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SSR Based Genetic Diversity Analysis in Diploid Algaroba (*Prosopis* spp.) Population

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Abstract

Algaroba (*Prosopis palida* and *Prosopis juliflora* species) provides important environmental and economic benefits for semi-arid regions of the world. These are resistant to drought, and its fruits are used in the manufacture of flour and algarobina syrup. In the present study, the chromosome number, the ploidy level, and the genetic diversity based on 40 microsatellite loci of *Prosopis* spp. were determined in samples of a Brazilian algaroba population. The cytogenetic analysis in the metaphase showed only diploid individuals ($2n = 28$), with multiple cells featuring two $CMA^3/DAPI^-$ heterochromatic blocks suggesting diploid level. However, polysomatism was found by the presence of some cells with four $CMA^3/DAPI^-$ blocks, showing the tetraploid level just in some somatic cells. Among all of the primers tested for cross-amplification in algaroba, 22 were selected to characterize the samples. Thirteen loci revealed allele polymorphisms in the population and are recommended for future population studies and genetic improvement. The mean values of the analyzes showed low genetic diversity (two alleles per locus and $H_E = 0.181$), reflecting the history of the introduction of algaroba in this sampled locality, and suggesting the genetic bottleneck and probable events of founders, as well as the characteristics of the species of this genera. However, amplified loci indicated low inbreeding (allelic fixation index of -0.007), although heterozygosis was higher than expected by the Hardy-Weinberg equilibrium. Therefore, this algaroba population is formed by diploid individuals and adjusts to the tendency of low number of alleles per locus SSR commonly observed in different species of *Prosopis*.

Keywords: cytogenetics, mesquite, algarroba, molecular marker, polysomatism, polyploid, *Prosopis juliflora*, *Prosopis pallida*

1. Introduction

Prosopis juliflora (Sw.) DC. is known as *algaroba* in Brazil, and as *algarroba* or *algarrobo* in some Spanish-speaking countries, as well as mesquite in African, Asian countries and others. *Prosopis juliflora* and *P. pallida* (H. & B. ex. Wild). H.B.K. are legumes, and they form a complex due to taxonomic issues not yet solved (Burkart, 1976; Pasiecznik et al., 2001). The species presents a basic chromosome number of x = 14, but the variable ploidy level present in most of the sample studies has caused cytotypes to be recognized as tetraploid (Hunziker et al., 1975; Pasiecznik et al., 2001, Nogueira et al., 2007; Trenchard et al., 2008). In this case, variation from 2n = 2x = 28 to 2n = 4x = 56 was principally in the genus *Prosopis* (Trenchard et al., 2008).

The species complex are distributed in Central and South America, Africa, Asia, and Oceania. Algaroba is widely found in the dry forests of Peru and was introduced in Brazil in 1940 (Azevedo, 1955; Burkart, 1976; Pasiecznik et al., 2001). Algaroba is a tropical species and has spread throughout the world for economic reasons and due to its ease of adaptation in arid and semi-arid regions, thus guaranteeing its survival for many centuries. The species is well adapted to regions that suffer periods of severe drought and unproductivity worldwide (Burkart, 1976). The algaroba has the potential to provide a wide range of products in the northeastern part of Brazil, where few useful species are found. It is one of the few economic resources for farmers and inhabitants of

the region. Algaroba is a food source for animals, an ingredient of breads, flour, sweets, and used as wood, firewood, and coal (Cruz, 1990; Silva, 1996; Felker, 1984; Figueiredo, 2000; Ribaski, 2009).

Considering the value of algaroba, the molecular diversity studies of *Prosopis* spp. are crucial for understanding the genetics of these invasive plants, their response to adverse conditions and events of genetic drift, and their use in studies on crop's genetic improvement.

Molecular methods for genetic detection of polymorphic microsatellite loci (SSR) can be performed by cross-amplification of markers from related species (Landeras et al., 2006). There are very limited molecular studies for *Prosopis* spp., and no polymorphic microsatellites were identified to evaluate genetic diversity in Brazilian algaroba populations. Cross-amplification of microsatellite markers was successful between distant taxa (Yasodha et al., 2005). Additionally, the algaroba chromosome number, the existence of species with variation in ploidy, and marker analysis of species with known ploidy will help on determination and evaluation of useful parameters. This phenomenon is commonly observed in plants with high adaptation.

SSR loci were originally developed for *P. chilensis* and *P. flexuosa* (Mottura et al., 2005), *P. alba* (Torales et al., 2013), *P. alba* and *P. chilensis* (Bessega et al., 2013), *P. rubriflora* and *P. ruscifolia* (Alves, 2014). Cross-species amplification of six microsatellites markers developed for *P. chilensis* have been tested in seven Prosopis species from Argentina: *P. alba*, *P. caldenia*, *P. ferox*, *P. hassleri*, *P. nigra*, *P. ruscifolia*, *P. torquata* and *P. brasiliensis* (Mottura et al., 2005). These six SRR loci were also used in cross-application of eight additional *Prosopis* species: *P. tamarugo* hybrid, *P. pallida*, *P. juliflora*, *P. laevigata*, *P. glandulosa* var. torreyana, *P. velutina*, *P. articulata*, *P. caldenia* (Sherry et al., 2011). So, cross-amplification with the six microsatellite loci in 15 different *Prosopis* species indicates that there must be a high level of similarity of flanking sequences from repetitive sites in *Prosopis* spp. However, these primers were not genetically characterized at the level of populations of these species; this is a necessary information to plan the use of the loci in population studies. In addition, the number of loci existing for each species is not sufficient for more comprehensive genetic studies in populations. Thus, the cross-amplification of SSR loci available in *Prosopis* is an alternative to characterize primers aiming studies of algaroba populations.

