

Genetic Diversity in *Mimosa tenuiflora* (Willd.) Poir.: A Multipurpose Plant Genetic Resource of Semiarid Brazil

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Abstract

Caatinga is the third largest biome in Brazil but little is known about the species diversity from this biotic community, despite of its social, economic and environmental importance for the semiarid region. Among the several typical plant species from Caatinga, *Mimosa tenuiflora* (Willd.) Poir. (black jurema) stands out because it plays a major role in the maintenance of this ecosystem, besides being widely used to recover degraded areas. Therefore, the goal of this study was to evaluate the genetic diversity and structural analysis from 10 populations of *M. tenuiflora* from the state of Bahia, northeastern Brazil, using 10 ISSR (Inter Simple Sequence Repeat) markers. A total of 117 fragments were obtained from 218 individuals with a mean number of 11.8 bands per primer. The mean population polymorphism was 85.0%, while the values of genetic diversity (H_e) and the Shannon index (I) were equal to 0.295 and 0.442, respectively. Most of genetic variation was observed (87.0%) but high F_{ST} values were observed (0.132), indicating the populations are genetically differentiated. Bayesian inference using Structure divided the populations into two groups while Geneland indicated five clusters that could be related to the fragmentation of Caatinga and to constraints in the dispersal of pollen and seeds. In conclusion, *M. tenuiflora* presents high levels of genetic diversity and natural populations might serve as potential sources for management and reforestation of degraded areas in Caatinga.

Keywords: Caatinga, genetic diversity, ISSR, reforestation

1. Introduction

Caatinga is a poorly known and highly threatened biome, restricted to Brazilian semiarid areas that have suffered from intensive deforestation for cattle and charcoal production as well as inappropriate irrigation systems, thus leading to desertification, sedimentation of rivers and soil erosion (Leal et al., 2005). However, the lack of detailed information about the regional biodiversity restrains effective conservation efforts in Caatinga. *Mimosa* L. is a species-rich genus in Leguminosae, comprising about 540 taxa (Simon et al., 2011) widespread throughout distinct phytophysognomies over tropical and subtropical regions in Americas. Brazil is a major center of origin for this genus (Barneby, 1991), with a high number of species (350), being 38 of them found in Caatinga (Queiroz, 2009; Dutra & Morim, 2015a).

Mimosa tenuiflora (popularly known as black jurema) represents an important natural resource in Caatinga along semiarid regions of northeastern Brazil, being found in the states of Alagoas, Bahia, Ceará, Paraíba, Pernambuco, Piauí, Rio Grande do Norte and Sergipe (Dutra & Morim, 2015b). Throughout its range, black jurema is widely used as timber for furniture, bridges, fences and studs, as charcoal in energy production (Maia, 2004; Riegelhaupt & Pareyn, 2010) and to feed cattle (Braga, 1989). On the other hand, the tree bark powder is popular in traditional medicine to treat burns, acne, and wounds, since it presents antibiotic, analgesic and astringent properties (Albuquerque, 2007) and antifungal activity in the control of *Alternaria alternata* in citrus (Pinto et al.,

2018). Furthermore, it is considered a sacred plant for native tribes from semiarid Brazil who prepare a wine from the bark to be used as a ceremonial drink, a tradition that was later expanded to some Afro-Brazilian religious activities (Maia, 2004). As a matter of fact, psychoactive effects are recognized in jurema wine because it is rich in the alkaloid N, N-dimethyltryptamine or DMT (Souza, 2008). This specie has been regarded as “high conservation priority” after presenting local risk of extinction in the southern mesoregion of the state of Ceará because of overexploitation for medical purposes and animal feeding. The small number of individuals can be also related to losses of genetic variability contributing to lower population sizes (Santos et al., 2017).

Besides the social, cultural and economic relevance, black jurema also plays a major role in the maintenance of forest cover and ecological processes (e.g., pollinator attraction) in Caatinga favored by its fast growth and propagation, being particularly recommended to recover degraded areas (Queiroz, 2009; Maia-Silva et al., 2012). Adaptative morphological features to arid and semiarid ecosystems, such as higher size and stem diameter, make *M. tenuiflora* suitable for recovering degraded areas in Caatinga (Lima et al., 2018). This plant is visited by moths, butterflies, flies and beetles, but bees represent the main visitors since black jurema produces pollen throughout most of the year, being particularly helpful to the survival of colonies raised by bee keepers during dry seasons (Maia-Silva et al., 2012; Silva et al., 2015).

