REVISTA BRASILEIRA DE FRUTICULTURA
Published by the Brazilian Society of Fruit Crops
ISSN 0100-2945

Genetics And Plant Breeding - Original Article- Edited by: Willian Krause

Genetic diversity among accessions of *Spondias mombin* L. collected in northeastern Brazil

Giovana Sarah Sales Batista[1], Samara de Brito Vieira[1], Leonardo Castelo Branco Carvalho[2], Sérgio Emílio dos Santos Valente[1], Lucio Flavo Lopes Vasconcelos[3], Paulo Sarmanho da Costa Lima[3]*

1 Universidade Federal do Piauí, Teresina-PI, Brazil.
2 Universidade Federal Rural da Amazônia, Belém-PA, Brazil.
3 Embrapa Meio-Norte, Teresina-PI, Brazil.
*Corresponding author: *paulo.costa-lima@embrapa.br*

Abstract: *Spondias mombin* is an arboreal species, the fruits of which possess a unique flavor and aroma and contain both nutritive and bioactive compounds. Considering that knowledge regarding the genetic variability in *S. mombin* is important for the establishment of a genetic improvement program, the aim was to characterize *S. mombin* accessions collected in the states of Maranhão and Piauí, northeastern Brazil, using molecular analysis with simple sequence repeats markers (ISSR). Twenty-six accessions maintained in the Embrapa Meio-Norte germplasm bank (Teresina, PI, Brazil) and two dwarf accessions collected from a private property in Teresina-PI were evaluated. The 12 ISSR markers employed presented strong discriminatory power as demonstrated by the high percentage of polymorphism (88.75%) and mean polymorphic information content (0.641). High genetic diversity among the accessions was demonstrated by the diversity indices of Nei (H=0.37) and Shannon (I=0.55), while gene flow between populations was considered moderate (Nm=0.6268 migrants per generation). The genetic diversity could be explained mainly by variability within-populations (88.71%) rather than between-populations (11.29%), although the genetic differentiation coefficient between-populations was moderate (GST=0.11) and statistically significant ($P < 0.01$). Unweighted pair group method with arithmetic mean (UPGMA) clustering and Population Structure analysis showed genetically distinct groups. The dwarf accessions were the most divergent and should be incorporated into the germplasm bank. Our study verified the existence of genetic differentiation between the tested *S. mombin* accessions and confirmed that such variability could be exploited through genetic improvement programs.

Index terms: cajazeira, genetic resources, molecular marker, germplasm bank.

Rev. Bras. Frutic., v.46, e-357 DOI: *https://dx.doi.org/10.1590/0100-29452024357*
Received 15 Jun, 2023 ● Accepted 05 Apr, 2024 ● Published Jul/Aug, 2024. Jaboticabal - SP - Brazil.

Diversidade genética entre acessos de *Spondias mombin* L. coletados no Nordeste do Brasil

Resumo - *Spondias mombin* é uma espécie arbórea cujos frutos possuem sabor e aroma únicos e contêm compostos nutritivos e bioativos. Considerando que o conhecimento sobre a variabilidade genética em *S. mombin* é importante para o estabelecimento de um programa de melhoramento genético, objetivou-se caracterizar acessos de *S. mombin* coletados nos Estados do Maranhão e Piauí, Nordeste do Brasil, utilizando análise molecular com repetições simples de sequência: marcadores (ISSR). Foram avaliados 26 acessos mantidos no banco de germoplasma da Embrapa Meio-Norte (Teresina-PI, Brasil) e dois acessos anões coletados em propriedade privada, em Teresina-PI. Os 12 marcadores ISSR empregados apresentaram forte poder discriminatório demonstrado pelo alto percentual de polimorfismo (88,75%) e conteúdo médio de informação polimórfica (0,641). A alta diversidade genética entre os acessos foi demonstrada pelos índices de diversidade de Nei (H=0,37) e Shannon (I=0,55), enquanto o fluxo gênico entre populações foi considerado moderado (Nm=0,6268 migrantes por geração). A diversidade genética pode ser explicada, principalmente, pela variabilidade dentro das populações (88,71%) e não entre populações (11,29%), embora o coeficiente de diferenciação genética entre populações tenha sido moderado (GST = 0,11) e estatisticamente significativo (P <0,01). O método de grupos de pares não ponderados com agrupamento de média aritmética (UPGMA) e a análise de estrutura populacional mostraram grupos geneticamente distintos. Os acessos anões foram os mais divergentes e deveriam ser incorporados ao banco de germoplasma. Nosso estudo verificou a existência de diferenciação genética entre os acessos testados de *S. mombin* e confirmou que tal variabilidade poderia ser explorada através de programas de melhoramento genético.

Termos para indexação: cajazeira, recursos genéticos, marcador molecular, banco de germoplasma.

Introduction

Species of the genus Spondiaceae (Anacardiaceae) are widely distributed in the tropical region of America, Africa and Asia (SILVA et al., 2017a). The arboreal species *Spondias mombin* L. is found extensively in the North, Northeast, Southeast and Midwest regions of Brazil, where it is commonly known as “cajazeira”. The deciduous trees, which can grow to a height of some 25 m, produce ovoid-shaped dupe-type fruits (3 to 4 cm in length) that are orange-yellow in color and have a thin pulp and an acidic taste (LOURENÇO et al., 2018). The fruits are appreciated for their pleasant flavor and aroma, as well as their nutritive potential by reason of their high content of vitamins B1 and C (ALOBI et al., 2017), and may be consumed *in natura* or processed as juice, jam or ice cream. In addition, extracts of *S. mombin* are used in traditional medicine for the treatment of a number of diseases (NWIDU et al., 2018). The native fruits are particularly valued in the North and Northeast regions of Brazil and have significant socioeconomic importance (SILVA et al., 2017a).