The genetic aspects of representative algaroba population in the state of Bahia, Brazil, was studied with the following objectives: i) To test the chromosomal number, ploidy level of this selected population to determine the use of chosen molecular markers; ii) To test the cross-amplification of selected simple sequence repeat/microsatellite loci in *Prosopis juliflora* and *P. pallida*; iii) To evaluate the polymorphism of the number of alleles per locus, heterozygosity and coefficient of inbreeding; iv) To determine the genetic diversity of the Brazilian algaroba population and to compare this diversity with previous studies of different populations.

2. Materials and Methods

2.1 Study Site and Plant Material

The samples were collected in a farm located in the municipality of Manoel Vitorino, Bahia, Brazil (Figure 1), in a semi-arid region characterized by the predominance of the *Caatinga* biome and bordered by the *De Contas* River. It is an area of the occurrence and cultivation of the *Prosopis* species for animal feed.

Leaves were collected from 20 representative genotypes of the population, and they were identified, stored, and taken to the Laboratory of the Center for Biotechnology and Genetics (CBG) at the State University of Santa Cruz (UESC) for the development of the molecular marker research.

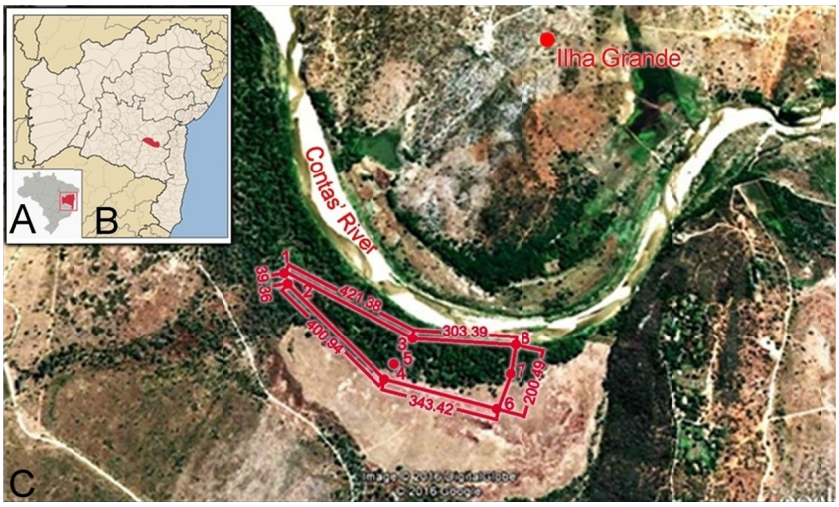

Figure 1. Location of the sampling area of algaroba population. (A) Positioning of Bahia in the Brazil map (red square) and (B) positioning of Manoel Vitorino municipality on the map of Bahia state (red spot). (C) Polygon of the area of algaroba collection, indicating the distances of some points sampled in meters. Number 1 to 8 are collection sites in RIOCON Farms in Contas' River (Source: Google Earth) (Drawing: Romildo Freitas). Collection sites and coordination are: (1) 13°50′28″ S, 40°43′29″ W; (2) 13°50′29″ S, 40°43′29″ W; (3) 13°50′35″ S, 40°43′17″ W; (4) 13°50′39″ S, 40°43′20″ W; (5) 13°50′38″ S, 40°43′19″ W; (6) 13°50′43″ S, 40°43′09″ W; 13°50′40″ S, 40°43′08″ W; 13°50′37″ S, 40°43′07″ W

2.2 Determination of the Level of Ploidy by Fluorochrome Banding

Algaroba's seeds were collected and germinated in Petri dishes with filter paper daily moistened with distillated water until root growth. Radicles were pretreated for 24 h at 8 °C with 0.002 mol.L^{-1} 8-hydroxyquinoline for well spread metaphase obtainment. After pretreatment radicles were fixed in freshly Carnoy 1 fixative [ethanol:acetic acid (3:1, v/v) (Johansen, 1940) for 24 h at room temperature and stored in the freezer (-22 °C) before cytological slide preparation. The cytological slides preparation were performed using radicles digested by cellulase and pectinase (2:20 v/v) for 1 h at 37 °C followed by squashing in 45% acetic acid according to Guerra and Souza (2002). Slides were aged for three days before and after the chromosome banding by the use of Cromomycin A_3 (CMA_3) and 4'6-diamidino-2-phenylindole (DAPI) fluorochromes. The banding was performed according to the protocol proposed by Schweizer & Ambros (1994), adding CMA_3 for 1 h followed by DAPI for 30 min with the subsequent slides mounting using McIlvaine-Glycerol 1:1 (v/v). Slides were analyzed using Olympus CX41 epifluorescence microscope and the best well spread cells were recorded with an Olympus DP25 digital camera.

