CROP BREEDING AND APPLIED BIOTECHNOLOGY

ARTICLE

Genetic diversity and structure of *Hancornia speciosa* Gomes populations characterized by microsatellites markers

Crop Breeding and Applied Biotechnology
22(2): e38012228, 2022
Brazilian Society of Plant Breeding.
Printed in Brazil
http://dx.doi.org/10.1590/1984-70332022v22n2a18

Ana Kelly S. Maia[1], Jackeline Terto[1], Iêda Ferreira de Oliveira[1], Wellington Ferreira do Nascimento[2], Cícero Almeida[3] and Edson Ferreira da Silva[1*]

Abstract: Hancornia speciosa *Gomes, a native fruit tree, plays an important socio-economic role in Brazil's traditional communities. The objective was to estimate the genetic structure of 176 individuals from eight locations in the Cerrado, Caatinga and Atlantic Forest biomes, based on six microsatellite markers. The analyzes revealed the formation of five population groups: A- Pernambuco, Paraíba and Sergipe (Atlantic Forest), B- Maranhão (Cerrado); both, var. speciosa, C- Bahia (Caatinga / Cerrado transition zone) and Ceará (Caatinga), D- Goiás (Cerrado) and E- Minas Gerais (Cerrado,) being groups C, D and E possibly var. pubescens. Most of the genetic variation is within the groups (65.61%, Fst = 0.34, p <0.001). Although group A had the highest Ho (0.66), it had a negative fixation index (f = -0.02), while the other had positive values. Our results revealed high levels of genetic diversity and provide support the development of more efficient conservation strategies for H. speciosa.*

Keywords: *Conservation genetics, native fruit, mangaba, Brazilian biomes*

INTRODUCTION

The mangabeira (*Hancornia speciosa* Gomes, Apocynaceae) is a tropical species of great socioeconomic importance and great potential for commercial exploitation in Brazil. Its fruits are highly appreciated *in natura* and in the production of juices, ice creams, jams and jellies (Silva Júnior et al. 2017). This species belongs to the Apocynaceae family, in which the monotypic genus is formed by six botanical varieties: *H. speciosa* var. *speciosa* Gomes that occurs predominantly in coastal and restinga plateaus, both belonging to the Atlantic Forest biome; *H. speciosa* var. *maximiliani* A. DC., *H. speciosa* var. *cuyabensis* Malme, *H. speciosa* var. *lundii* A. DC., *H. speciosa* var. *gardneri* (A. DC.) Muell. Arg. and *H. speciosa* var. *pubescens* (Nees and Martius) Muell. Arg., all widely distributed in the Cerrado of the Midwest of Brazil (Monachino 1945) and also in North (Koch et al. 2015, Silva Júnior et al. 2017). However, currently, only two varieties are recognized: *pubescens* and *speciosa*, according to Koch et al. (2015).

Regarding the reproductive and phenological aspects, mangaba is facultative autogamous, self-incompatible and has the pollination carried out by insects of the many families (Darrault and Schlindwein 2005). This species is in the domestication process (Silva et al. 2019); however, significant parts of its genetic resources are being lost due to the reduction of native vegetation in the area

***Corresponding author:**
E-mail: silvaedsonf@hotmail.com
ORCID: 0000-0001-6071-4011

Received: 02 July 2021
Accepted: 23 February 2022
Published: 31 May 2022

[1] Universidade Federal Rural de Pernambuco, Rua Dom Manuel de Medeiros, s/n, Dois Irmãos, 52.171-900, Recife, PE, Brazil
[2] Universidade Federal do Maranhão, BR 222, km 4, s/n, Boa Vista, 65.500-000, Chapadinha, MA, Brazil
[3] Universidade Federal de Alagoas, Av. Manoel Severino Barbosa, s/n, Bom Sucesso, 57.309-005, Arapiraca, AL, Brazil

of occurrence, mostly in the Cerrado, Caatinga and Atlantic Forest biomes (Costa et al. 2015). Due to its great economic importance, wide natural distribution and mainly because it is under intense anthropic pressure, it is essential to know the genetic diversity and structure of the remaining populations mangaba in order to elaborate efficient conservation strategies both *in situ* and *ex situ* (Costa et al. 2015).

Several molecular markers are available to estimate the level of diversity and genetic structure of plant populations. Among the available molecular markers, microsatellites (SSR-Simple Sequence Repeats) have been widely used. These are regions of DNA that have one to ten base pairs repeated in tandem, distributed throughout the genome of most eukaryotes; they are still highly polymorphic, multi-allelic, codominant and have high reproducibility (Vieira et al. 2016, Moura et al. 2017).