Despite the economic and medicinal potential of *S. mombin*, little information is available concerning the genetic diversity of the species, although some studies have been performed with random amplified polymorphic DNA (RAPD) (LIMA et al., 2011; GOIS

et al., 2014) and inter simple sequence repeats (ISSR) (SILVA et al., 2017b) markers. However, broader studies are required in order to conserve and manage the species more efficiently and to exploit better the genetic resources available. In this context, genetic markers are important tools for expediting the characterization and selection of genotypes with desirable traits, particularly in the case of perennial species (SILVA et al., 2017b). The ISSR technique affords a number of advantages in that it is simple, rapid and cost-effective, and does not require prior knowledge of the nucleotide sequences of the genome (BARUAH et al., 2017). The high reproducibility and species-specificity of the procedure are due to the size of the microsatellite primers and the stringency of the annealing temperature that can minimize non-specific primer annealing and the generation of undesired products. The abundance of microsatellites allows the detection of large numbers of polymorphic loci and the differentiation of closely related genotypes (BUHROO et al., 2018).

Considering that knowledge regarding the genetic relationship among *S. mombin* accessions is important for the establishment of a genetic improvement program targeting the conservation and management of the species, we aimed to characterize 28 accessions of cajazeira collected from the states of Maranhão and Piaui, northeastern Brazil, using ISSR markers.

