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Diversity and genetic structure of mangabeira (*Hancornia speciosa* Gomes), a fruit species from Cerrado

Diversidade e estrutura genética de mangabeira (*Hancornia speciosa* Gomes), uma fruteira do Cerrado

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Abstract

Hancornia speciosa Gomes, popularly known as mangabeira, is a fruit tree belonging to the Apocynaceae family, native to the Brazilian Cerrado. The fruit is widely used by the local population as an alternative source of income. Limited information is available about this species, which increases the difficulty of conserving its genetic resources and exploiting mangabeira as an economic resource. The objective of this research was to evaluate the genetic diversity and genetic structure of *H. speciosa* from Chapada dos Guimarães. Twenty-four trees and ten inter simple sequence repeats (ISSR) primers were evaluated. Of the 57 bands obtained, 33 (57.89%) presented polymorphism. The analysis using Structure defined two different clusters (K = 2), which were consistent with the unweighted pair group method with arithmetic mean (UPGMA) clustering analysis. The number of observed alleles (Na = 1.58), number of effective alleles (Ne = 1.29), Nei's genetic distance (He = 0.18) and Shannon index (I = 0.27) were considered low among the population. The analysis of molecular variance (AMOVA) revealed that the variability within the cluster (83.39%) was higher than among clusters (16.61%). ISSR primers proved effective for detection of genetic polymorphism in *H. speciosa*, and could be utilized for strategies that aim at conservation, plant breeding programs, and commercial use.
Key words: Mangaba. Molecular analysis. ISSR.

Resumo

Hancornia speciosa Gomes, conhecida popularmente como mangabeira, é uma fruteira pertencente à família Apocynaceae que ocorre espontaneamente no Cerrado brasileiro. O fruto da mangabeira é bastante utilizado pela população local como fonte alternativa de renda e sua exploração é feita de forma extrativista. As informações sobre esta espécie são escassas, o que dificulta a conservação dos recursos genéticos e sua exploração econômica. Neste trabalho foi estudada a diversidade e estrutura genética de uma população de mangabeira, tendo sido avaliada um total de 24 árvores matrizes localizadas em Chapada dos Guimarães-MT por meio de 10 *primers* ISSR (inter repetições de sequências simples) que revelaram 57 bandas. Destas, 33 (57,89%) apresentaram polimorfismo. A análise utilizando o *Structure* definiu dois grupos diferentes (K = 2), resultados que foram consistentes com os da análise

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de agrupamento UPGMA. O número de alelos observados (*Na* = 1,58), número de alelos efetivos (*Ne* = 1,29), índice de diversidade de Nei (*He* = 0,18) e o índice de Shannon (*I* = 0,27) foram relativamente baixos dentro da população. A AMOVA revelou que a variabilidade dentro dos grupos (83,39%) foi mais alta do que entre os grupos (16,61%). A utilização de iniciadores ISSR mostrou-se eficaz para detecção de polimorfismo genético em mangabeira, servindo de aporte para estratégias que visem à conservação, melhoramento genético e exploração econômica da espécie.
Palavras-chave: Análise molecular. Mangaba. ISSR.

Introduction

Hancornia speciosa Gomes (Apocynaceae), popularly known as mangabeira, is a species native to the Brazilian Cerrado. Owing to its fruit, the mangaba, this tree is as an alternative source of family income for the local population. It has a potential for cultivation because it is easy to obtain the seeds and the species has adapted to grow in marginal soils. Some orchards designated for cultivation of mangabeira for commercial use are found in the midwestern and northeastern Brazilian (PEREIRA et al., 2010).

The plant is medium-sized, ranging from 5 to 15 m high, with an irregular canopy and numerous tortuous branches. The optimal vegetative growth is achieved during the period with high temperatures and annual rainfall between 750 and 1,600 mm. Considered a typical allogamous species, its flowers are hermaphrodite and self-incompatible. The flowering occurs during the rainy season (SILVA JUNIOR; LÉDO, 2006). Insects belonging to family Euglossinae, Hesperiidae, Urbaninae, Nymphalidae, Sphingidae, and Sphinginae families are the pollinators for this plant species (DARRAULT; SCHLINDWEIN, 2005).