It is worth mentioning that no study, as far as we know, has been carried out covering mangaba populations from different biomes. In this context, the objectives of this study were: 1) to analyze the polymorphism of microsatellite loci in *H. speciosa*; 2) to estimate genetic diversity and population structure of mangaba within and between the Atlantic Forest, Cerrado and Caatinga biomes; and 3) to propose appropriate strategies for the collection and conservation of *H. speciosa*.

MATERIAL AND METHODS

Sampling and DNA extraction

Young leaves from adult individuals were collected in eight locations distributed between the Midwest (Cerrado biome) and Northeast (Atlantic Forest and Caatinga biomes) regions of Brazil (red in Figure S1). A total of 176 individuals were sampled between the states of Paraíba (24), Pernambuco (24), Sergipe (24) and Maranhão (24), all possibly from var. *speciosa*; and Goiás (20), Minas Gerais (20), Bahia (20) and Ceará (20), all possibly from var. *pubescens*. The leaves packed in silica and stored in a -30 °C were used for DNA extraction according to the protocol of Doyle and Doyle (1990). The quantification and quality of DNA were analyzed after electrophoresis on 1% agarose gel.

PCR amplification and genotyping

Six specific SSR primers, developed by Rodrigues et al. (2015), were tested (Table S1). PCR amplifications were performed in the final 50 µL volume containing: 1 µL of genomic DNA at 20 ng μL^{-1}, 5 µL of reaction buffer, 1.5 mM MgCl2, 0.2 mM dNTP, 1.25 U Taq DNA polymerase, 0.5 µL forward and reverse primers (10 pmol each) labeled with 6-FAM. The amplification program used had an initial denaturation phase at 95 °C for 10 min, followed by 15 cycles under the following conditions: 30s for denaturation at 94 °C, annealing temperature of 55-60 °C for 1 min, an additional 20 cycles at 89 °C for 1 min and annealing at 55-60 °C for 1 min, and extension at 72 °C for 1 min. The final extension was performed at 72 °C for 1 hour. The PCR reactions were performed in a thermocycler (BioCycler), and the PCR products subjected to capillary electrophoresis in a 3500 genetic analyzer (Applied Biosystems Inc., Foster City, CA, USA), using GeneScanTM 600 Liz® (Applied Biosystems) as a standard size.

Analysis of diversity and population genetic structure

The allele number (*Na*), expected heterozygosity (H_E), observed heterozygosity (H_O) and Hardy-Weinberg balance deviation (HWE) were obtained using the R Pegas package (Paradis et al. 2017) and the R PopGenReport 3.0 package (Gruber and Adamack 2017). The Principal Component Discriminant Analysis (DAPC) was performed in the R adegenet 2.0 package (Jombart and Ahmed 2011) using the K-means and α-score method to retain the best number of PCAs, considering at least 70% probability of ancestry in one of the clusters ($q \geq 0.7$). The population genetic structure was estimated using the R Geneland 2.0.9 package (Estoup et al. 2007). The spatial analysis of molecular variance was performed using the Spatial Analysis of Molecular Variation (SAMOVA) software (Dupanloup et al. 2002). This approach uses simulations to determine groups of populations (k) that are geographically homogeneous and that maximize differences between groups. SAMOVA analyzes were performed using 1000 permutations for $k = \{2, ..., 8\}$ groups. In addition, STRUCTURE 2.3.4 (Hubisz et al. 2009) was used to estimate the data log-likelihood for predefined k values. The k populations were determined using the Geneland package (Estoup et al. 2007). Gene flow (*Nm*) between populations was estimated using the values obtained from *Fst*, based on the formula $Nm = (1 - Fst)/4Fst$ (Slatkin and Barton 1989).

We estimated effective population size (*Ne)* using the molecular co-ancestry method of Nomura (2008), as implemented in NeEstimator V2.1 (Do et al. 2014). The frequency of null alleles was calculated for each locus using Chakraborty et al. (1994), implemented in R PopGenReport 3.0 package (Gruber and Adamack 2017) and Dempster et al. (1977) using GENEPOP (Raymond and Rousset 1995).