2.3 Extraction of Genomic DNA

Deoxyribonucleic acid (DNA) extraction was performed randomly in four of the 20 foliar samples of algaroba of approximately 200 ng for testing and standardization of the extraction protocol. Among the protocols tested, the selected protocol was Sorbitol/CTAB 2.0% (Storchová et al., 2000). The DNA extracted from the samples was quantified based on comparative assessments of the observed band standards and the Lambda (standard molecular DNA λ) marker (Invitrogen).

2.4 Cross-Amplification of Microsatellite Markers

Cross-amplification was performed using 40 microsatellite or simple sequence repeat (SSR) primers developed for the *Prosopis* species selected in the literature (Suplementary material). A total of 11 SSR primers from *Prosopis alba* described by Torales et al. (2013), 23 primers for *Prosopis rubriflora* and *Prosopis ruscifolia* by Alves et al. (2014), and six primers for *Prosopis chilensis* and *Prosopis flexuosa* by Mottura et al. (2005).

The SSR loci amplifications were done using a final volume of 13 µL composed of 3 µL of DNA (2.5 ng/µL); 1.3 µL of PCR buffer 10X pH 8.5 (Tris-HCl 10 mmol L^{-1}, KCl 50 mmol L^{-1}); 0.39 µL of $MgCl_2$ (25 mmol L^{-1}); 0.26 µL of dNTP (25 mmol L^{-1}); 1.3 µL of BSA (2.5 µg/mL); 0.26 µL of primer (10 µmol L^{-1}); 0.52 µL of primer labeled with specific fluorochromes (1 µmol L^{-1}); and 0.2 µL of Taq DNA polymerase (5 U/µL) (Phoneutria).

The amplifications were performed with the following PCR program: 30 cycles of 94 ºC for 45 s, specific

annealing for each primer for 45 s, 72 ºC for 45 s; and 10 cycles of 94 ºC for 1 min, 53 ºC for 45 s, 72 ºC for 45 s, final extension with 72 ºC for 5 min. The amplification products were visualized by GelGreen™ stained 1% agarose gel electrophoresis in conjunction with 1Kb DNA ladder as standard marker (Invitrogen®). Genotyping was performed on ABI 3500XL (Applied Biosystems®) with the aid of GeneMapper® software (Applied Biosystems®).

The characterization of SSR loci followed the estimation of genetic parameters: the number of alleles per locus (*A*); observed heterozygosity (H_O); expected heterozygosity (H_E); and inbreeding coefficient (*I*). These parameters were estimated according to Wright's statistics (1951, 1965) in the GenAlEx 6.5 software (Peakall & Smouse, 2012), which indicates the ability to identify the individual by its genotype (Paetkau et al., 1995), using polymorphic information (PIC); paternity exclusion index (Q) (Kalinowski et al., 2007); and linkage disequilibrium (LD) with Genepop 4.2 software (Rousset, 2008). The Hardy-Weinberg equilibrium (HWE) was also evaluated using the formula $(H_E - H_O)/H_E$ within population, which was calculated using GenAlEx 6.5 software (Peakall & Smouse, 2012). The dispersion and clustering of population access was performed by Principal Components Analysis (PCA) in GenAlEx 6.5 software (Peakall & Smouse, 2012).

The comparative study analysis revealed diversity in the populations by different researchers in the characterization of *Prosopis* sp using SSR primers. In this analysis, the estimated mean H_O and H_E parameters of each population used in the SSR characterization developed for *Prosopis* were obtained as follows: (i) data obtained from previously published articles that presented them in the results (Pomponio et al., 2015); (ii) parameters calculated as the mean of the expected heterozygosity (H_E) and heterozygosity observed (H_O) values of each locus characterized in the previous works (Bessega et al., 2013; Alves et al., 2014); and (iii) original data from this study concerning a Brazilian population.

3. Results

3.1 Ploidy Level and Chromosome Banding

All analyzed genotypes in our study have the majority of cells with the diploid chromosome number $2n = 28$ (Figure 2). All chromosome counts were performed in very scattered metaphases and no indication of chromosome loss due to mechanical damage. However, some polyploid cells with $2n = 56$ were simultaneously found. This result was confirmed with the presence of some giants nucleus together with the majority of smalls nucleus.

The analysis of the CMA_3 and DAPI banding showed that diploids cells ($2n = 28$) have two $CMA_3^+/DAPI^-$ terminal blocks (Figure 2A). However, four $CMA_3^+/DAPI^-$ terminal blocks were found in few amount of polyploidy cells with $2n = 56$ chromosome (Figure 2B). In some cases, the $CMA_3^+/DAPI^-$blocks were distanced from the chromosomes, but it can be clearly observed by photo overlaps. The number of $CMA_3^+/DAPI^-$ blocks was confirmed by the number of CMA_3^+ blocks on interphasic nucleus.