The knowledge about population structure is essential to conservation, genetic improvement, sustainable management, and selection of seed trees to recover deforested areas (Kageyama et al., 2003). The genetic diversity of *M. tenuiflora* was analyzed from 15 natural population located in state of Rio Grande Norte, revealing higher genetic variation within populations and the formation of four genetic groups. This study suggested that conservation efforts should focus on six populations, such as those with the highest and the lowest levels of genetic variation. In particular, the population of Espírito Santo (Rio Grande do Norte) was selected as a priority for conservation, as it presents the highest genetic distinctiveness in relation to the other population (Chagas, 2018). Hence, identification of genetically rich and divergent populations helps defining operational units that should be prioritized in ex situ or in situ conservation programs (Diniz-Filho & Telles, 2002).

Forest fragmentation might eventually lead to decreased population effective size and high inbreeding levels, thus depleting the original genetic variation of natural populations that hence become more susceptible to environmental, demographic and genetic changes (Rajora & Mosseller, 2001) even in widespread species such as *M. tenuiflora*. Several methods of molecular analyses have been used to assess the genetic diversity in natural populations of plants, including ISSR (Inter Simple Sequence Repeats) markers (Almeida et al., 2009). In spite of their dominant behavior, ISSR markers are advantageous to assess the genetic diversity in wild plants because they disregard previous information about genomes, are easily obtained, indicate variation within unique regions of the genome at several loci at the same time and provide reproducible results with high levels of polymorphism (Reddy et al., 2002; Kumar & Yadav, 2018).

Therefore, the goal of this study was to evaluate the genetic diversity and population structure in *M. tenuiflora* along the state of Bahia, northeastern Brazil, based on ISSR markers. The present information is useful to the management and conservation of this species that represents a major plant resource in Caatinga.

2. Material and Methods

2.1 Sampling

Leaf samples from 218 individuals of *M. tenuiflora* were collected in 10 sites from the state of Bahia, which comprises the largest area within the natural range of this species, comprising about 564 thousand km² (Table 1, Figure.1). To avoid the sampling of closely related individuals, a minimum distance of 10 m was applied among individual samples. A transect was defined across the state based on two major roads (BR-116 and BR-242) that cross the region and, thus, the sampling efforts comprised about 1,200 km from east to west and from north to south. Each sampled individual of *M. tenuiflora* was labeled *in situ* with a number code for identification and each sampled location was georeferenced using Garmin 12 GPS (Global Position System) receiver. The leaf samples were stored at -20 °C in the Laboratory of Molecular Genetics at UESB and voucher samples from each population were stored in the Herbarium from Universidade Estadual do Sudoeste da Bahia (HUESB) to confirm the species identification. The geographic distance among sampled populations was measured (Table 2) using the software DIVA-GIS (Hijmans et al., 2001) based on the coordinates of each collection site (Table 1). The coordinates were transformed into UTM (Universal Transverse Mercator) values to calculate the distance among localities.

Table 1. Localities, coordinates, altitude (A) and number of samples (N) per population of *Mimosa tenuiflora* (Willd.) Poir. in the state of Bahia

Localities	Abbreviation	Coordinates	A (m)	N
Vitória da Conquista	VC	14°51'48" S; 40°50'48" W	874	24
Poções	PO	14°40'17" S; 40°18'42" W	911	20
Jequié	JE	13°49'18" S; 40°05'51" W	218	15
Itaberaba	IT	12°44'25" S; 40°19'53" W	272	21
Seabra	SE	12°24'58" S; 41°46'09" W	812	21
Ibotirama	IB	11°59'40" S; 43°12'50" W	115	25
Barreiras	BA	12°8'50" S; 44°59'40" W	454	21
Santo Amaro	SA	12°32'26" S; 38°42'39" W	8	20
Serrinha	SR	11°39'43" S; 39°00'32" W	379	25
Euclides da Cunha	EC	10°30'29" S; 39°00'47" W	472	26
Total	-	- -	-	218