Materials and Methods

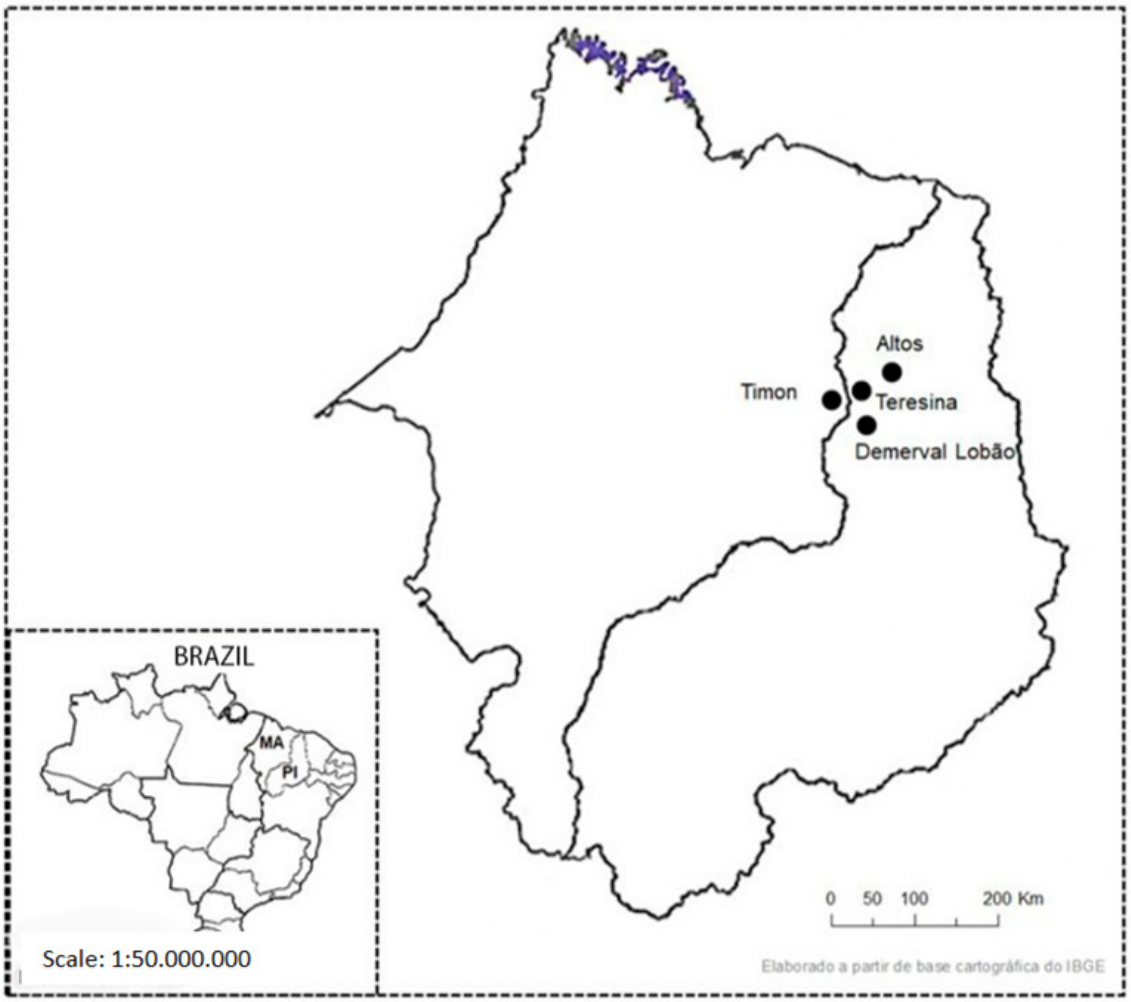
Plant material

Twenty six accessions of S. mombin from 4 different sample locations currently maintained in the active germplasm bank (AGB) of Embrapa Meio-Norte (Teresina, PI, Brazil), together with two dwarf accessions collected from a rural property in the municipality of Teresina, were selected for analysis. Therefore, 5 study populations from 4 different locations were considered: 1) Timon-MA, with 2 accessions; 2) Altos -PI, with 2 accesses; 3) Teresina-PI (a), with 20 accesses; 4) Teresina-PI (b), with 2 dwarf accessions; and 5) Demerval Lobão-PI, with 2 accesses.

Figure 1 presents the sampling sites in the states of Maranhão (Timon) and Piauí (Altos, Demerval Lobão and Teresina). Fresh leaves of each of the accessions were collected, wrapped in paper towels, placed in an ice box for transportation to the Laboratory of Molecular Biology at Embrapa Meio-Norte, and stored at -20 °C until required for DNA extraction.

DNA extraction

Leaves were macerated in a Precellys® 24 tissue homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France) and genomic DNA extracted using DNeasy Plant Mini Kits (Qiagen, Venlo, Netherlands) following the recommendations of the manufacturer. Extracted DNA was resuspended in TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) and kept at -20 °C until required for analysis. In order to quantify the DNA, extracts and lambda DNA of known concentration (0.1 µg/µL) were subjected to 0.8% agarose gel electrophoresis in 0.5 X TBE running buffer (44 mM Tris-HCl, 44 mM boric acid and 1 mM EDTA, pH 7.5) for 1 h at 80 V, followed by staining with GelRed® Nucleic Acid Stain (Biotium, Fremont, CA, USA). Gels were visualized on a transilluminator and images captured and digitized using the MiniBis Pro gel documentation system (DNR Bio Imaging System, Neve Yamin, Israel). DNA samples were diluted with sterile ultrapure water to a standard concentration of 10 ng/µL and stored at -20 °C until required for ISSR analysis.



Populations	Accessions
Timon, MA	BGC03 and BGC13
Altos, PI	BGC04 and BGC05
Teresina, PI	BGC07, BGC09, BGC10, BGC11, BGC12, BGC14, BGC15, BGC17, BGC18, BGC19, BGC20, BGC21, BGC22, BGC23, BGC24, BGC25, BGC26, BGC27, BGC28, BGC29, P1* and P8*
Demerval Lobão, PI	BGC 31 and BGC 32

Figure 1. Sampling points of *Spondias mombin* accessions in the Brazilian states of Maranhão (MA) and Piaui (PI), northeastern Brazil. Asterisks indicate the dwarf genotypes.

ISSR analysis

ISSR primers were selected from among the 50 included in the UBC (University of British Columbia, Vancouver, BC, Canada) primer set #9 based on polymorphism and band resolution obtained by PCR of four randomly selected DNA extracts (Table 1). The reaction mixture contained 1 X amplification buffer [20 mM Tris-HCl pH 8.0, 0.1 mM EDTA, 1 mM DTT; 50% (v/v) glycerol], 2.0 mM $MgCl_2$, 0.8 mM dNTPs, 0.8 mM ISSR primer, Taq DNA polymerase (1U/ µL; Invitrogen), 1 µL of genomic DNA (7 ng/µL) and ultrapure water to a total volume of 10 µL. Amplifications were carried out in a 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, EUA) under the following conditions: initial denaturation at 94 °C for 1 min, followed by 40 cycles each with denaturation at 94 °C for 40 s, annealing at 50 - 57 °C (primer dependent) for 45 s, extension at 72 °C for 2 min, and a final extension at 72 °C for 7 min. Amplicons from all accessions, together with 1 kb lambda DNA ladders (Kasvi, São José dos Pinhais, PR, Brazil), were submitted to electrophoresis on 1.5% agarose gel pre-stained with 1 µL

GelRed™ in 0.5 X TBE running buffer for 4 h at 110 V. Gels were visualized on a transilluminator and images were captured and digitized using the system previously described.

Table 1. ISSR primer sequences and banding patterns of 28 *Spondias mombin* (cajazeira) accessions collected in northeastern Brazil.

Primers	Primer sequence 5'- 3'	PIC	TNB (n)	NPB (n)	PPB (%)
UBC-807	$(AG)_8T$	0.623	11	11	100.00
UBC-808	$(AG)_8C$	0.489	8	8	100.00
UBC-810	$(GA)_8T$	0.764	16	14	87.50
UBC-811	$(GA)_8C$	0.637	13	10	73.92
UBC-826	$(AC)_8C$	0.691	16	16	100.00
UBC-827	$(AC)_8G$	0.484	13	09	69.23
UBC-836	$(AG)_8YA$	0.744	13	12	93.30
UBC-842	$(GA)_8YG$	0.817	18	17	94.44
UBC-856	$(AC)_8YA$	0.424	10	08	80.00
UBC-857	$(AC)_8YG$	0.661	12	12	100.00
UBC-890	VHV $(GT)_7$	0.618	15	12	80.00
UBC-891	HVH $(TG)_7$	0.527	15	13	86.66
Mean		**0.641**	**13.41**	**11.83**	**88.75**
Total			**161**	**142**	

Abbreviations: PIC: polymorphic information content; TNB: total number of bands; NPB: number of polymorphic bands; PPB: percentage of polymorphic bands; A: adenine; T: thymine; G: guanine; C: cytosine; Y: C or T; V: A, G or C; H: A, C or G.