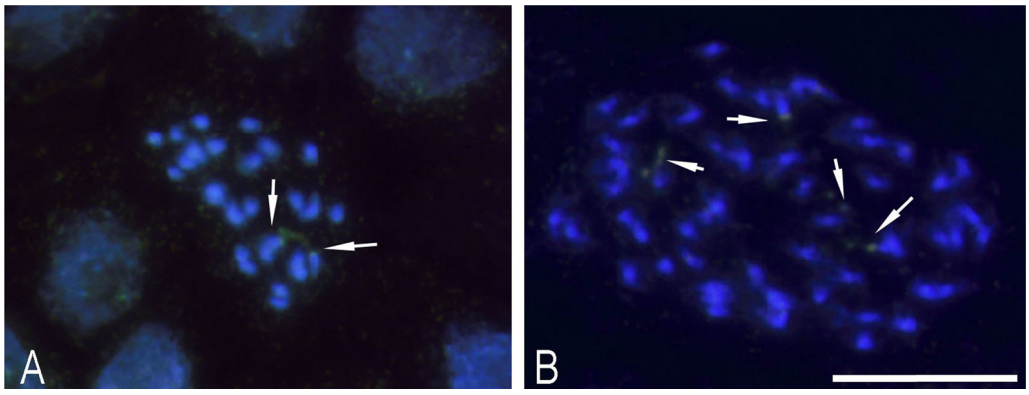

Figure 2. Metaphases of the algaroba complex. (A) overlap $CMA_3^+/DAPI^-$ in diploid cell. (B) Overlapping $CMA_3^+/DAPI^-$ in polyploid cell. Arrows indicate $CMA_3^+/DAPI^-$ blocks related to secondary constrictions and satellites. Bar = 10 μm

3.2 Cross-Species Amplification of SSR

The source of the SSR primers used in the cross-amplification test to algaroba are five *Prosopis* species of

natural populations concentrated in arid, semi-arid (*P. alba*, *P. chilensis*, and *P. flexuosa*), and subtropical (*P. ruscifolia* and *P. rubriflora*) regions of South America.

Among the 40 primers tested, 34 (85%) were cross-amplified. However, only 22 primers were selected as a functional with ideal minimum size of 100 base pair (bp) fragment for genotyping, excluding the possibility of false allele interpretations in reading the peaks (Table 1). Of these amplified primers, at least one allele per locus was verified. Amplicons in the 176 bp and 371 bp range were detected. The amplification temperature of most of the primers (16) was at 56 °C, and the others required specific temperatures of 52 °C to 58 °C.

Table 1. SSR primers derived from *Prosopis* species cross-amplified to algaroba complex with allele amplitude and optimized hybridization temperature

Locus	Forward 5′-3′ sequence	Reverse 5′-3′ sequence	Amplicon	TM ºC
I-P06286b	TGACAACCCATCTTCTTCTTCA	ATTTGCACAAGGGTAAAGATGG	176-246	52
I-P06639	CATCCCGTTCAAGTCCAAGT	AGCCCCCTTCCAACTTCTAA	184-260	57
I-P10500	CTCCGACAGATTCAGCATCA	TTCTTTCAAACTCGCCATCA	230-305	56
I-P07653	AGTGATGATTCGGATCCTGG	GAGAGACGAGGACTTGGTGC	136-250	56
I-P00930d	TCGAGATTTTCTTGGGGTTG	AAATTCCCTCCTCCTCCAAA	146-208	56
I-P00930c	TATGGCGCTATTTTTGGAGG	TCATGCTCCTCACAATCTGC	206-270	56
I-P00930b	GCAACAGCACTGCTTCAAA	AAAATAGCGCCATAGTTTGCTC	230-298	56
S-P1DKSFA	GTTTACCCATTGCAGGTCGT	CCCCATATGCAGAATCACCT	132-193	55
I-P03211	TTGCTTCAGAAAGCTGCTCA	AACCCTCGAAGATGATGGTG	160-229	56
Mo05	AATTCTGCAGTCTCTTCGCC	GATCCCTCGTGACTCCTCAG	184-248	56
Mo07	GAAGCTCCCTCACATTTTGC	CTATTTGCGCAACACACAGC	155-243	56
Mo08	TATCCTAAACGCCGGGCTAC	TCCCATTCATGCATACTTAAACC	174-252	56
Mo09	ATTCCTCCCTCACATTTTGC	CATTATGCCAGCCTTTGTTG	173-275	56
Prb9	TTCTTCTCCTTCTTCATCTTCCTCC	ACAACGTTGATCCCAAAACCTAAG	137-205	56
Prsc1	AATGGAGTTTGTTTGTGTCTGTGG	ATTACGGATACATCGAGCCTTCTT	243-327	56
Prsc3	CCACAAGCACACGCACACTCAGAC	CCAGCACTAGACTTCGCCACCAAC	126-190	58
Prsc4	CAAAATCCAACAAATAAACACACC	GGCGGATTCTTGGCTCTCT	188-262	57
Prsc5	CGCGTTAAGTCTGCCTTGCTTT	CTCATGGTATTTCCCTTGTCGTCC	190-270	56
Prsc6	CGAGCGGCGAAAAATGATAAA	GCTGCTTCCCATAATCCTCTCCT	154-240	56
Prsc10	AACGCAACGGCCGCAACTAT	ACAAAACGCTCGAATACTGGGGG	230-314	56
Prsc11	CCCGGCAACTCAAATCAACTTCATA	GTCTAATTCTATTGGTGGGCTCTCTGG	199-421	57
Prsc12	GGGGTGCATGTTGGGGATTG	TTTGGCCGGATTAAAACAGAGCA	155-253	56

3.3 Genetic Characterization of SSR Loci

The genetic data analysis in 20 algaroba genotypes for polymorphic loci is described (Table 2). Among 22 primers, 13 amplified more than one allele, four of which were the maximum number of alleles found per locus, and a total of 46 alleles were distributed among the 20 accessions of the algaroba. The loci showed a mean H_O that was greater than the H_E, and the mean of the PIC was 0.167, having values lower than or similar to those of the species to which the loci were developed.