RESULTS AND DISCUSSION

The microsatellite regions studied in the populations, when submitted to DAPC analysis, enabled the identification of two large groups (Figure 1): I- individuals from the states of Paraíba, Pernambuco and Sergipe (Atlantic Forest, *H. speciosa* var. *speciosa*) and Maranhão (Cerrado, *H. speciosa* var. *speciosa*); II- individuals from the states of Goiás (Cerrado), Bahia (Cerrado / Caatinga transition zone), Minas Gerais (Cerrado) and Ceará (Caatinga); possibly belonging the var. *pubescens.* With the exception of individuals from Maranhão (Cerrado, possibly var. *speciosa*) who are in group I, all individuals from Cerrado and Caatinga (Goiás, Minas Gerais, Bahia and Ceará), seem to be closer genetically. While the individuals from the Atlantic Forest (Paraíba, Pernambuco and Sergipe) seem to be more divergent.

The analysis of population structure resulted in five genetic groups: A- individuals from the states of Pernambuco, Paraíba and Sergipe (Atlantic Forest), B- individuals from the state of Maranhão (Cerrado); both var. *speciosa*, C- individuals from the states of Bahia (transition zone between Caatinga and Cerrado) and Ceará (Caatinga), D- individuals from the states of Goiás (Cerrado) and E- individuals from the state of Minas Gerais (Cerrado); probably var. *pubescens* (Figure 2). In this analysis, except for Maranhão, the populations of the Cerrado and Caatinga are closer genetically, while the populations of the Atlantic Forest

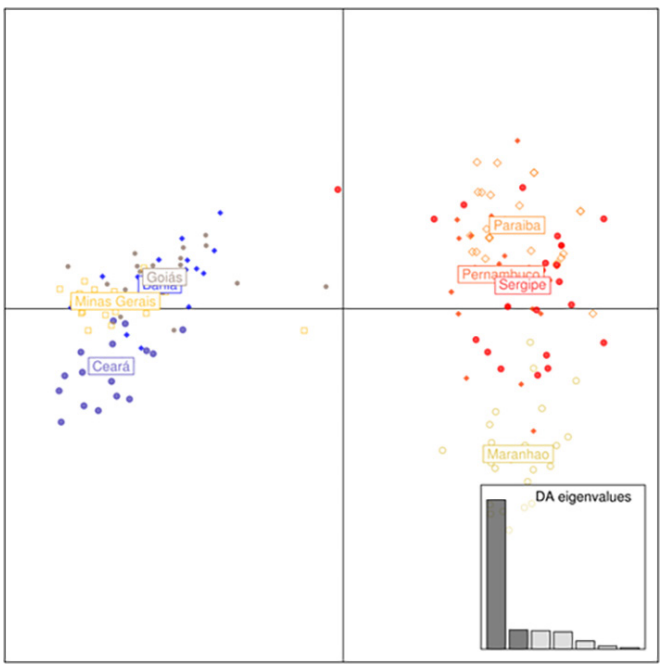

Figure 1. The discriminant analysis of principal components (DAPC) carried out based on six microsatellite loci for populations from eight states: Pernambuco, Paraíba and Sergipe (Atlantic Forest,), Maranhão (Cerrado); *H. speciosa* var. *speciosa*, Bahia (Cerrado/Caatinga Transition zone), Ceará (Caatinga,), Goiás (Cerrado) and Minas Gerais (Cerrado); probably belonging *H. speciosa* var. *pubescens*. The colours represent the groups determine by DAPC.

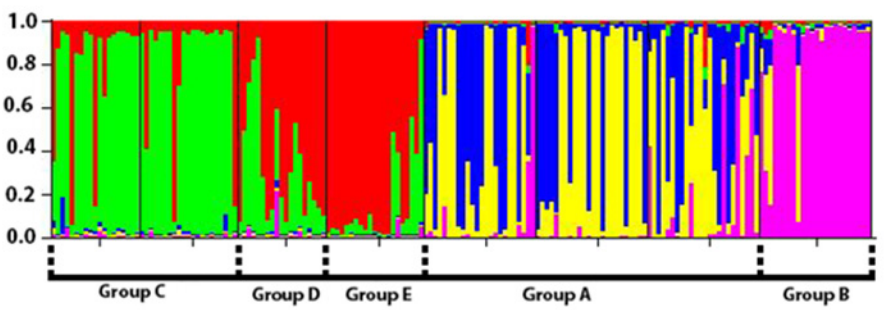

Figure 2. Population genetic structure obtained by structure software. The groups A to E were determined by SAMOVA and Geneland analyses, being: A - individuals from the states of Pernambuco, Paraíba and Sergipe (Atlantic Forest, *H. speciosa* var. *speciosa*), B - individuals from the states of Maranhão (Cerrado), C - individuals from the states of Bahia (Transition zone Cerrado/Caatinga) and Ceará (Caatinga), D - individuals from the states of Goiás (Cerrado, *H. speciosa* var. *pubescens*) and E- individuals from the states of Minas Gerais (Cerrado); probably belonging the *H. speciosa* var. *pubescens*. The colours represent the groups determine by structure.