Studies on the genetic diversity of mangabeira populations are scarce. The knowledge of the genetic structure of natural populations is essential for their domestication and breeding and for Genbank sampling, because it may directly affect the long-term viability of the population (MOURA et al., 2011). Owing to the reduction of areas where our study species occurs naturally, i.e., the Cerrado and the Atlantic Forest, the identification, collection, preservation, and characterization of the available genetic resources is of utmost importance (SILVA JUNIOR; LÉDO, 2006).

Molecular markers are widely used to evaluate the genetic variability in natural populations of plants (REDDY et al., 2002; SOARES et al., 2008; ZUCCHI et al., 2005). They are more advantageous than morphological markers because molecular markers are not affected by the environment and they present a high number of polymorphisms, allowing studies at any developmental stage of the plant (FALEIRO, 2007). Inter-simple sequence repeat (ISSR) is a useful tool to study genetic diversity and structure (JIMENEZ et al., 2015).

Therefore, the aim of this study was to use ISSR markers to evaluate the diversity and characterize the genetic structure of mangabeira from the Chapada dos Guimarães/MT.

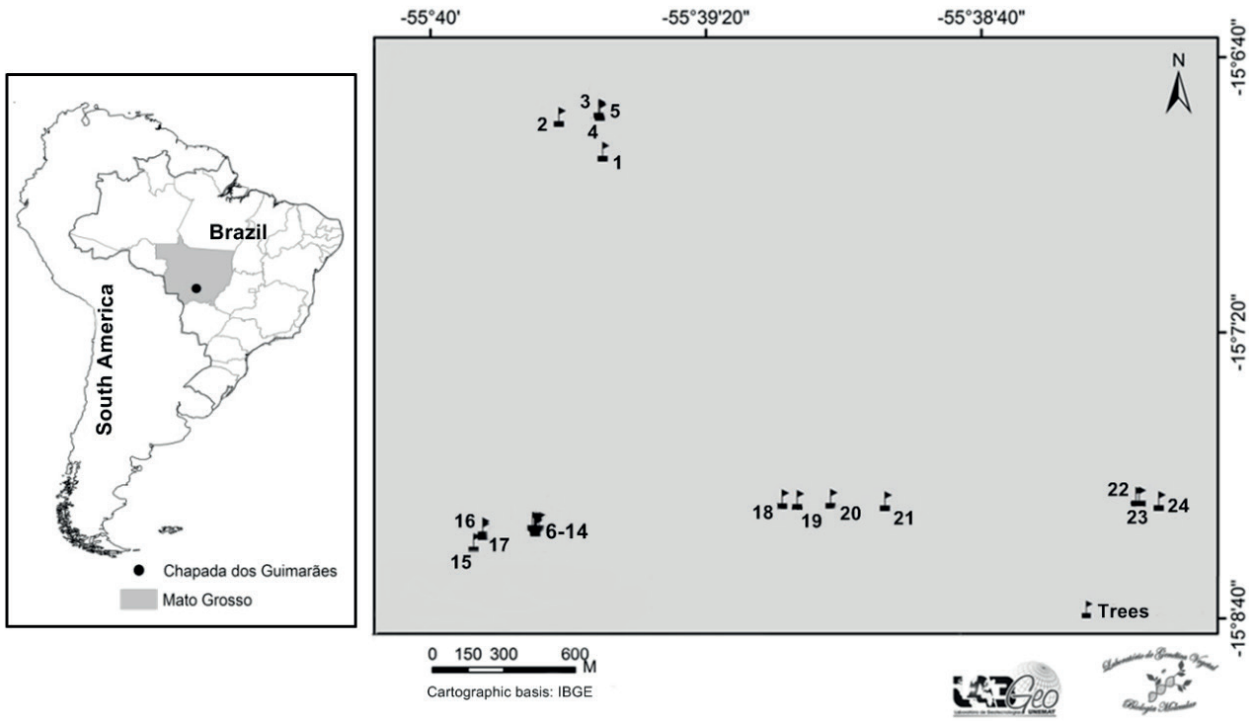
Material and Methods

Plant material and extraction of genomic DNA

Young leaves from 24 mangabeira mother trees were collected in the Chapada dos Guimarães/MT (Figure 1) and stored at −20°C in the Laboratory of Genetics and Molecular Biology of the University of Mato Grosso (UNEMAT) in Alta Floresta/Mato Grosso. DNA was extracted according to the CTAB protocol described by Doyle and Doyle (1987) with the following modifications to the extraction buffer: 5% CTAB (100 mM of Tris-HCl, pH 8; 20 mM of EDTA, pH 8; and 1.4 M of NaCl), 1.5% β-mercaptoethanol, 4% PVP, and the addition of 3.5 µL of Proteinase K (20 mg mL^{-1}) per sample.