Table 2. Descriptive genetics of microsatellites analyzed in algaroba

Locus	**N**	**A**	H_O	H_E	**PIC**	**HWE**	***F***
I-P06286b-A	20	1	0.000	0.000	0.000	NS	-
I-P06639-A	20	1	0.000	0.000	0.000	NS	-
I - P10500-A	20	1	0.000	0.000	0.000	NS	-
I-P07653-A	20	1	0.000	0.000	0.000	NS	-
I-P00930d-A	20	1	0.000	0.000	0.000	NS	-
I-P00930c-A	20	3	0.650	0.454	0.371	NS	-0.433
I-P00930b-A	20	2	0.050	0.049	0.048	NS	-0.026
S-P1DKSFA-A	20	1	0.000	0.000	0.000	NS	-
I-P03211-A	20	3	0.950	0.524	0.410	*	-0.814
Mo05-A	20	4	0.200	0.269	0.256	NS	0.256
Mo07-A	20	3	0.450	0.366	0.326	NS	-0.229
Mo08-A	20	1	0.000	0.000	0.000	NS	-
Mo09-A	20	2	0.000	0.095	0.090	NS	1.000
Prb9-A	20	1	0.000	0.000	0.000	NS	-
Prsc1-A	20	3	0.400	0.516	0.406	NS	0.225
Prsc3-A	20	2	0.050	0.049	0.048	NS	-0.026
Prsc4-A	20	3	1.000	0.524	0.410	**	-0.909
Prsc5-A	20	2	0.200	0.320	0.269	NS	0.375
Prsc6-A	20	3	0.050	0.096	0.094	NS	0.481
Prsc10-A	18	3	0.222	0.204	0.194	NS	-0.091
Prsc11-A	20	3	0.100	0.096	0.094	NS	-0.039
Prsc12-A	8	2	0.375	0.430	0.337	NS	0.127
Total	-	46					
Mean	-	2.09	0.214	0.181	0.167	-	-0.007

Note. N = number of individuals with cross-amplified loci; A = number of alleles; PIC = informative content of polymorphism; H_O = observed heterozygosity; H_E = expected heterozygosity; HWE = Hardy-Weinberg equilibrium; *F* = inbreeding. NS = non-significant deviation * $p < 0.05$ ** $p < 0.01$.

The mean for the combined exclusion of the loci (Q) was 0.96, and the coefficient of identity (*I*) estimate was 0.00022963.

In the analysis of linkage disequilibrium, no non-random association was found for the great majority of pairs of microsatellite loci, and loci with only one allele were not computed. The associated pairs were (Mo07, Prsc5), (Prsc5, Prsc10), and (Prsc1, Prsc11).

3.4 Grouping of Individuals in the Population

In the analysis of the main components, three groups were observed: one group with eight individuals, one group with 11 individuals, and another with just one individual (Figure 3).

3.5 Characteristics of Markers

In the analysis of the genetic diversity of different populations of the genus of *Prosopis* characterized by the microsatellite loci of several studies previously established in the literature, it can be observed that the mean values of H_O and H_E of all populations were intermediate or low (Table 3).

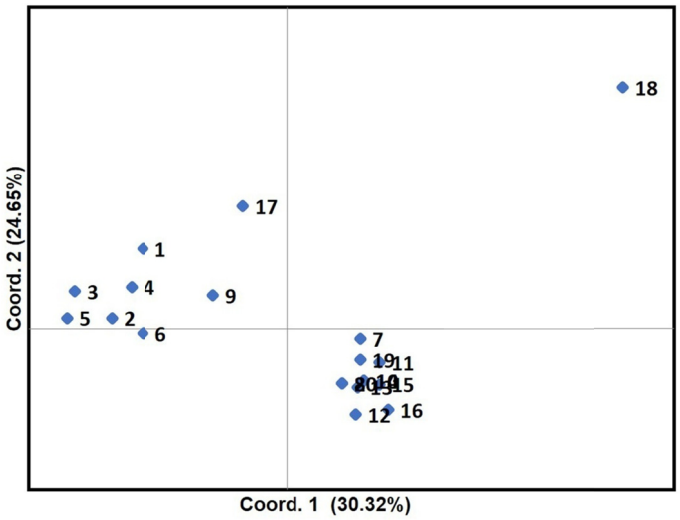

Figure 3. Dispersion of 20 algaroba individuals according to principal component analysis (PCA) based on genetic distance matrix calculated on the basis of microsatellite markers

Table 3. Mean values of H_O and H_E estimates of different populations of *Prosopis* spp.