Table 1. Genetic diversity parameters for the five population groups determined by Structure, SAMOVA and Geneland analyses: A- individuals from the states of Pernambuco, Paraíba and Sergipe (Atlantic Forest); B- individuals from the states of Maranhão (Cerrado); C- individuals from the states of Bahia (Transition zone Cerrado/Caatinga) and Ceará (Caatinga); D- individuals from the states of Goiás (Cerrado); and E- individuals from the states of Minas Gerais (Cerrado)

Groups*	Microsatellite loci							
	Samples	Nr. Alleles	Average alleles	Shared alleles (%)	H_O	H_E	*f***	N_e
A	72	62	10.33	61.3	0.66	0.65	-0,02	5
B	24	42	7.00	73.8	0.49	0.57	0.14	infinite
C	21	48	7.17	77.1	0.60	0.69	0.13	25
D	40	50	8.00	64.0	0.53	0.69	0.23	infinite
E	19	43	8.33	72.1	0.57	0.80	0.29	40.8
Average	35.2	49	8.17	69.7	0.57	0.68	0.15	-

* H_O: Observed heterozygosity; H_E: expected heterozygosity; *f*: Wright's fixation index; N_e: effective population size.
** Not significant.

have more divergent genotypes, corroborating with the DAPC. These groups were used to estimate the parameters of genetic diversity.

The genetic diversity of the population groups obtained revealed an average of 49 alleles (Table 1), of which group A had an average of 10.33 alleles. In comparison, the other groups had an average between 7 and 8.33 alleles. The average H_O ranged from 0.49 (group B) to 0.66 (group A), and the H_E ranged from 0.57 (group B) to 0.80 (group E). The value of *f*, whose average was 0.15, ranged from -0.02 (group A) to 0.29 (group E). Only population group A had a negative fixation index, while the other groups had positive values.

There was an average of 69.7% of alleles shared between population groups, ranging from 61.3% (group A) to 77.1% (group C) (Table 1). Group A showed 24 private alleles, groups D and E showed 18 and 12, respectively, while groups B and C revealed 11 private alleles (Figures 3A and 3B). It was observed that groups A and B, formed by individuals from the Atlantic Forest and Cerrado from Maranhão, shared more alleles among themselves, while groups C, D and E, formed by individuals from Caatinga and Cerrado revealed more shared alleles.

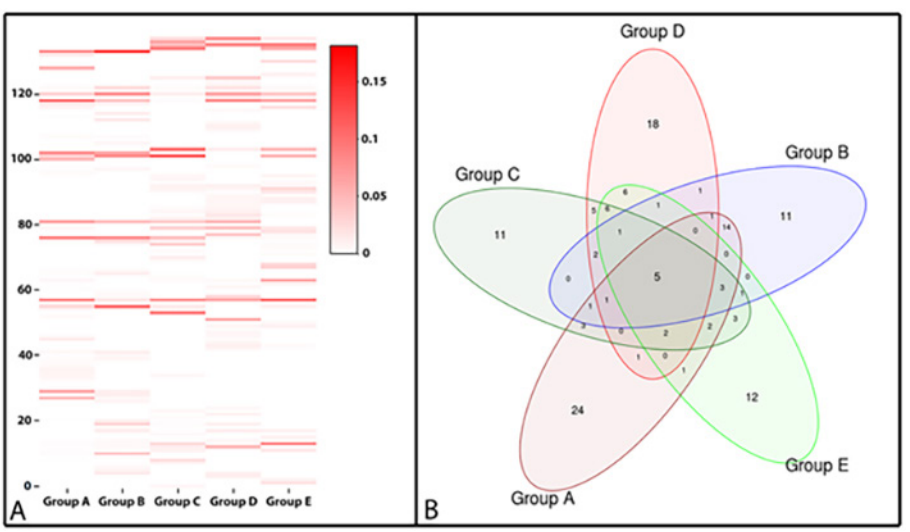

Figure 3. Distribution of the microsatellite alleles by heatmap (A) and Venn diagram (B). The groups A to E were determined by SAMOVA and Geneland analyses, being: A- individuals from the states of Pernambuco, Paraíba and Sergipe (Atlantic Forest), B- individuals from the states of Maranhão (Cerrado,); belonging *H. speciosa* var. *speciosa*, C- individuals from the states of Bahia (Transition zone Cerrado/Caatinga) and Ceará (Caatinga), D- individuals from the states of Goiás (Cerrado) and E- individuals from the states of Minas Gerais (Cerrado,); probably belonging the *H. speciosa* var. *pubescens*. The colours represent the groups determined by the structure.