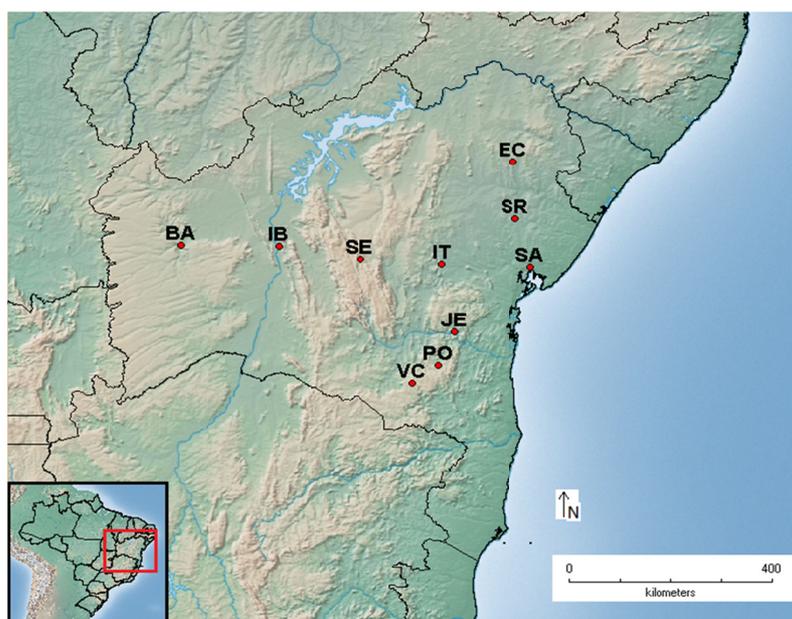


Figure 1. Mapping of the populations. Geographic distribution of the 10 sampled populations of *Mimosa tenuiflora* from the state of Bahia, Brazil (Table 1)

Table 2. Geographic distance among the populations of *Mimosa tenuiflora* collected in the state of Bahia, Brazil

	VC	PO	JE	IT	SE	IB	BA	SA	SR	EC
VC	0									
PO	63.0	0								
JE	143.0	97.0	0							
IT	235.0	238.0	187.0	0						
SE	288.0	297.0	244.0	160.76	0					
IB	412.0	433.0	401.0	322.01	166.0	0				
BA	542.0	582.0	568.0	510.2	353.0	196.0	0			
SA	349.0	294.0	210.0	180.83	336.0	496.0	685.0	0		
SR	408.0	366.0	274.0	188.31	313.0	456.0	656.0	107.0	0	
EC	523.0	484.0	391.0	285.74	371.0	487.0	680.0	232.0	130.0	0

2.2 DNA Extraction and Amplification

The total DNA was isolated from leaves of *M. tenuiflora* according to the optimized protocol by Arruda et al. (2017). After isolation, the DNA samples were quantified in 0.8% agarose gel using L-Quant spectrophotometer

(Loccus). Forty ISSR primers provided by UBC (University of British Columbia) were tested and 10 of them were selected based on their repeatability and high-quality of band profiles (Table 3).

Table 3. ISSR primers and their respective sequences, annealing temperature (T_m), total number of loci, and number of polymorphic loci

Primer	Sequence (5'-3')	T _m (°C)	Number of loci	Number of polymorphic loci
UBC-807	AGAGAGAGAGAGAGT	53	10	8
UBC-811	GAGAGAGAGAGAGAC	53	11	9
UBC-812	GAGAGAGAGAGAGAA	52	10	9
UBC-815	CTCTCTCTCTCTCTG	55	14	12
UBC-827	ACACACACACACACG	53	16	14
UBC-836	AGAGAGAGAGAGAGYA	52	10	5
UBC-845	CTCTCTCTCTCTCTRG	53	11	10
UBC-854	CTCTCTCTCTCTCTRG	54	16	15
UBC-856	ACACACACACACACYA	53	12	10
UBC-864	ATGATGATGATGATGATG	53	7	7
Total	-	-	117	99
Mean	-	-	11.8	9.9

The DNA amplification via PCR (Polymerase Chain Reaction) was performed using 10 mM Tris-HCl pH 8.3, 2.5 mM of MgCl₂, 1 mM of dNTPs, 0.2 M of each primer, 5 U of Taq DNA polymerase (Biotools) and 40 ng of template DNA and ultrapure water to a final volume of 25 µL in a MG108+ thermocycler. The PCR conditions were: first denaturation step at 94 °C for 3 min, followed by 40 cycles of 1 min at 92 °C, 2 min at optimum annealing temperature (Table 3), 2 min at 72 °C, plus a final extension at 72 °C for 7 min. The amplified products were run in 1.2% agarose gel for 2h and 30 min at 100 volts and photodocumented under UV light using L-Pix system (Loccus). A 1000-bp ladder was used to estimate the fragment size while poorly define or weakly stained bands were disregarded from the present analyses.