Statistical analysis

The accesses were initially clustered using R software (R CORE TEAM, 2022), *pvclust* package (SUZUKI; SHIMODAIRA, 2006). Well-resolved amplicons produced by the same ISSR primer and present in the same location on gels of different accessions were considered to belong to the same gene locus and were scored visually as absent (0) or present (1) for each of the 28 genotypes. Polymorphic information content (PIC) was calculated for each of the ISSR markers according to Eq. 1 in which fi is the frequency of the i^{th} amplified allele (BOTSTEIN et al., 1980).

$$PIC = 1 - \sum fi^2$$

Eq. 1

A binary similarity matrix was constructed from the ISSR marker scores and a distance matrix obtained using Jaccard's coefficients of genetic similarity. Hierarchical cluster analysis was performed by applying the unweighted pair-group method with arithmetic averages (UPGMA) to produce a dendrogram from the distance matrix. The cophenetic correlation coefficient (*r*) and the bootstrap confidence index were calculated from the binary matrix of amplified fragments and the dendrogram following 1000 replicates.

Genetic diversity parameters were estimated using POPGENE version 1.32 software (YEH, et al., 1999), including the number of polymorphic bands (NPB), percentage of polymorphic bands (PPB), Nei genetic diversity index (H) (NEI, 1987), Shannon diversity index (I) (SHANNON; WEAVER, 1949), genetic differentiation coefficient (G_{ST}), and the number of migrants (Nm) from the estimated value of G_{ST}. Based on ISSR marker patterns, the distribution of the genetic variation within and between populations was determined by analysis of molecular variance (AMOVA) using the software Arlequin version 3.5.1.2 (EXCOFFIER; LISCHER, 2010).

The population structure analysis was obtained using the Structure software (v.2.3.4) (PRITCHARD; STEPHENS; DONNELLY, 2000). The mixture model with correlated allele frequencies was adopted, so that 10 indepen-

dent runs were performed for each K value, which ranged from 1 to 10; with 1,000,000 simulations in MCMC (Markov Chain Monte Carlo) and 500,000 burnin generations. The most likely K was determined using the ΔK values according to the methodology of Evanno, Regnaut and Goudet (2005) obtained in Structure Harvester v. 0.6.9 (EARL; VONHOLDT, 2012) and through the cross-entropy method (FRICHOT; FRANÇOIS, 2015).

Results and Discussion

A detailed knowledge of the variability within and between populations is fundamental in designing breeding strategies that exploit available genetic resources effectively. The PIC and PPB values presented in Table 1 demonstrate that the ISSR markers were efficient in assessing genetic diversity and relationships among the 28 accessions of *S. mombin* from five assumed populations, four of which were maintained in the AGB of Embrapa Meio-Norte.

The 12 selected ISSR primers produced a total of 161 bands of which 142 (88.75%) were polymorphic. Primer UBC-842 generated the highest NPB while primers UBC-808 and UBC-856 produced the lowest. The mean PPB for the primers used in this study (88.75%) was considerably higher than that reported by Silva et al. (2017b) for the same species (64.65%), indicating that the studied genotypes embody high genetic diversity. The parameter PIC is an indicator of the discriminatory ability of a molecular marker and, according to Botstein et al. (1980), a quality marker would be characterized by a PIC > 0.5. The PIC values obtained in the present study ranged from 0.424 (UBC-856) to 0.817 (UBC-842) with a mean of 0.641, indicating that the ISSR markers exhibited strong discriminatory power for individuals of the studied species.

The existence of large genetic diversity among the individual populations of *S. mombin* was confirmed by the values of the diversity indices H, which ranged from 0.13 to 0.35, and I, which ranged from 0.18 to 0.51 (Table 2). Considering all populations together, the diversity indices were 0.37 for H and 0.55 for I. The Teresina population presented the highest value for PPB (93.17%) while the Demerval Lobão population exhibited the lowest (26.71%). Overall polymorphism at the species level was 99.39%. The significant genetic diversity among the evaluated accessions is compatible with the high PPB detected for the ISSR markers. Silva et al. (2017b) also reported the existence of considerable genetic diversity (H = 0.26; I = 0.39) among populations of *S. mombin* from three geographical areas. Considering that *S. mombin* is undergoing domestication, a large diversity among accessions is to be expected (LIMA et al., 2011) in contrast to domesticated species that tend to be more genetically uniform because of successive artificial selection processes.

Table 2. Genetic diversity of five populations of *Spondias mombin* (cajazeira) collected in northeastern Brazil.

Population (municipality, state)	H	I	NPB (n)	PPB (%)	Nm
Timon, MA	0.16	0.22	52	32.20	
Altos, PI	0.19	0.27	64	39.75	
Teresina, PI	0.35	0.51	150	93.17	
Demerval Lobão, PI	0.13	0.18	43	26.71	
Teresina, PI (dwarf type)	0.22	0.30	71	44.10	
All populations together	0.37	0.55	160	99.39	0.6268

Abbreviations: H: Nei's genetic diversity index; I: Shannon's diversity index; NPB: number of polymorphic bands; PPB: percentage of polymorphic bands; Nm: gene flow.