Table 2. Spatial Analysis of Molecular Variance (SAMOVA) within and between mangaba population groups obtained by structuring based on six simple sequence repeats (ncSSR): Group A (Mata Atlântica - Paraíba, Pernambuco, Sergipe); Group B (Cerrado - Maranhão); Group C (Transição Cerrado/Caatinga - Bahia, Caatinga - Ceará); Group D (Cerrado - Goiás); Group E (Cerrado - Minas Gerais)

Variation Source	df	SS	VC	PV	F Statistics
Among groups*	4	39.133	0.139	31.5	$F_{ST} = 0.34$*
Among population within groups	3	2.585	0.013	2.89	
Within populations	344	98.976	0.288	65.61	
Total	351	140.693	0.438	100%	

SS: Sum of squares; VC: Variance Components; PV: Percentage of variation; * Significant at p<*0.001* (999 permutation).

The spatial analysis of the molecular variation (SAMOVA) demonstrated that most of the genetic variation is distributed within the groups (65.61%), although significant differences are shown between the population groups obtained (31.5%, F_{ST} = 0.34, p <0.001) (Table 2). When comparing pairs of population groups using the F_{ST} statistic, the genetic structure was observed between them (Table S2). The greatest structuring was observed between groups B (Cerrado of Maranhão) in relation to D (Cerrado of Goiás) and E (Cerrado of Minas Gerais), both with F_{ST} equal to 0.65, while the less divergent groups were C (zone of transition between Cerrado/Caatinga in Bahia and Caatinga in Ceará) and E (Cerrado of Minas Gerais), with F_{ST} equal to 0.07.

The *Nm* values also estimated from genetic differentiation (F_{ST}) between population groups show low gene flow between groups B in relation to D and E (*Nm* = 0.14), while groups C and E showed gene flow rate very high (*Nm* = 3.32) (Table S2). High values of gene flow were also observed among the populations of Bahia and Ceará (group C), Goiás (group D) and Minas Gerais (group E), with *Nm* = 1.68.

Dynamics of genetic variability in mangaba populations

The results obtained in this study made it possible to understand the diversity and genetic structure of eight populations of mangaba from three different Brazilian biomes (Caatinga, Cerrado and Atlantic Forest). These populations comprised individuals located in the occurrence area of two possibly botanical varieties (*H. speciosa* var. *speciosa* and *H. speciosa* var. *pubescens*) described initially by Monachino (1945). The two major genetic groups (possibly from the two varieties mentioned above) were also observed in the DAPC analysis, whose populations in Pernambuco, Paraíba, Sergipe and Maranhão, where the only var. *speciosa* was reported, formed a group, while the populations of Goiás, Bahia, Minas Gerais and Ceará, possibly var. *pubescens*, formed another group (Figure 1). Although the genetic resources of the species are widely distributed in Brazil, there seems to be a correlation between botanical varieties and the Brazilian states where the populations are found. However, it is necessary to highlight that the genetic variation between populations does not necessarily correspond to the morphological variation between individuals; this is the basis for the classification of botanical varieties proposed by Monachino (1945).

Although there is evidence of a correlation between the botanical varieties and the Brazilian states where the populations are found, this study did not observe a clear correlation between the genetic structure of the varieties and biomes (Figures 2 and 3), because despite being constituted by populations of var. *pubescens*; possibly, group C was formed by individuals from Caatinga (Ceará) and from the transition zone between Cerrado and Caatinga (Bahia). The populations of the Cerrado of Goiás and Minas Gerais formed two distinct groups, D and E, respectively. Also, groups A (Paraíba, Pernambuco and Sergipe) and B (Maranhão), despite being formed by populations from var. *speciosa*, come from the Atlantic Forest (sandbank and coastal plateaus) and the Cerrado, respectively. Still based on the genetic structure, except for the population of the Cerrado from Maranhão, which again forms a distinct group, the populations of the Caatinga and Cerrado are genetically closer. The populations of the Atlantic Forest, on the other hand, have more divergent genotypes.