Species	N	H_O	H_E	Pop	Source
Algaroba	13	0.214	0.181	CC	Data of this study
P. alba	11	0.366	0.414	PN	Pomponio et al., 2015
P. flexuosa	7	0.286	0.354	PN	Pomponio et al., 2015
Híbridos	8	0.286	0.344	PN	Pomponio et al., 2015
P. chilensis	8	0.310	0.319	PN	Pomponio et al., 2015
P. denudans	6	0.357	0.421	PN	Pomponio et al., 2015
P. hassleri	10	0.300	0.333	PN	Pomponio et al., 2015
P. rubriflora	8	0.545	0.592	FSM	Alves et al., 2014
P. rubriflora	9	0.463	0.529	FSV	Alves et al., 2014
P. ruscifolia	12	0.390	0.537	FRC	Alves et al., 2014
P. ruscifolia	12	0.418	0.687	ECD	Alves et al., 2014
P. alba	12	0.570	0.595	PN	Bessega et al., 2013
P. chilensis	8	0.607	0.647	PN	Bessega et al., 2013
P. flexuosa	6	0.486	0.725	PN	Mottura et al., 2005
P. chilensis	6	0.525	0.508	PN	Mottura et al., 2005

Note. N = Number of loci used. CC = Commercial cultivation. PN = Natural population. FSM and FSV = (*Fazenda Retiro Conceição*) remaining areas of the Chaco region in Brazil. ECD = (*Estação do Carandazal*) remnant population highly anthropized. FRC = area recovered from a 15-year depression on a farm. Obs. In Pomponio et al. (2015), we did not consider loci with zero value in the calculation of H_O and H_E.

4. Discussion

In the present study, the chromosome number and diversity and genetic variability in microsatellite loci were evaluated in a Brazilian algaroba population. Our experimental data demonstrated that this population is formed by diploid individuals, which have polysomatism events, and present microsatellite loci with low polymorphism. Additionally, the analysis were based on the evaluation of algaroba diversity in combination with the allele diversity data in the literature, confirming the hypothesis regarding a low diversity of alleles in SSR loci as a common route in *Prosopis* species.

The level of polyploid of algaroba has been investigated using both chromosome count (Nogueira et al., 2007) and flow cytometry (Trenchard et al., 2008). However, the identification of chromosomal markers such as CMA_3^+ blocks and other cytological markers may contribute to the discovery of polyploid species and/or cytotypes by the multiplicity of these markers compared to diploid genotypes (Melo et al., 2014, 2017). The chromosomes of algaroba could be better analyzed by the application of the base-specific fluorochromes CMA_3

and DAPI, which facilitated the visualization and definition of the chromosome due to its small size, in comparing to Giemsa staining in our previous studies.

In the genus *Prosopis* the most common chromosome number found has been 2n = 28, based on the basic number x = 14 we can consider these diploid species. However, the *P. juliflora* taxon, found naturally in both North and South America, has been shown to be tetraploid with 2n = 4x = 56 (Trenchard et al., 2008). However, in Brazil, algaroba can present both diploid and polyploid cytotypes (Hunziker et al., 1975). It also corroborates the findings of Nogueira et al. (2007), which analyzed a population of *P. juliflora* from three municipalities of different Brazilian states. Only one of the accessions sampled in one of the populations was found in the tetraploid form while the others were diploids. Therefore, although the algaroba population analyzed in the present study is considered locally as *P. juliflora*, this population should be *P. pallida*.

The occurrence of polysomatism in the species consisting of cells or organs of the same individual with levels of ploidy was verified. Barow (2006) reports that polysomatism is common in well-adapted plants and is important for accelerated plant growth, as well as to support certain physiological cellular functions. This event differs from polyploidy, which consists of the entirety of the individual's cells having extra chromosome sets. The erroneous interpretations of polyploidy for *P. juliflora* have been explained by the polysomatism that commonly occurs in root tissues that are used in analyses, or even by non-accurate counting errors (Burkart, 1976). Additionally, the low frequency with which tetraploid cells are reported in the literature could be understood as possible polysomatism and not necessarily the appearance of polyploid individuals. Specifically, in relation to the results obtained in the present study, it has been proven to be a case of polysomatism.

Prosopis juliflora polyploidy origin is still unknown (Pasiecznik, 2001). Trenchard et al. (2008) found only tetraploid individuals for this species from different source, indicating that *P. juliflora* would be the only tetraploid specie related, whereas *P. pallida* and several other species of *Prosopis* would be diploid, since 32 species from the genera has been cytological analyzed. However, the algaroba population evaluated in the present study, known among those who cultivate *P. juliflora*, is a true diploid, with rare polysomatism. Introductions of *P. pallida* were made in Brazil, but there were no records or information on the places of these introductions (Burkart, 1976). This divergence proves the taxonomic complexity of the group, and even though there were errors of identification in the native populations of Peru and the Pacific coast. These data suggest the need for future taxonomic, cytogenetic, and phylogenetic studies from their native scale that can clarify both the history and the identification of consensus for these *Prosopis* materials in Brazil.

Our analyses using molecular markers revealed that loci I-P03211 and Prsc4 showed a deviation to the proportions expected by the Hardy-Weinberg equilibrium considering the 95% and 99% confidence interval, respectively. This result is due to the number of heterozygotes observed to be much higher than expected.

Despite the low allelic diversity, the average inbreeding coefficient or loss of heterozygotes (*F*) was considered to be optimal because the population presented a low level of heterozygosity (-0.007). On the other hand, the means for the combined exclusion of the loci (Q) and the estimation of the coefficient of identity (*I*) were low.