2.3 Data Analysis

A binary matrix from the band profile for each ISSR primer was built in which “0” and “1” indicated the absence and the presence of a particular band, respectively. Based on these data, we estimated the Shannon’s (I) and Nei’s (He) genetic diversity (Nei, 1973), Nei’s genetic distance (Nei, 1978), gene differentiation between populations (F_{ST}) and gene flow (*Nm*) levels using the software TFPGA 1.3 (Miller, 1997). The pairwise Nei’s genetic distances was estimated using the software GenAlEx 6.5. In order to verify whether the genetic and geographic distances were correlated or not, we performed the Mantel’s test (Manly, 1997) with 10,000 permutations, using AIS 1.0 (Miller, 2005). In the software Arlequin 3.0 (Excoffier & Schneider, 2005), we carried out the analysis of molecular variance (AMOVA) to estimate how the genetic variation was partitioned using 10,000 bootstrap permutations to test its significance.

To estimate the degree of admixture among sampled populations, a Bayesian inference of population structure was obtained in the software STRUCTURE 2.2.3 (Falush et al., 2007). The number of tested populations (K) ranged from 1 to 11, with 10 iterations and 1 million MCMC (Markov Chain Monte Carlo) generations with a burn-in of 100,000 generations. After adjustments in the software for dominant markers, we determined the best admixture model and assumed that the allele frequencies were uncorrelated among populations (Wang et al., 2012). The most likely K value was calculated using the platform Structure Harvester Web v.0.6.9 following the parameters established by Evanno et al. (2005). A second Bayesian model was tested using the GENELAND R package in order to analyze the geographic distribution of the genetic variation (Guillot, 2012). The number of clusters (K) was defined within an interval from 1 to 10 to infer the actual K value comprising the maximum number of populations following 1 million MCMC generations and a burn-in of 1,000 generations. A Principal Component Analysis (PCA) was also performed using the software PAST (Hammer et al., 2001) to visualize the degree of relatedness between populations based on the matrix of genetic distance for the 117 ISSR alleles.

3. Results

3.1 Genetic Diversity

A total of 117 bands were obtained from the 218 individuals of *M. tenuiflora*. The number of amplicons per ISSR primer ranged from 7 to 16 (UBC-864 and UBC-827, respectively), with a mean number of 11.8 bands/primer

(Table 3) and fragment sizes between 250 and 2000 base pairs (pb). At a population level, the mean percentage of polymorphic bands per locality was 85.0%, being the lowest and the highest values observed in Jequié (65.0%) and Serrinha (94.9%), respectively (Table 4). The mean values of genetic diversity for the 10 populations were estimated in 0.295 (He) and 0.442 (I). On the other hand, these values were highly differentiated among populations, ranging from He = 0.239 and I = 0.354 in Jequié (JE) to He = 0.369 and I = 0.538 in Serrinha (SR) (Table 4).

Table 4. Nei's genetic diversity (He), Shannon's index (I) and percentage of polymorphic loci (PLP) in populations of *Mimosa tenuiflora* from the state of Bahia, Brazil

Population	He	I	PLP (%)
VC	0.299	0.443	82.0
PO	0.315	0.466	84.6
JE	0.239	0.354	65.0
IT	0.339	0.496	88.0
SE	0.347	0.509	91.4
IB	0.348	0.510	90.6
BA	0.294	0.434	79.5
SA	0.305	0.450	81.2
SR	0.369	0.538	94.9
EC	0.368	0.536	94.0
Mean	0.322	0.474	85.0

3.2 Genetic Structure in Natural Populations of *Mimosa tenuiflora*

The AMOVA revealed that most of genetic variation was found within populations (87.0%) of *M. tenuiflora* (Table 5). Nonetheless, high and significant levels of interpopulation genetic differentiation were detected ($F_{ST} = 0.132$; $p = 0.001$) along with estimates of less than one migrant individual per generation ($Nm = 0.87$; $p < 0.001$). In fact, Mantel's test revealed significant correlation between genetic and geographic distances in the analyzed populations ($r = 0.19$, $p = 0.001$). The lowest values in pairwise genetic distance were observed between the samples from VC \times PO (0.074) and PO \times JE (0.087), while the populations from VC and EC showed the highest genetic divergence (0.225) (Table 6).