These results demonstrate that the populations of *H. speciosa* var. *speciosa* from the Atlantic Forest are more genetically distant from the populations of the Cerrado, despite forming a single group, precisely because they are subject to different selection pressures. The populations of the Cerrado and Caatinga (most of the possibly var. *pubescens*) were genetically closer, although the collection areas had a greater geographical distance. The environmental conditions in the Cerrado and Caatinga, including in the transition zone between these two biomes, are more similar, which reduces

the selection pressure between the populations of these two biomes. It is worth mentioning that the mangaba is a native species of different ecosystems, as it adapts to different types of soil and climate (Silva Júnior and Ledó 2006).

High levels of genetic diversity were observed in all population groups obtained in this study, with H_O values greater than 0.49 and H_E values greater than 0.57 (Table 1). With the exception of group A, formed by Paraíba, Pernambuco and Sergipe (f = -0.02), all other population groups showed H_E values higher than the H_O values, as expected for a population in Hardy-Weinberg equilibrium. The positive intrapopulation index obtained in most population groups indicates the lack of random mating between individuals, crossing between related genotypes and increased self-fertilization rate. The average values of H_O, H_E and f obtained in this study are within the standards reported for other populations of *H. speciosa* (Amorim et al. 2015) and are in accordance with the parameters expected for tree and allogamous species, such as mangaba (Santos et al. 2011, Alves et al. 2013).

The SAMOVA analysis revealed that despite the existence of genetic variation between groups (31.5%), the greatest variability was found within populations (65.61%, F_{ST} = 0.34) (Table 2). These results also agree with those obtained by Amorim et al. (2015) when they analyzed the remnants of six mangaba populations in the Northeastern states of Brazil (Ceará, Pernambuco and Sergipe), and also observed a higher percentage of variation within the populations (83.18%).

The F_{ST} pair-by-pair matrix in the present study (Table S2) revealed moderate (F_{ST} = 0.13) to high (F_{ST} above 0.24) genetic differentiation between most population groups. Low differentiation was found only between the populations of Bahia, Ceará and Minas Gerais (F_{ST} = 0.07), all from the Cerrado and Caatinga. Therefore, despite the low level, the difference was relevant. This difference may be important for future adaptation of populations with low levels of genetic variability among individuals (Diniz-Filho et al. 2012).

Despite the low *Nm* values among the majority of the mangaba population groups (Table S2), the high level of genetic variation within populations suggests an intense intrapopulation gene flow, indicating that the genetic distance between mangaba populations in Brazil does not necessarily imply isolation by geographical distance or environmental conditions. This fact can be confirmed in the high values of *Nm* among the populations of the Cerrado and Caatinga distributed among the States of Bahia, Ceará, Goiás and Minas Gerais, probably attributed to the displacement of fruits between the two groups made by the inhabitants of these regions. Collevatti et al. (2018), when trying to unveil the patterns of genetic differentiation of mangaba varieties described by Monachino, based on seven microsatellite loci, observed a high mixture between varieties and between populations. The genetic differentiation between varieties was less than between populations within the varieties, with greater inbreeding within the population. These results suggest that there was high historical connectivity between populations of this species, but due to the fragmentation of the biomes, this flow may be lost over time.

The population structure of central Brazil using the Geneland approach was different from that obtained by Collevatti et al. (2018), who found five clusters, while this study was found two groups, probably because the sample in the Cerrado was smaller in the present study. Based on the result of the present study, structuring and differentiation F_{ST} values among population groups, it is in agreement with the recent classification into only two botanic varieties (Koch et al. 2015), in which the populations of northeast Brazil (groups A and B) correspond to *H. speciosa* var. *speciosa* and groups C, D and E correspond possibly the botanical variety *H. speciosa* var. *pubescens*.

Implications for conservation

The conservation of genetic resource should be carried out using the knowledge of the diversity and genetic structure of its populations. Genetic diversity can also be used to select more divergent individuals according to the research study objective (Nunes et al. 2021) and is also the basis for genetic improvement (Fajardo et al. 2018). However, some species have particularities concerning the physiologic aspect, reproductive system, etc., which also is very important and demand specific strategies to conserve their genetic resources.

Mangaba is a perennial, cross pollination and self-incompatible species (Darrault and Schlindwein 2005); thus, their individuals are heterozygous and genetically distinct in natural populations. Therefore, the choice or sampling of individuals by seeds does not reproduce the same characters as the parent due to genetic recombination. Thus, if it is intended to maintain some phenotypic characteristics of the plant, it is necessary to collect vegetative parts for direct

propagation or grafting. In addition, mangaba has recalcitrant seeds making it impossible to maintain a seed bank. In this sense, a reasonable strategy for *ex situ* conservation of the mangaba's genetic resources would be the installation of orchards, which requires a large area to maintain the adequate number of accesses; as the adult tree is a few meters tall, a minimum of 10 square meters is required for each tree. Considering these particularities and the wide distribution of populations in different biomes and ecological niches, *in situ* conservation is more appropriate for mangaba and should be prioritized, in parallel with the enrichment of the already installed germplasm banks (orchards), as well as the creation of new banks. According to Nass et al. (2012), this is a way to reconcile the conservation of diversity with sustainable development.