According to Govindaraju (1989), gene flow can be classified as low (Nm < 0.25 migrants per generation), moderate (Nm = 0.25 - 0.99 migrants per generation) or high (Nm > 1 migrants per generation). On this basis, the Nm coefficient of 0.6268 migrants per generation estimated for the studied populations of *S. mombin* (Table 2) indicates moderate gene flow, suggesting that the populations were not genetically isolated although the movement of genes from one population to another was not strong enough to reduce genetic differences between populations. Gene flow is driven by a variety of agents, the most important of which are animal pollinators (SLARKIN, 1985). Species of stingless bees are regular visitors and potential pollinators of *S. mombin*, although they have a short flight radius and their foraging activities are of low intensity (FREITAS; BOMFIM, 2017). The Nm index obtained in this study indicates that, despite being close, the distances between the locations of the studied accessions are greater than the radius reached by the pollinators. The Nm index of 2.0684 recorded by Silva et al. (2017b) for natural populations of *S. mombin* collected from three different municipalities in the state of Mato Grosso is considerably higher than that reported herein. This disparity can be explained by the fact that the accessions evaluated in this study were collected from urbanized areas, and urbanization is a barrier that isolates populations of species and reduces gene flow.

According to AMOVA, 88.71% of the total variability of *S. mombin* was found within-populations while only 11.29% was found between-populations (Table 3). Nevertheless, the between-population genetic diversity was considered moderate (G_{ST} = 0.11) and statistically significant (P < 0.01). The Nei G_{ST} coefficient is analogous to Wright's fixation index (F_{ST}; (WRIGHT, 1978) and is defined as the proportion of genetic diversity that resides among populations regardless of the size of the assumed populations. The G_{ST} coefficient obtained for *S. mombin* in the present study indicates that there is a moderate level of genetic differentiation between the Timon, Altos, Demerval Lobão and the two Teresina populations since, according to Wright (1978), F_{ST} values ranging from 0.05 to 0.25 are considered intermediary. Silva et al. (2017b) also reported that the genetic diversity between the three *S. mombin* populations in Mato Grosso could be explained mainly by the variability within-populations (77.38%) rather than between-populations (22.62%). A high intrapopulation variability is expected in *S. mombin* because sexual reproduction in this species is preferentially allogamous (cross-fertilization). Indeed, Adler and Kielpinski (2000) demonstrated that the breeding system in several *S. mombin* populations from Panama favored allogamy by identifying patterns of seasonal and synchronous flowering.

Table 3. Analysis of molecular variance (AMOVA) based on ISSR markers in five populations of *Spondias mombin* collected in northeastern Brazil.

Source of variation	Degrees of freedom	Sum of squares	Variance component	Percentage of total variability	G_{ST}	P value
Between- populations	4	167.43	3.75	11.29	0.11	0.0000**
Within- populations	23	678.75	29.51	88.71		

Abbreviation: G_{ST}, genetic differentiation coefficient (fixation measure).

Jaccard's coefficients of genetic similarity varied between 0.27 and 0.84 with a mean of 0.52, demonstrating not only the existence of large variability among the 28 accessions but also the efficiency of the ISSR markers in detecting the genetic diversity at the species level. UPGMA clustering analysis generated a dendrogram (Figure 2)

that faithfully preserved the data points of the initial similarity matrix according to the cophenetic coefficient (r = 0.88), statistically significant (P<0,01). By adopting the maximum variation between the branches as the cut-off point, the accessions could be divided into three groups as follows: group I comprised only one accession (P8), group II comprised BGC-04 and BGC-14, while group III encompassed all of the remaining accessions (89.28%). Group III could be subdivided into sub-groupings and sub-groups showing varying degrees of similarity and containing representatives of the five populations studied.