Table 5. Population structure and genetic divergence in populations of *Mimosa tenuiflora* from the state of Bahia based on 10 ISSR markers

Source of variation	AMOVA ^a (%)	<i>p</i> -value*	F_{ST} ^b	<i>p</i> -value*	Nm ^c
Among populations	13.0	<0.000	0.132	<0.001	0.870
Within populations	87.0	<0.000			
Total	100				

Note. ^a = analyses of molecular variance; ^b = index of genetic fixation; ^c = gene flow; * significance level.

Table 6. Pairwise Nei's genetic distance in populations of *Mimosa tenuiflora* from the state of Bahia, Brazil

	VC	PO	JE	IT	SE	IB	BA	SA	SR	EC
VC	0.000									
PO	0.074	0.000								
JE	0.192	0.087	0.000							
IT	0.182	0.134	0.098	0.000						
SE	0.178	0.135	0.140	0.134	0.000					
IB	0.148	0.122	0.104	0.088	0.123	0.000				
BA	0.200	0.165	0.147	0.149	0.175	0.151	0.000			
SA	0.207	0.150	0.167	0.166	0.161	0.164	0.138	0.000		
SR	0.216	0.171	0.154	0.119	0.171	0.139	0.220	0.158	0.000	
EC	0.225	0.218	0.215	0.180	0.167	0.210	0.199	0.193	0.165	0.000

Accordingly, the Bayesian inference of population structure confirmed that the $K = 2$ is the most suitable number of genetic groups in *M. tenuiflora* (Figure 2) (see Appendix A1 for details). The first group (green) comprised the populations VC, BA, SA and EC and the second (red) grouped the populations PO, JE, IT, SE, IB and SR. The analysis based on GENELAND separated the 10 populations into five clusters (Figure 3). The first cluster (dark green) was formed by samples from BA; the second group (light green) comprised the VC and PO populations; the third group (yellow) encompassed the SA and SR samples; the fourth group (beige) was formed by EC; and the fifth group (grey) grouped the samples from IB, SE, IT and JE. Moreover, the PCA discriminated only the population from BA in relation to the others, which composed a large group (Figure 4).

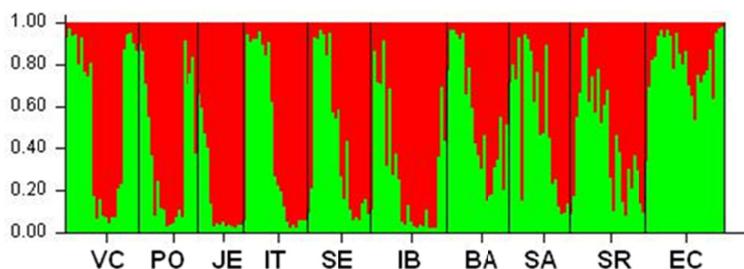


Figure 2. Result of population analysis of *Mimosa tenuiflora* showing the genetic relatedness of 10 populations of *Mimosa tenuiflora* (Table 1) estimated by STRUCTURE based on ISSR markers ($K = 2$; Mean (LnProb) = -14143.590). The vertical lines indicate the specimens and the colors represent the allele frequencies