The enrichment of natural populations in situ, mainly the least vulnerable with the participation of the local population, and forest restoration in Conservation Units using propagules from different areas, especially those with high genetic diversity, are good strategies to conserve a greater volume of the resource genetics of mangaba.

In this sense, the results of this study indicate that the population of group E, from Minas Gerais, should be prioritized for conservation because it presents greater diversity (H_E = 0.80) (Table 1) and the consequently greater probability of finding genotypes with characteristics of agronomic interest. To enrich the population of Minas Gerais (group E), Maranhão genotypes (group B) are more suitable. This is because they were more divergent (F_{ST} = 0.65) and had a lower estimate of genetic flow (*Nm* = 0.14), which should provide more diversified genetic recombination in subsequent generations (Table S2). Based on the *FST* and *Nm*, group B genotypes are also indicated to enrich populations in groups C and D. For the same reason, to enrich the population in group B, individuals in groups C, D and E are option more suitable.

It is also noteworthy that for the *in situ* conservation of the mangaba genetic resources, special attention is needed to cultural aspects, considering the different customs of the local populations, mainly of communities that use mangaba populations as an extractivism of their fruits. In this context, the transmission of technical knowledge and training of local populations that do extractivism in mangaba populations is pointed out as good practices for the sustainable conservation of the genetic diversity of natural populations (Silva Júnior et al. 2017). Strategies for *in situ* conservation of the mangaba genetic resource in Brazil need to be associated with the National Nature Conservation System, which is currently one of the most notable systems of conservation units in the world, according to Padua (2018). As mangaba has wide occurrence in different biomes, the conservation of its genetic resources should be with efforts to conserve as many natural populations as possible with less vulnerability.

ACKNOWLEDGMENTS

This work was supported by the project CNPq (process number 484847/2013-9) that the authors gratefully acknowledge. Additional Tables S1 and S2 can be required to the author by e-mail (silvaedsonf@hotmail.com).

REFERENCES

Alves MF, Barroso PA, Ciampi AY, Hoffmann LV, Azevedo VCR and Cavalcante U (2013) Diversity and genetic structure among subpopulations of *Gossypium mustelinum* (Malvaceae). **Genetic Molecular Resources 12**: 597-609.

Amorim JAE, Mata LR, Lédo AS, Azevedo VCR and Silva AVC (2015) Diversity and genetic structure of mangaba remnants in states of northeastern Brazil. **Genetics and Molecular Research 14**: 823-833.

Chakraborty R, Zhong Y, Jin L and Budowle B (1994) Nondetectability of restriction fragments and independence of DNA fragment sizes within and between loci in RFLP typing of DNA. **American Journal of Human Genetics 55**: 391-401.

Collevatti RG, Rodrigues EE, Vitorino LC, Lima-Ribeiro MS, Chaves LJ and Telles MPC (2018) Unravelling the genetic differentiation among varieties of the Neotropical savanna tree *Hancornia speciosa* Gomes. **Annals of Botany 122**: 973-984.

Costa DF, Vieira FA, Fajardo CG and Chagas KPT (2015) Diversidade genética e seleção de iniciadores ISSR em uma população natural de mangaba (*Hancornia speciosa* Gomes) (Apocynaceae). **Revista Brasileira de Fruticultura 37**: 970-976.

Darrault RO and Schlindwein D (2005) Limited fruit production in *Hancornia speciosa* (Apocynaceae) and pollination by nocturnal and diurnal insects. **Biotropica 37**: 381-388.

Dempster AP, Laird NM and Rubin DB (1977) Maximum likelihood from incomplete data via the EM algorithm. **Journal of the Royal Statistical Society, Series B (Methodological) 39:** 1-38.

Diniz-Filho JA, Collevatti RG, Chaves LJ, Soares TN, Nabout JC, Range, TF, Melo DB, Lima JS and Telles MPC (2012) Geographic shifts in climatically suitable areas and loss of genetic variability in *Dipteryx alata* ("Baru" Tree; Fabaceae). **Genetic Molecular Resources 11**: 1618-1626.

Do C, Waples RS, Peel D, Macbeth GM, Tillett BJ and Ovenden JR (2014) NeEstimator V2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. **Molecular Ecology Resources 14**: 209-214.