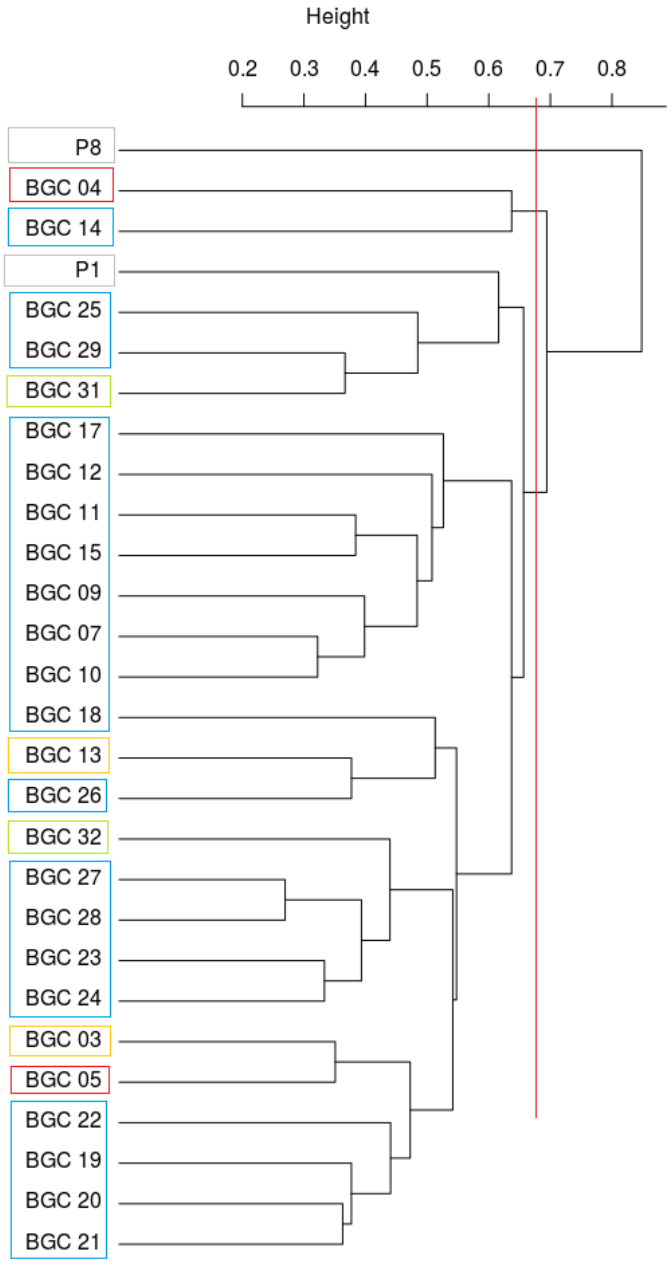


Figure 2. Dendrogram based on ISSR polymorphism and unweighted pair-group method with arithmetic averages (UPGMA) clustering showing the genetic similarities between 28 *Spondias mombin* accessions. Genetic distances were determined through the Jaccard coefficients. The locations of origin of the accessions are represented by rectangles of different colors.

The Bayesian analysis performed in the Structure software (Figure 3) reveals that there was no correspondence between the observed genetic variability and the geographic origin of the S. mombin accessions since individuals from different collection lo-

cations presented a high possibility belonging to the same genetic group. The S. mombin accessions were separated into two distinct genetic groups, as the most likely value of ΔK was K=2. According to the entropy method to determine the ideal value of K, the value of K=2 was also found. Only accessions BGC-03, BGC-18, BGC-20, BGC 25, and P1 presented less than 80% of the constitution of a single genetic group. Accessions BGC-11, BGC-12, BGC-24, BGC 28, BGC-29, and P8 showed uniformity in their genetic structure.

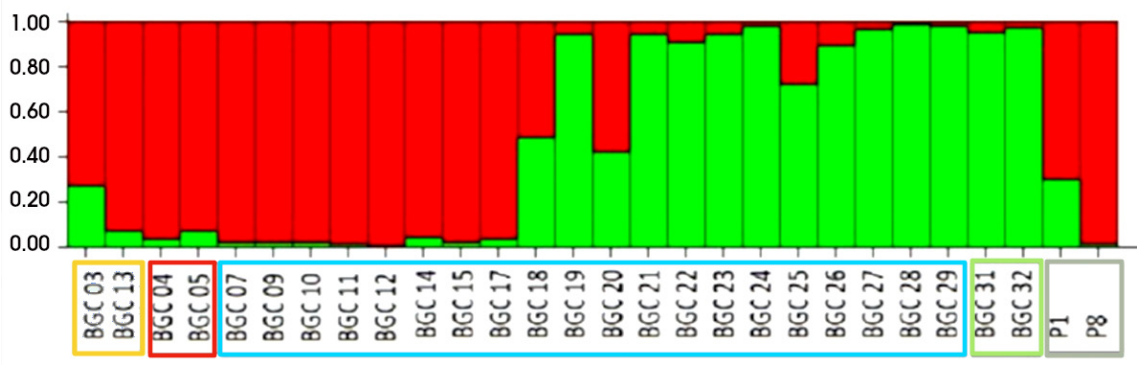


Figure 3. Population structure of S. mombin accessions. The probability of an access belonging to group 1 or group 2 is represented by different colors, red and green, respectively. The locations of origin of the accessions are represented by rectangles of different colors.

This finding suggests that there is large genetic diversity in *S. mombin* at species level. Although the accessions could be classified into distinct genetic groups, there was significant allele exchange between them so that the assumed populations could not be structured based on geographic location. It was noteworthy that more than 80% of the accessions formed a single cluster and this finding indicates that, despite their genetic divergence, there is a certain degree of connection among the accessions. This is strongly supported by the moderate Nm index found while, according Chhajer et al. (2018), an independent grouping demonstrates great genetic variability.

Although both dwarf accessions were collected in Teresina, their actual origin is uncertain since the producer cultivates seeds without control of origin. This justifies the allocation of P8 and P1 into different groups together with other accessions from Teresina and other locations. The genetic distance values of these two dwarf accessions were above the mean distance of all accessions. It is suggested, therefore, that P1 and P8 be integrated into the AGB of Embrapa Meio-Norte with the purpose of enriching the stored and conserved variability. Previous studies on the genetic diversity of *Spondias* species carried out using ISSR analysis also found that accessions collected from the same location were grouped separately (SANTANA et al., 2011; YAMAMOTO et al., 2017). Moreover, a study carried out by Lima et al. (2011) involving RAPD analysis of many of the accessions studied herein revealed that the majority (84.37%) were included in a single group and that the generated dendrogram was similar to that presently reported. These researchers also emphasized that the geographic locations of the sampling sites were not determinant in forming groups.