The 13 amplified loci with more than one allele are useful tools for future population and genetic diversity studies of algaroba, although they are not recommended for studies aimed at plant identification and protection due to the low values of the "Q" and "I" indices.

The microsatellite loci "Mo05" developed by Mottura et al. (2005) for *P. chilensis* and *P. flexuosa* detected more alleles in the algaroba, to which it was cross-amplified, than in the species to which it was developed. In addition, the same series of loci (Mo05, Mo07, Mo08, Mo09, Mo13, and Mo16) developed by Mottura et al. (2005) were cross-amplified in six other *Prosopis* species (*P. alba*, *P. caldenia*, *P. ferox*, *P. hassleri*, *P. nigra*, *P. ruscifolia*, and *P. torquata*) and the range of alleles found from zero to five, was very similar to that of our algarroba population that ranged from zero to four alleles per locus. Similarly, the loci developed by Alves et al. (2014) for *P. ruscifolia* and *P. rubriflora* detected between one and five alleles per locus, and only one locus (Prb9) of *P. rubriflora* amplified to algaroba with just one allele, probably due of the genetic distance between them. In a study carried out by Bessega et al. (2013), microsatellite loci developed for *P. alba* and *P. chilensis* also obtained a higher concentration between two and five alleles per locus. These loci were not used in this work for algaroba.

Although the loci developed by Torales et al. (2013) for *P. alba* have amplified algaroba DNA of the species tested, these loci did not show data of allelic diversity. Thus, the reduced number of alleles detected in the present study is explained by the low allelic diversity of the species of the genus *Prosopis*.

Despite the low allelic diversity, low inbreeding levels were found even in the phase of the introduction, history of the species, with possible genetic bottleneck events and founder effects. This fact can be explained on the

basis of two hypotheses. The first one is related to the fact that the "F" evaluates the result of the crossings of individuals from one generation to another; thus, this indicates that there was no intersection between relatives in the previous generation. The second hypothesis that can have a joint action with the first is the reproductive system of the species that favors heterozygosis, because it is allogamous and self-incompatible.

As for the associated pairs in the analysis of linkage disequilibrium, we suggest the use of X, Y, and Z only because they contain a higher ICP or because they have a greater number of alleles.

The data of the grouping of the individuals evidences the dispersion of the accessions sampled by the genetic distance. Of the three groups, one has individuals that were more closely connected, indicating the possibility of having origins of the same region in cases of cultivated individuals or of individuals those have been naturally dispersed by the region. It is possible that these individuals may be the fruit of crossing individuals that are physically close and the other groups otherwise. Despite the genetic distance shown in the analysis, the 18 followed the same pattern of the other accessions collected.

The results of this study add value to the literature, showing the importance of having molecular tools from species with a close phylogenetic relationship, because the algaroba (*P. juliflora-P. pallida*) has no SSR developed and the present work contributed 22 cross-amplified SSR loci.

The results of the diversity of the different populations of *Prosopis* may be related to the low number of alleles found in the *Prosopis* species, for which molecular data are known. Another observation is that, among these species, only the population used in the present study presented an estimate of H_O higher than that of H_E. Because the area sampled in the present study is a growing area, introductions of different accessions may have favored the observed heterozygosity, even with low allelic diversity.

In addition to our comparison with the literature, we added new genotyping data of a Brazilian population of algaroba. However, there are populations of algaroba in different states of northeastern Brazil. Therefore, the following question remains open for future investigation: are the different Brazilian populations formed by diploid individuals? Do these populations have the general tendency of low polymorphism in SSR loci? There were multiple introductions of algaroba in Brazil? The microsatellite loci that showed cross-amplification and PIC in algaroba in the present work are useful to delineate new studies aimed to answer these questions.

Although the different studies carried out at distinct areas such as natural populations, anthropic areas and recovered areas, there was no correlation pattern with H_O values. The natural population of *P. chilensis* showed the highest mean value of H_O (0.607) of the presented studies, however it was lower than the mean H_E value (0.647). This information reinforcing that the low genetic diversity is not strictly related to the environmental conditions of the study area, but with a natural characteristic of the genus.

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Appendix A

Characteristics of the 40 SSR primers obtained in the literature of different species of *Prosopis* for cross-amplification in algaroba DNA

