geographically located in the northern Atacama Desert, whereas Cluster III grouped Central (SP, CALA) and Southern individuals (COP, AC and VIC); as well as the CHA individuals coming geographically

from the North. On the other hand, the analysis showed that PACH and AZA populations were separated from the rest of populations with a high support of bootstrap value (75) (Fig. 1B). A similar clustering of AZA and PACH individuals was observed in a MDS analysis (Fig. 2).



**Fig. 1. A:** NJ dendrogram based on Nei's genetic distance showing the relationships of 84 *Geoffroea decorticans* individuals; bootstrap value percentages are indicated. **B:** UPGMA dendrogram showing the relationships of 8 *Geoffroea decorticans* populations; bootstrap value percentages are indicated.

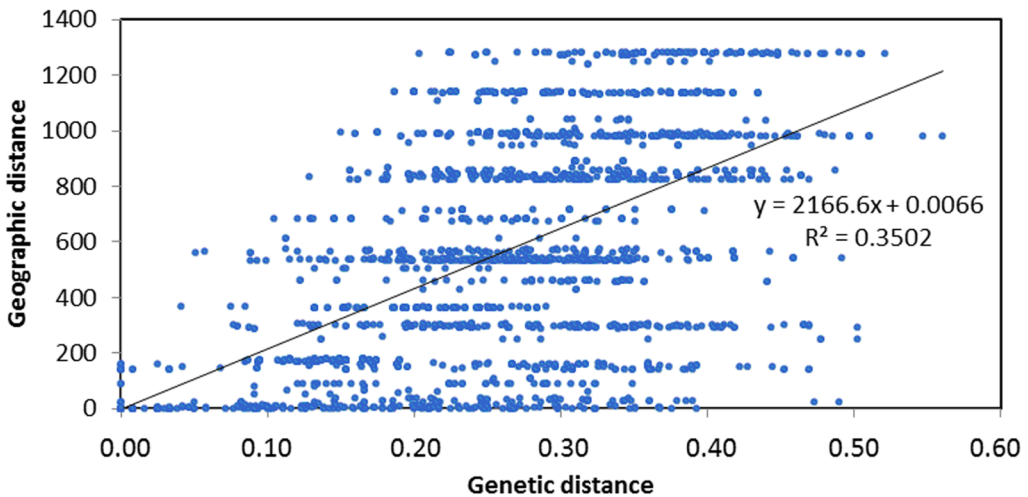


**Fig. 2.** Metric multidimensional scaling (MDS) analysis grouping on 84 individuals using 5 SSR markers.

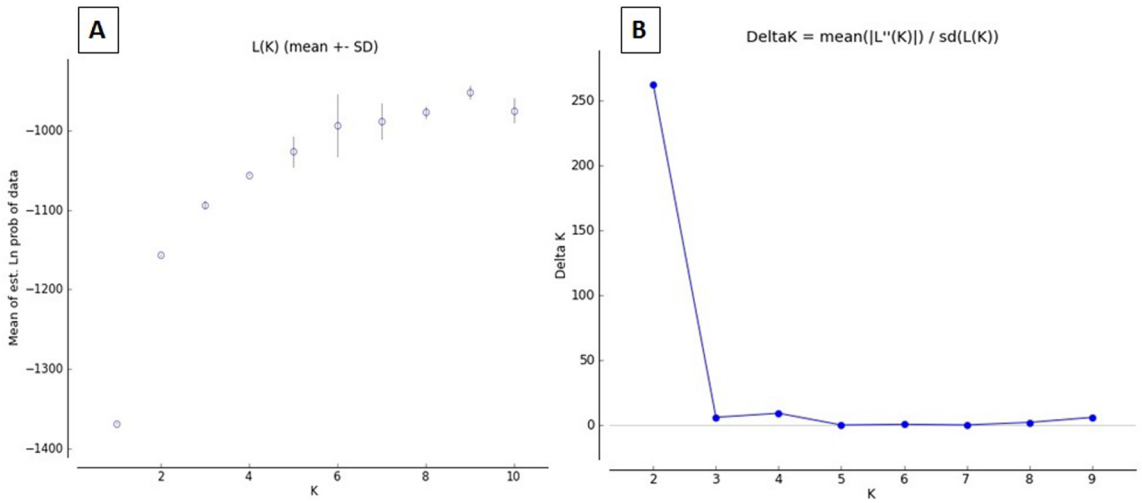
Fig. 3 shows a significant correlation between Nei's genetic distance and the geographic distance among the 84 *G. decorticans* individuals evaluated ( $r^2 = 0.35$ ,  $P = 0.01$ ) using the Mantel test (Fig. 3).