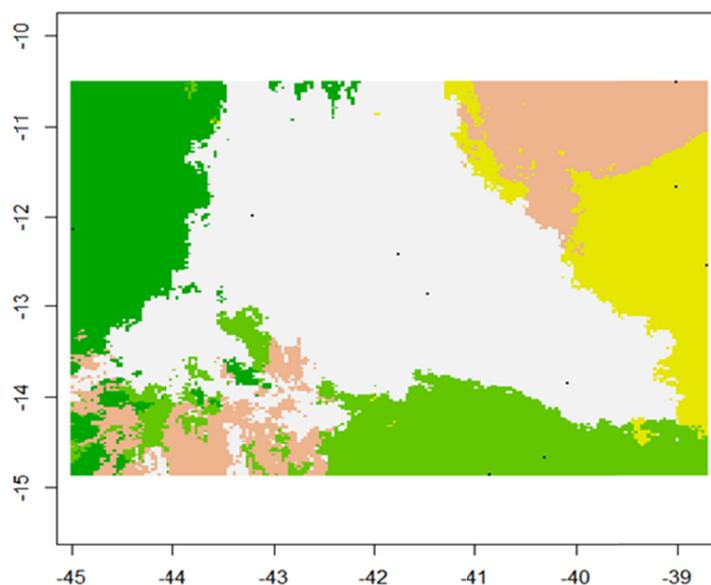


Figure 3. Mosaic of the spatial distribution of genetic groups in *Mimosa tenuiflora* based on the algorithm implemented in GENELAND ($K = 5$). The dots indicated the geographic location of populations and each color represents the genetic clusters, as follows: 1 (light green) comprising the BA samples, 2 (dark green) encompassing VC and PO, 3 (yellow) including SA and SR, 4 (beige) composed of EC and 5 (grey) comprising JE, IT, SE and IB

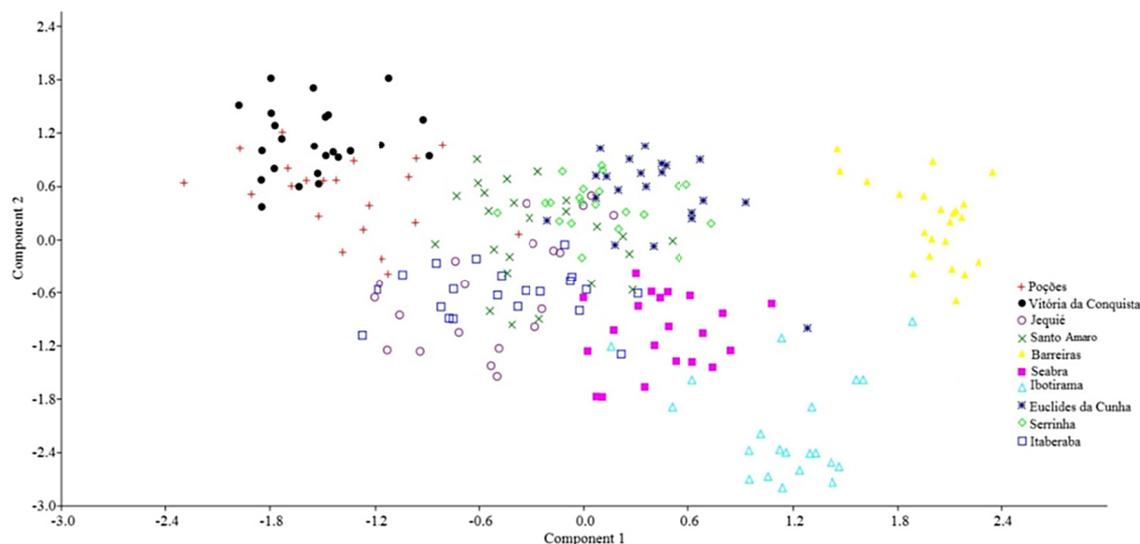


Figure 4. Graph of the PCA showing the position of each population along the axis. Principal Component Analysis (PCA) in populations *Mimosa tenuiflora* sampled in the state of Bahia, Brazil, based on the matrix of genetic distance of 117 ISSR alleles

4. Discussion

4.1 Genetic Diversity

A high percentage of polymorphic loci (mean value around 85.0%) was detected in the present study, being superior to that reported in *M. tenuiflora* (65.3%) (Chagas, 2018) and *Mimosa caesalpiniaefolia* Benth (52.7%) (Araújo et al., 2016). The present values are typical of cross-fertilized plants as commonly observed in Fabaceae species, like *Enterolobium contortisiliquum* (Moreira et al., 2015) and *Erythrina velutina* Willd (Gonçalves et al., 2014). Increased levels of polymorphism have also been described in plant species from heterogeneous desert habitats, such as *Medicago ruthenica* (L.) Trautv. (Fabaceae) (Li et al., 2013), *Haloxylon ammodendron* (Sheng et al., 2004) and *H. salicornicum* Moq (Amaranthaceae) (Salameen et al., 2018). Similarly, Caatinga is a semiarid biome characterized by habitats with differences in sunlight incidence, mean temperatures, rates of evapotranspiration, and rainfall, usually restricted to short seasonal periods (Leal et al., 2003). As a result, species living in differentiated habitats under stressful conditions usually present high values of genetic variation across their range related to local adaptation processes (Martínez-Palacios et al., 1999; Shrestha et al., 2002). The lowest polymorphism observed in the populations from JE (65.0%) is likely to reflect the high degree of deforestation for agriculture and timber exploitation in this area, determining population decline and high levels of inbreeding eventually reducing local genetic variation.