Our findings demonstrate that the exchange of alleles, as expressed by the Nm coefficient, favored the homogenization of the different populations of *S. mombin* and increased intrapopulation differentiation. Furthermore, our study verified the existence of genetic differentiation between the studied *S. mombin* accessions and confirmed that such variability could be exploited through genetic improvement programs.

Conclusions

ISSR analysis revealed the presence of large genetic variability among the 28 accessions of *S. mombin*. Such diversity cannot be attributed to the spatial distribution of the accessions but to individual differences originating from both the non-domesticated status of the species and to allogamous reproduction. Within-population variability outweighed between-population variability. Although the accessions were separated into distinct genetic groups, they are strongly related, and the five sampling points did not constitute subpopulations since they were not subjected to reproductive isolation, as demonstrated by the moderate exchange of alleles (moderate Nm) and intermediate genetic differentiation between the assumed populations (moderate G_{ST}). The dwarf accessions were the most divergent of the entire collection.

Acknowledgements

The authors wish to thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior for the scholarships awarded to G. S. S. B.

References

ADLER, G.H.; KIELPINSKI, K.A. Reproductive phenology of a tropical canopy tree, *Spondias mombin*. **Biotropica**, Lawrence, v.32, n.4, p.686-92, 2000. Available from: *https://doi.org/10.1111/j.1744-7429.2000.tb00516.x*

ALOBI, N.O.; BASSEY, G.A.; KALU, R.E.; ENYI-IDOH, K.H. Comparison of the phytochemical and nutritional properties of leaf, stem and root of *Spondias mombin* L. **International Journal of Current Microbiology and Applied Sciences**, Tamilnadu, v.6, n.6, p.804-10, 2017. *https://doi.org/10.20546/ijcmas.2017.606.094*

BARUAH J.; GOGOI, B. DAS, K.; AHMED N.M.; SARMAH, D.K.; LAL, M.; BHAU, B.S. Genetic diversity study amongst *Cymbopogon* species from NE-India using RAPD and ISSR markers. **Industrial Crops and Products**, Amsterdam, v.95, p.235-43, 2017. *https://doi.org/10.1016/j.indcrop.2016.10.022*

BOTSTEIN, D.; WHITE, R.L.; SKOLNICK, M.; DAVIS, R.W. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. **American Journal of Human Genetics**, Chicago, v.32, p.314-31, 1980. Disponível em: *https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1686077/pdf/ajhg00189-0020.pdf*

BUHROO, Z.I.; BHAT, M.A.; GANAI, N.A.; KAMILI, A.S.; BALI, G.K.; AZIZ, A. An efficient protocol for the inter-simple sequence repeat (ISSR) marker approach in population genetic studies. **Journal of Entomology and Zoology Studies**, New Delhi, v.6, n.4, p.597-600, 2018. Disponível em: *https://www.entomoljournal.com/archives/2018/vol6issue4/PartK/6-4-96.pdf*

CHHAJER, S.; JUKANTI, A.K.; BHATT, R.K.; KALIA, R.K. Genetic diversity studies in endangered desert teak [*Tecomella undulata* (Sm) Seem] using arbitrary (RAPD), semi-arbitrary (ISSR) and sequence based (nuclear rDNA) markers. **Trees**, Crawley, v.32, n.4, p.1083-1101, 2018. *https://doi.org/10.1007/s00468-018-1697-9*

EVANNO, G.; REGNAUT, S.; GOUDET, J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. **Molecular Ecology**, Oxford, v.14, n.8, p. 2611-20, 2005. *https://doi.org/10.1111/j.1365-294X.2005.02553.x*

EARL, D.A.; VONHOLDT, B.M. Structure Harvester: a website and program for visualizing structure output and implementing the Evanno method. **Conservation Genetics Resources**, Dordrecht, v. 4, p. 359-61, 2012. *https://doi.org/10.1007/s12686-011-9548-7*

EXCOFFIER, L.; LISCHER, H.E.L. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. **Molecular Ecology Resources**, Oxford, 3, p.564-67, 2010. *https://doi.org/10.1111/j.1755-0998.2010.02847.x*

FRICHOT, E.; FRANÇOIS, O. LEA: An R package for landscape and ecological association studies. **Methods in Ecology and Evolution**, Hoboken, v.8, p.925-9, 2015. *https://doi.org/10.1111/2041-210X.12382*

FREITAS, B.M.; BOMFIM, I.G.A. Meliponíneos e polinização: a abelha jandaíra e outros meliponíneos na polinização agrícola no semiárido. *In*: IMPERATRIZ-FONSECA, V.L.; KOEDAM, D.; HRNCIR, M. (ed.). **A abelha jandaíra**: no passado, presente e no futuro. Mossoró: EdUFERSA, 2017. p.213-20.