A peak  $K = 2$  value was determined using to the statistical model described by Evanno *et al* (2005)

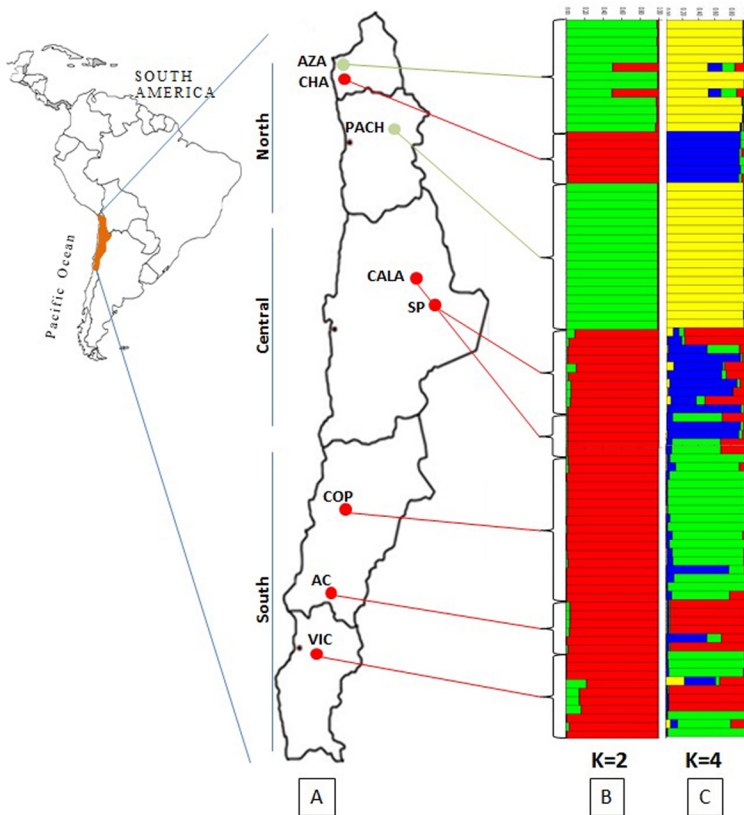
(Fig. 4). The STRUCTURE analysis revealed an optimal number of subpopulations  $K = 2$ , confirming the existence of at least two different groups among the eight studied populations (Fig. 5A). According to the Fig. 5B, most individuals of AZA population and all individuals of PACH



**Fig. 3.** Pairwise genetic distance and geographical distance obtained of 168 SSR fragment data from 84 *Geoffroea decorticans* individuals sampling in different zones from northern Chile. The probability value was obtained after 1000 permutations.



**Fig. 4.** Relationships between the number of clusters ( $K$ ) and the corresponding  $L(K)$  statistics. **A:** Relationships between the number of clusters ( $K$ ) and the corresponding  $\Delta K$  statistics. **B:** All values calculated according to STRUCTURE analysis.



**Fig. 5.** Map of Atacama Desert from Chile and population assignments by STRUCTURE. **A:** Distribution of *Geoffroea decorticans*' populations across Northern Chile. Structure indicated two genetic clusters ( $K=2$ ) (**B**) and four genetic clusters ( $K=4$ ) (**C**), where the proportion of colors in each bar indicates the assignment probabilities of *G. decorticans* individuals to each group.

population present different structure to the rest of populations. Moreover, a small signal at  $K = 4$  showed a different distribution in the structure of the populations (Fig. 5C). Figures 5B and 5C show an interesting phenomenon, a small subset of individuals within the AZA population (Northern zone) are closer to populations of the Southern zone and vice versa. Indeed, the CHA population located in the Northern zone displays a genetic structure that resembles Southern populations (Fig. 5B), specifically the SP and CA populations (Fig. 5C). In summary, the STRUCTURE analysis, and NJ and UPGMA genetic relationship analyses strongly suggest the existence of differentiated groups from Atacama Desert.