The Nei's genetic distance and Shannon index values in *M. tenuiflora* were similar to those observed in other leguminous plants (Fabaceae), such as *M. ruthenica* (Li et al., 2013) and *Adesmia bijuga* (Guerra et al., 2018). According to Cole (2003), non-rare plant species usually show increased genetic diversity, since genetic drift is affected by small populations, resulting in decreased heterozygosity levels. This statement corroborates the present study since *M. tenuiflora* is an abundant species with high levels of genetic polymorphism. It should be pointed out that the genetic diversity is a key feature to the evolutionary potential of species inasmuch as it allows selecting adaptive genotypic combinations to specific environmental conditions (Sebbenn et al., 2000; Freeland et al., 2011).

4.2 Genetic Structure and Gene Flow

The present data revealed low levels of gene flow among populations of *M. tenuiflora* ($N_m = 0.87$) and moderate population genetic structure ($F_{ST} \sim 0.13$; $p = 0.001$). In plants, F_{ST} values above 0.15 are regarded as evidence of high population structure (Frankham et al., 2002), being inversely proportional to gene flow levels (Salameen et al., 2018). Most of genetic variation in *M. tenuiflora* was found within populations, as similarly observed in a previous report with this species in state of Rio Grande do Norte (Chagas, 2018) and other cross-fertilized plants such as *M. ruthenica* (Li et al., 2013), *Caryocar brasiliense* Camb. (Melo Jr. et al., 2012), *H. ammodendron* (Sheng et al., 2004), and *H. salicornicum* (Salameen et al., 2018). Nonetheless, the reproductive biology of *M.*

tenuiflora, an essential aspect to infer the population genetic structure (Hamrick, 1989; Salameen et al., 2018), remains largely unknown.

As observed in other biomes, Caatinga has been impacted by human activities (e.g., uncontrolled deforestation) thus leading to habitat fragmentation which restrains the gene flow among the populations of *M. tenuiflora*. Previous reports suggest that values of gene flow equal or higher than 1.0 are able to prevent the genetic differentiation among populations (Slatkin & Barton, 1989). According to the present cluster analyses and Nm values, the gene flow among the samples of *M. tenuiflora* are insufficient to avoid the population structure, putatively favored by the effects of low connectivity among individuals from fragmented habitats. Nonetheless, other features such as genetic isolation by geographic distance and genetic drift should also be taken into account (Khierallah et al., 2014). *M. tenuiflora* usually occur in aggregates with high population density, besides presenting reduced life cycles (~20 years) and short-distance seed dispersal, being thus more susceptible to limited gene flow and high genetic population structure (Wright, 1943).

The groups formed evidenced by the cluster analysis (Figure 2 and Figure 4) may result from the increased gene flow among nearby individuals along the distribution of populations, following the isolation-by-distance model by Wright (1943). Considering the high fragmentation of the Caatinga biome, gene flow can also be limited by the pollination by insects that usually fly over short distances. To date, there are no studies about the pollen dispersal in *M. tenuiflora*, but bees, wasps and flies have been regarded as their main pollinators (Maia-Silva et al., 2012). For instance, bees (*Apis mellifera* and *Bombus morio*) have been observed as effective pollinators of *M. bimucronata* (Silva et al., 2011) while domestic goats (*Capra hircus*) have been reported as occasional dispersers of *M. luisana*, because the infestation of bruchids in seeds can reduce the amount of seeds in the excrement from goats (Giordani, 2008). The Mantel's test corroborated the isolation model since a low but significant correlation between genetic and geographic distance was observed ($r = 0.19$, $p < 0.001$).