N	Locus	Forward 5′-3′ sequence	Reverse 5′-3′ sequence	TM	Motif
1	I-P06286b	TGACAACCCATCTTCTTCTTCA	ATTTGCACAAGGGTAAAGATGG	-	(TC)5
2	I-P03325a	CGTGCATGAATGTCACAGAC	AGGGTGAGATCAGAAGGCAA	-	(CA)5
3	I-P06639	CATCCCGTTCAAGTCCAAGT	AGCCCCCTTCCAACTTCTAA	-	(AT)5
4	I-P10500	CTCCGACAGATTCAGCATCA	TTCTTTCAAACTCGCCATCA	-	(TTC)6
5	I-P07653	AGTGATGATTCGGATCCTGG	GAGAGACGAGGACTTGGTGC	-	(GTT)4
6	I-P00930d	TCGAGATTTTCTTGGGGTTG	AAATTCCCTCCTCCTCCAAA	-	(AAC)6
7	I-P00930c	TATGGCGCTATTTTTGGAGG	TCATGCTCCTCACAATCTGC	-	(GTC)4
8	I-P00930b	GCAACAGCACTGCTTCAAA	AAAATAGCGCCATAGTTTGCTC	-	(ACC)5
9	S-P1DKSFA	GTTTACCCATTGCAGGTCGT	CCCCATATGCAGAATCACCT	-	(TTTA)3
10	S-P1EPIV2	TAAGCATTCATAGCCAGCCC	GACCAGGTCCTGTTTACCGA	-	(TAA)4
11	I-P03211	TTGCTTCAGAAAGCTGCTCA	AACCCTCGAAGATGATGGTG	-	(AAT)4
12	Mo05	AATTCTGCAGTCTCTTCGCC	GATCCCTCGTGACTCCTCAG	64	(CT)3T(CT)2
13	Mo07	GAAGCTCCCTCACATTTTGC	CTATTTGCGCAACACACAGC	59	(GC)8
14	Mo08	TATCCTAAACGCCGGGCTAC	TCCCATTCATGCATACTTAAACC	59	(AC)9
15	Mo09	ATTCCTCCCTCACATTTTGC	CATTATGCCAGCCTTTGTTG	59	(TG)17
16	Mo13	TTGATTAGAGTTGCATGTGGATG	TGCAGTCCCAAGTGTCAGAG	58	(GT)10CT(GT)2
17	Mo16	CATTGCCCCAATATCACTCC	GGGTCCATCCAGAGTAGTGG	60	(CA)12
18	Prb1	AACTACCGCAGCACTTTTCAGA	ACTACTTGGAGATGCCGTGGA	62.7	(GT)7
19	Prb2	GAAAGCCGCGCTCCTAAG	ATTCTTTTGTGTCTTGTCTTCTCG	61	(GC)4(AC)7
20	Prb3	TCCAAAGACCGCAAGAAGAT	AGGCCAAAAAGGACTCAAAAT	61	(CA)7
21	Prb4	ATCCGATAAATACACCTTCTGG	GGTGTATCGTAAAAGCCTGG	61	(CA)8
22	Prb5	TTTAAACATTGCACGTGAACCTAT	TTCACCCCTAAACCCCCTT	56.4	(AC)9
23	Prb6	CATCTCTCAAAGAAAACGCACTC	CCGCAGAGAAGCCCCTACATA	56.4	(TG)10
24	Prb7	GGCTTAGCATCACCCTCCAT	CTTACCCTTTCAGTCCATTTACCA	61	(AC)8
25	Prb8	CAACACCAAAACGGCGAGATGAT	TTCGCCAAACGCCAGCATTAG	61	(GT)13
26	Prb9	TTCTTCTCCTTCTTCATCTTCCTCC	ACAACGTTGATCCCAAAACCTAAG	62.7	(AC)9
27	Prb10	TTTTGGTGGATTTGATAGAGCC	GAGTGGGGTCAAGAAAGAACAG	56.4	(TCA)5
28	Prsc1	AATGGAGTTTGTTTGTGTCTGTGG	ATTACGGATACATCGAGCCTTCTT	56.5	(AC)9(CT)5
29	Prsc2	GCGGAATTCCAAACGACAA	ACAGCAACACCCTCACTCTCAA	64.7	(AC)9
30	Prsc3	CCACAAGCACACGCACACTCAGAC	CCAGCACTAGACTTCGCCACCAAC	64.7	(CA)6
31	sc4	CAAAATCCAACAAATAAACACACC	GGCGGATTCTTGGCTCTCT	63.9	(CAA)2(GA)4
32	Prsc5	CGCGTTAAGTCTGCCTTGCTTT	CTCATGGTATTTCCCTTGTCGTCC	59	(GT)8
33	Prsc6	CGAGCGGCGAAAAATGATAAA	GCTGCTTCCCATAATCCTCTCCT	63.9	(GT)8
34	Prsc7	AGGGATTTAATCTCTTTGGTGTAG	ACAAGCTGGAAAGAGTCGCA	59	(TG)8(GTGG)2(GT)5
35	Prsc8	AGTGACGTGAACACGCTGAGG	TGCTGATGTGTGTGGTTTTGAGAT	62.7	(TG)10
36	Prsc9	TCAGACTCCCGTGAACCAG	CGCACTCGAGCAGCATCT	59	(TG)9
37	Prsc10	AACGCAACGGCCGCAACTAT	ACAAAACGCTCGAATACTGGGGG	56.5	(CA)7(CT)7
38	Prsc11	CCCGGCAACTCAAATCAACTTCATA	GTCTAATTCTATTGGTGGGCTCTCTGG	62.7	(AC)11
39	Prsc12	GGGGTGCATGTTGGGGATTG	TTTGGCCGGATTAAAACAGAGCA	59	(GT)10
40	Prsc13	CTTCACCATCACCGATTTCCCTT	GCAACGAAGCAGCTGAAGAACAC	62.7	(CTT)5

Note. N, number of the primer, in which species and references of SSR loci are as follow: 1-11 *P. alba* (Torales et al., 2013); 12-17 *P. chilensis* and *P. flexuosa* (Mottura et al., 2005); 18-40 *P. rubriflora* and *P. ruscifolia* (Alves et al., 2014).

TM is melting temperature in ºC.