## DISCUSSION

We performed a genetic analysis in *G. decorticans* using transferability of five SSR markers described by Naciri-Graven *et al.* (2005). Our study did not find HWE in chañar populations, indicating an excess of heterozygotes, with the exception of the SP population that displayed a significant positive HWE. Accordingly, studies in *G. spinosa*, also found significant positive HWE in some populations (Naciri-Graven *et al.*, 2005), in agreement with populations from the Galapagos and Perú (Caetano *et al.*, 2012). The observed excess of *G. decorticans* heterozygotes in our study could be explained by the fact that this species prefers cross-pollination to self-pollination. Interestingly, studies on the biology of flowering and pollination suggest that *G. decorticans* is xenogamous under certain conditions. Indeed, this species is capable of self-pollination, but it produces more fruit when the pollination is xenogamous (Eynard & Galetto, 2002). Hence, heterozygote excess noted in the chañar from the Atacama Desert could have been triggered by the species aiming to avoid inbreeding depression. A good example of a mechanism to prevent inbreeding in flowering plants is the self-incompatibility system. This was previously evidenced in a study that characterized *Flourensia cernua* DC at the Chihuahuan Desert (Ferrer *et al.*, 2009).

The SSR markers in our study displayed a high genetic variation among the analyzed individuals. The 5 amplified SSRs in our study had a high level of polymorphism with 5-12 alleles per locus and 1.8 to 5.4 detected alleles in the analyzed populations.

In contrast, the study by Naciri-Graven *et al.* (2005) used 11 microsatellites across 6 *G. spinosa* populations (3 from Peru, 2 from Paraguay, and one from Argentina) and reported up to 19 alleles per population. Another study by Caetano *et al.* (2012) used 5 microsatellites to analyze 11 populations and found 1 - 12 alleles. Analyzed populations were from the Peruvian Pacific coast and Inter-Andean valleys, and the Galapagos islands. This study also demonstrated high levels of PIC (range: 0.640 to 0.822) across the 5 *G. decorticans* SSR loci, suggesting a high genetic diversity. In general, PIC values higher than 0.5 indicate a highly polymorphic locus (Botstein *et al.*, 1980). The high values of PIC indicate the validity of the markers used for this study. Likewise, we found a high genetic diversity in certain *G. decorticans* populations from the Atacama Desert using 8 ISSR and 3 RAPD molecular markers (Contreras *et al.*, 2018). The high genetic diversity observed in *G. decorticans* is even more surprising when we take into consideration the extreme environmental conditions in the Atacama Desert that favor isolation and limit migration of organisms, in sharp contrast with *G. spinosa*'s habitat preferentially located near the Amazon jungle.

As pointed out, observed heterozygosity levels ( $H_o = 0.649$ ) were much higher than initially expected ( $H_e = 0.550$ ) demonstrating HWE loss across the analyzed loci. In contrast, Naciri-Graven *et al.* (2005) and Caetano *et al.* (2012) consistently report HWE in the majority of *G. spinosa* populations studied. Importantly, our study analyzed 3 loci that were different from the 5 reported by Caetano *et al.* (2012). Furthermore, studies in *Populus euphratica* Olivier, a species not related to the Fabaceae family that grows in the desertic Northeast China also showed an imbalanced HW across several loci and populations (Wang *et al.*, 2011).

As described above, chañar populations in the Atacama Desert are usually separated by large distances and must face a natural desertic barrier that difficults the migration of any organism. Consequently, here we demonstrated significant differences among the selected populations studied. These differences were further confirmed by AMOVA, which indicated that 29% of the molecular variation could be attributed to population diversity. Moreover, ISSR and RAPD analyses showed that

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35% of the variation was attributed to population diversity (Contreras *et al.*, 2018). On the other hand, studies in *G. spinosa* from Perú, Paraguay, Argentina (Naciri-Graven *et al.*, 2005) and Galapagos Island (Caetano *et al.*, 2012) demonstrated a significant genetic differentiation among different populations. In contrast, *Populus euphratica* species from desert areas in China did not show high levels of population differentiation, despite the evident isolation conditions present in their habitat (Wang *et al.*, 2011).