Doyle JJ and Doyle J L (1990) A rapid total DNA preparation procedure for fresh plant tissue. **Focus 12**: 13-15.

Dupanloup I, Schneider S and Excoffier L (2002) A simulated annealing approach to define the genetic structure of populations. ***Molecular Ecology 11***: 2571-2581.

Estoup A, Guillot G and Santos F (2007) Geneland: simulation and MCMC inference in landscape genetics. R package version 2.0.5. Available at <http:/cran.r-project.org/>. Accessed on December 20, 2020.

Fajardo CG, Costa DF, Chagas KPT and Vieira FA (2018) Genetic diversity in natural populations of mangabeira (*Hancornia speciosa* Gomes): implications for conservation of genetic resources. **Ciência e Agrotecnologia 42**: 623-630.

Gruber B and Adamack A (2017) PopGenReport: A simple framework to analyze population and landscape genetic data. Available at <https://github.com/green-striped-gecko/PopGenReport>. Accessed on December 15, 2019.

Hubisz MJ, Falush D, Stephens M and Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. **Molecular Ecology Resources 9**: 1322-1332.

Jombart T and Ahmed I (2011) Adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. **Bioinformatics 27**: 3070-3071.

Koch I, Rapini A, Simões AO, Kinoshita LS, Spina AP and Castello ACD (2015) Apocynaceae. In Lista de espécies da flora do Brasil. Jardim Botânico do Rio de Janeiro. Available at <http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB26640>. Accessed on December 15, 2019.

Monachino JA (1945) A revision of *Hancornia* (Apocynaceae). **Lilloa 11**: 19-48.

Moura RF, Dawson DA and Nogueira DM (2017) The use of microsatellite markers in neotropical studies of wild birds: A literature review. **Anais da Academia Brasileira de Ciências 89**: 145-154.

Nass LL, Sigrist MS, Ribeiro CSC and Reifschneider FJB (2012) Genetic resources: the basis for sustainable and competitive plant breeding. **Crop Breeding Applied Biotechnology 12**: 75-86.

Nomura T (2008) Estimation of effective number of breeders from molecular coancestry of single cohort sample. **Evolutionary Applications 1**: 462-474.

Nunes VV, Silva-Mann R, Souza JL and Calazans CC (2021) Geno-phenotypic diversity in natural population of *Hancornia speciosa* Gomes: implications for conservation and improvement. **Genetic Resources and Crop Evolution 68**: 2869-2882**.**

Padua JG (2018) Conservation of crop genetic resources in Brazil in the context of the target 9 of the global strategy for plant conservation. **Rodriguésia 69**: 1557-1565.

Paradis E, Gosselin T, Goudet J, Jombart T and Schliep K (2017) Linking genomics and population genetics with R. **Molecular Ecology Resources 17**: 54-66.

Raymond M and Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. **Journal of Heredity 86**: 248-249.

Rodrigues AJL, Yamaguishi AT, Chaves LJ, Coelho ASG, Lima JS and Telles MPC (2015) Development of microsatellites markers for *Hancornia speciosa* Gomes (Apocynaceae). **Genetics and Molecular Research 14**: 7274-7278.

Santos KL, Ducroquet JPHJ and Nodari RO (2011) Caracterização genética de populações naturais de goiabeira-serrana (*Acca sellowiana*) com marcadores microssatélites heterólogos. **Biotemas 24**: 75-83.

Silva AVC, Vitória MF, Azevedo VRR, Sá AJ, Nascimento ALS, Cardoso MN, Soares ANR, Silva Júnior JF and Ledo AS (2019) Genetic diversity of the mangaba GeneBank using microsatellites. **Genetics and Molecular Research 18**: gmr18108.

Silva Júnior JF and Ledó AS (2006) **A cultura da mangaba**. Embrapa Tabuleiros Costeiros, Aracaju, 253p.

Silva Júnior JF, Mota DM, Lédo AS, Muniz AVCS, Schmitz H and Rodrigues RFA (2017) Mangaba (*Hancornia speciosa* Gomes). In Burle M and Faleiro FG (eds) **Frutales nativos con importancia actual y potencial para del Cono Sur.** Procisur, Montevideu, 27p.

Slatkin M and Barton NH (1989) A comparison of three indirect methods for estimating average levels of gene flow. **Evolution 43**: 1349-1368.

Vieira MLC, Santini L, Diniz AL and Munhoz CF (2016) Microsatellite markers: what they mean and why they are so useful. **Genetics and Molecular Biology 39**: 312-328.