The number of clusters differed between both Bayesian approaches, what could be related to the distinct parameters from each analysis. It should be pointed out that Geneland takes into consideration the geographic and genetic values while Strucutre considers only the genetic estimates. According to the results based on Geneland, the cluster 1 included the first record of *M. tenuiflora* in cerrado (a Brazilian savannah biome), represented by the samples from BA. The discrimination of BA individuals in two out of the three cluster analyses should be related to the fact that this is the only population of this species found in cerrado and separated by the São Francisco River (Figure 1), thus placing this locality apart from all the other populations and putatively acting as a barrier to gene flow. The group 2 (VC and PO) comprised the populations separated by short geographic distances, high altitude and similar vegetation, favoring gene flow within this cluster. Similarly, the populations in group 3 (SA and SR) represented nearby localities in the northern portion of Bahia. The group 4 (EC) was separated from the others, representing the population located in the northern range of this species. The populations from the group 5 (IT, SE, IB, and JE) are geographically close to each other and inhabit areas that share similar altitudes, landscape and rainfall indexes (Tables 1 and 2). A similar separation of clusters was observed in the analysis using Structure, inasmuch four (IT, SE, IB and JE) out of six populations in group 2 (IT, SE, IB, JE, PO and SR) were clustered in both Bayesian approaches.

Once the genetic diversity is directly related to the survival of species in face of environmental changes (Freeland et al., 2011) and little is known about the genetic structure in most organisms from dry regions, the present data in populations of *M. tenuiflora* from semiarid regions are essential to the proper restoration of forest cover in degraded biomes, such as Caatinga. The use of pioneer plants such as *M. tenuiflora* enables the subsequent establishment of other species, the stabilization and increase of the biological activity of the soil (Chaves et al., 2006), thus being a key species to maintaining biodiversity and ecosystem functionality. In general, the populations presented high levels of genetic variation, being relevant to species conservation. Moderate population structure in black jurema is possibly related to the remarkable fragmentation of Caatinga and biological features of the analyzed species that favor the genetic differentiation among populations of *M. tenuiflora* across their range. Therefore, the local conservation of genetically divergent populations is strongly recommended. In particular, special attention should be drawn to those populations with the highest levels of polymorphism to be used in reforestation programs in Caatinga or as sources for ex situ and in situ conservation of *M. tenuiflora*.

Then, we indicate the SR, EC and IB populations was the best options to management and conservation plans inasmuch as they present high rates of genetic diversity, therefore, being the most genetically represented populations. We also point out the importance of conserving the populations from JE, BA and SA since they share low levels of genetic variation in order to avoid furthers genetic losses.

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Appendix A

Graphs identifying the optimal number of populations following Evanno's methods

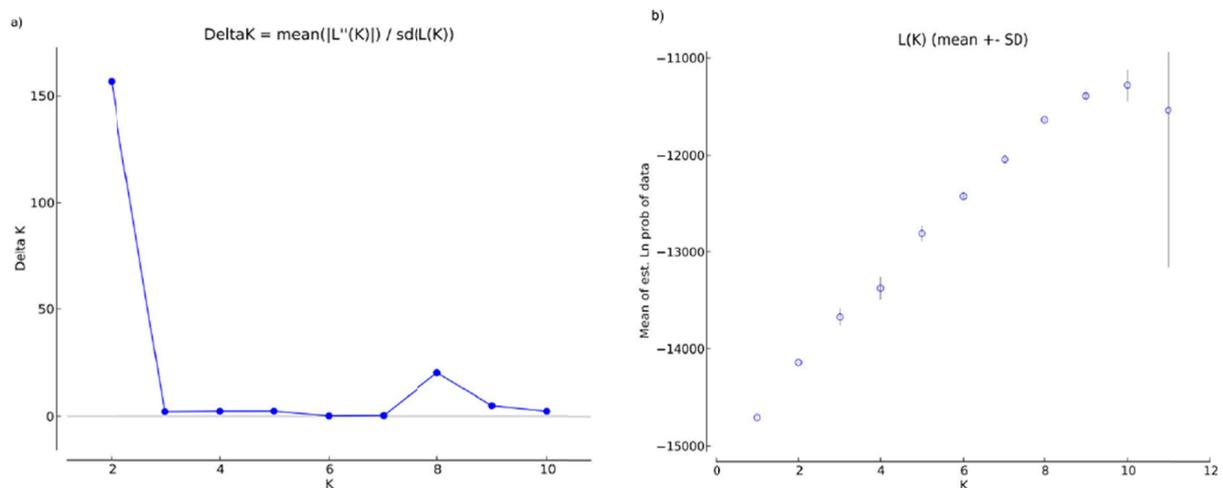


Figura A1. Graph used to demonstrate the DeltaK value (a) and the L(K) value (b), a single K value out of a range of K values, which captures the uppermost level of structure (Evanno et al., 2015)

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