Regarding SSR markers, the PACH population displayed the highest genetic differentiation and the lowest gene flow among all analyzed populations with a Shannon's Information Index equal to 0.509. This population also had low genetic diversity levels confirmed by ISSR and RAPD markers (Contreras *et al.*, 2018). In this scenario, and considering its small size (~30 individuals), we could forecast a notable increase in the genetic drift within this population in the near future. This is not even taking into consideration the pressure derived from the copper mining industry that can further reduce the chañar populations. In sharp contrast to the population described above, the VIC population had the highest number of private alleles, *I* index, gene flow, allelic richness along with the lowest genetic differentiation. This population also had low genetic diversity by ISSR and RAPD, a result that could be explained by the small sample size (two individuals) used for analysis (Contreras *et al.*, 2018). Despite being located in the Atacama Desert, precipitation for the Vicuña population is higher (29°S and 30°S) (Azua-Bustos *et al.*, 2012) with more water reservoirs available along with a better plant coverage (Squeo *et al.*, 2008). In fact, the driest areas of the Atacama Desert are located between 22°S and 26°S (Gómez-Silva *et al.*, 2008). Undoubtedly, more favorable conditions for Vicuña also mean more pollinators and a higher gene flow among individuals.

Our study shows a positive correlation between genetic and geographic distance. Therefore we hypothesize that significant geographic restriction acts as a barrier to gene flow among populations. The high genetic differentiation  $F_{ST}$  levels observed across populations confirm our hypothesis. ISSR and RAPD markers further evidenced the isolation by distance in *G. decorticans* populations (Contreras *et al.*, 2018).

In the cluster analysis, 84 individuals of chañar studied did not show grouping according to their localities and there is no clear occurrence of grouping

among them, except the individuals of PACH population. For example, all individuals of AZA are not grouped in the same cluster; two individuals are separated from the rest. It is possible that the same alleles are present in the different populations that explain the observed inconsistencies. When analyzing the cluster by populations, no groupings with consistent supports were observed, with the exception of AZA and PACH that are separated from the rest of the populations with good support.

STRUCTURE and cluster analyses suggested the existence of groups of chañar individuals formed by Northern populations of the Atacama Desert (located at AZA and PACH), and groups that included central and southern populations (SP, CALA, COP, AC and VIC). Interestingly, the Northern CHA population shows a different genetic structure when compared to neighbouring populations. On the other hand, it is quite possible that Northern populations could receive the influence of the pollen and the direct contact of chañar populations from Bolivia and/or Peru. Unfortunately, to the best of our knowledge there is no genetic evidence from those countries in order to support such hypothesis. Maybe, the groups formed by central and southern populations could be influenced by Argentinian populations, despite the geographical barrier formed by the Andes Mountains. According to McRostie *et al.* (2017) *P. alba* was brought from Argentina and established in Chile by human groups of settlers. Therefore, we could speculate that these groups also brought chañar from Argentina. However, to date there are no genetic studies that could support this hypothesis.

Our genetic structure analyses suggest that the CHA population is surprisingly similar to SP and CALA (Fig. 5C); interestingly, these populations are separated by a deserted area of approximately 167,849 km<sup>2</sup>, where few organisms can survive.

For future genetic studies focusing on *G. decorticans*, a deeper study will be required with a sampling of a greater number of individuals, a greater number of markers and a greater number of localities that cover all the regions.

In summary, microsatellite studies have provided valuable information on the genetic diversity and structure of *G. decorticans* populations. The majority of the analyzed populations from the Atacama Desert displayed a high genetic diversity, with the exception of PACH. Indeed, the characteristics observed in this population suggest a genetic drift, due to their low

genetic diversity, limited gene flow and high genetic differentiation.

Chañar populations also displayed a high genetic differentiation and a moderate gene flow given by the natural barrier imposed by the Atacama Desert. The eight chañar populations studied were separated in groups from Northern and Southern regions in the Atacama Desert. Variability and diversity parameters provide valuable baseline information to understand the genetic diversity and structure of *G. decorticans* populations at the Atacama Desert.

## AUTHOR CONTRIBUTIONS

RC conceived and planned the experiments, contributed to the design and implementation of the research, carried out the analysis of the results and the writing of the manuscript. VP carried out the experiments, contributed to sample preparation, performed the measurements of assays. FA performed the measurements of assays and contributed to sample preparation.

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