GOIS, I.B.; FERREIA, R.A.; SILVA-MANN, R.; BLANK, M.F.A.; SANTOS NETO, E.M. Diversidade genética entre indivíduos de *Spondias lutea* L. procedentes do baixo São Francisco sergipano, por meio de marcadores RAPD. **Revista** Árvore, Viçosa, MG, v.38, n.2, p.261-9, 2014. *https://doi.org/10.1590/S0100-67622014000200006*

GOVINDARAJU, D.R. Variation in gene flow levels among predominantly self-pollinated plants. **Journal of Evolutionary Biology**, Oxford. v.2, n.3, p.173-81, 1989. *https://doi.org/10.1046/j.1420-9101.1989.2030173.x*

LIMA, A.T.B.; SOUZA, V.A.B.; GOMES, R.L.F., LIMA, P.S.C. Molecular characterization of cajá, *Spondias mombin* (Anacardiaceae), by RAPD markers. **Genetics and Molecular Research**, Ribeirão Preto, v.10, n.4, p.2893-904, 2011. Disponível em: *https://ainfo.cnptia.embrapa.br/digital/bitstream/item/48679/1/gmr1216.pdf*

LOURENÇO, M.A.M.; BRAZ, M.G.; AUN, A.G.; PEREIRA, B.L.B.; FIGUEIREDO, A.M.; SILVA, R.A.C.; KAZMAREK, E.M.; ALEGRE, P.H.C.; BACHIEGA, T.F.; ZANATI, S.G.; AZEVEDO, P.S.; POLEGATO, B.F.; FERNANDES, A.A.H.; PAIVA, S.A.R.; ZORNOFF, L.A.M.; MINICUCCI, M.F. *Spondias mombin* supplementation attenuated cardiac remodelling process induced by tobacco smoke. **Journal of Cellular and Molecular Medicine**, Oxford, v.22, n.8, p.3996-4004, 2018. *https://doi.org/10.1111/jcmm.13683*

NEI, M. **Molecular evolutionary genetics**. New York: Columbia University Press, 1987. *https://doi.org/10.7312/nei-92038*

NWIDU, L.L.; ELMORSY, E.; OBOMA, Y.I.; CARTER, W.G. Hepatoprotective and antioxidant activities of *Spondias mombin* leaf and stem extracts against carbon tetrachloride-induced hepatotoxicity. **Journal of Taibah University Medical Sciences**, Medina, v.13, n.3, p.262-71, 2018. *https://doi.org/10.1016/j.jtumed.2018.03.006*

PRITCHARD, J.K.; STEPHENS, M.; DONNELLY, P. Inference of population structure using multilocus genotype data. **Genetics**, Bethesdsa, v.155, n.2, p.945-59, 2000. *https://doi.org/10.1093/genetics/155.2.945*

R CORE TEAM. **R**: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2022. Disponível em: *https://www.R-project.org/*

SANTANA, I.B.B.; OLIVEIRA, E.J.; SOARES FILHO, W.S.; RITZINGER, R.; AMORIM, E.P.; COSTA, M.A.P.C.; MOREIRA, R.F.C. Variabilidade genética entre acessos de umbu-cajazeira mediante análise de marcadores ISSR. **Revista Brasileira de Fruticultura**, Jaboticabal, v.33, n.3, p.868-76, 2011. *https://doi.org/10.1590/S0100-29452011005000090*

SHANNON, C.E.; WEAVER, W. **The mathematical theory of communication**. Urbana: University of Illinois Press, 1949.

SLARKIN, M. Gene flow in natural populations. **Annual Review of Ecology and Systematics**, Palo Alto, v.16, n.1, p.393-430, 1985. *https://doi.org/10.1146/annurev.es.16.110185.002141* **(1)**

SILVA, B.M.; ROSSI, A.A.B.; DARDENGO, J.F.E.; TIAGO, A.V.; SILVEIRA, G.F.; SOUZA, S.A.M. Genetic divergences between *Spondias mombin* (Anacardiaceae) genotypes found through morphological traits. **Revista de Biología Tropical**, San Jose, v.65, n.4, p.1337-46, 2017a. *https://doi.org/10.15517/rbt.v65i4.25765*

SILVA, B.M.; ROSSI, A.A.B.; TIAGO, A.V.; SCHMITT, K.F.M.; DARDENGO, J.F.E.; SOUZA, S.A.M. Genetic diversity of cajazeira (*Spondias mombin* L.) in three geographic regions. **Genetic and Molecular Research**, Ribeirão Preto, v. 6, n.1, 2017b. *https://doi.org/10.4238/gmr16018946*

SUZUKI, R.; SHIMODAIRA, H. Pvclust: an R package for assessing the uncertainty in hierarchical clustering. **Bioinformatics**, Oxford, v.22, n.12, p.1540-2, 2006.

WRIGHT, S. **Evolution and the genetics of populations.** Chicago: University of Chicago Press, 1978. v.4.

YAMAMOTO, E.L.M.; HOLANDA, I.S.A.; MORAIS, P.L.D.; LEMOS, M.S.; ARAUJO, E.O.A. Morphological and molecular characterization of umbu-cajá tree in the northeast region of Brazil. **Biotecnología Vegetal**, Villa Clara, v.17, n.2, p.77-90, 2017. Disponível em: *https://revista.ibp.co.cu/index.php/BV/article/view/544/pdf*

YEH, F.C.; YANG, R-C.; BOYLE, T. **POPGENE version 1.31. Microsoft window-based freeware for population genetic analysis**. Quick User Guide. Alberta: University of Alberta and Centre for International Research, 1999. Disponível em: *https://sites.ualberta.ca/~fyeh/popgene.pdf*