The amount and integrity of DNA samples were verified on 1% agarose gel stained with ethidium bromide (10 mg mL^{-1}) and subjected to horizontal electrophoresis for approximately 2 h at 80 V. DNA was visualized under UV light using a transilluminator and quantified by comparison with a known standard molecular weight using the bacteriophage λ DNA (10 ng μL^{-1}). Samples were diluted in autoclaved distilled water, standardized to a concentration of 5 ng μL^{-1}, and stored at −20°C.

Figure 1. Distribution of 24 mangabeira mother trees in the sampled population in Chapada dos Guimarães/MT.



PCR and electrophoresis

Ten ISSR primers (University of British Columbia, Vancouver, Canada) were used for genomic DNA amplification. The mixture comprised 2.5 µL of 10X buffer (without Mg^{++}), 1 µL of $MgCl_2$ (50 mM), 1 µL of DMSO (100%), 1 µL of dNTPs (1 mM each), 2 µL of primer (0.2 mM), 0.12 µL Taq polymerase (5 U), 2 µL of genomic DNA (5 ng) and autoclaved distilled water to a final volume of 25 µL. Amplification conditions were one initial denaturation for 4 min at 94°C, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 49.8 to 53°C for 1 min, and extension at 72°C for 2 min, plus a final extension step at 72°C for 7 min.

Amplification products were separated on 2% agarose gel stained with ethidium bromide and immersed in 1X TBE buffer (89 mM Tris-base, 89 mM boric acid, and 2 mM EDTA) for about 4 h at 80 V. The DNA ladder of 100 bp (Kasvi) was used as a molecular weight standard. Gels were visualized in UV light and photographed using a digital camera.

Statistical analysis

The data of presence (1) and absence (0) of bands were used to estimate the genetic distance across the arithmetic complement of the Jaccard coefficient using the software Genes (CRUZ, 2013). The dendrogram of genetic dissimilarity based on

the unweighted pair group method with arithmetic mean (UPGMA) hierarchical method was generated in the software R, version 3.2.4 (R CORE TEAM, 2016), with 1,000 simulations for a grouping consistency test.

The Bayesian analysis was performed using the software Structure (PRITCHARD et al., 2000). The number of *K* clusters was adjusted to variations from 1 to 5, with 20 independent interactions, each using 250,000 burn-ins and 750,000 Markov chain Monte Carlo (MCMC) simulations. The genetic structure was analyzed using the software Structure Harvester (EARL; VONHOLDT, 2012) following criteria described by Evanno et al. (2005) for determining the optimal number of clusters. The genetic variation between clusters was calculated by Nei's genetic distance (*He*) and Shannon index (*I*) using the software Popgene (YEH et al., 1997). The analysis of molecular variance (AMOVA) was calculated using the software Arlequin 3.5 (EXCOFFIER; LISCHER, 2010). The significance of differentiation was tested with 1,000 permutations, where P denotes the probability of observing a random value greater than or equal to the observed value.

Results and Discussion

The set of ISSR primers used produced 57 bands, 5.7 bands per primer, ranging from 2 (UBC 812) to 8 (UBC 809 and UBC 835) (Table 1). Among all amplified bands, 33 (57.89%) were polymorphic, indicating that these markers may be used to distinguish mangabeira populations and trees. Studies with this species using the ISSR technique have detected 47.62% (COSTA et al., 2015) to 89.27% (JIMENEZ et al., 2015) of polymorphism.

Table 1. ISSR primers with their nucleotide sequences, annealing temperatures (Ta), total number of bands (TNB), polymorphic bands (PB), and percentage of polymorphic bands (PPB).

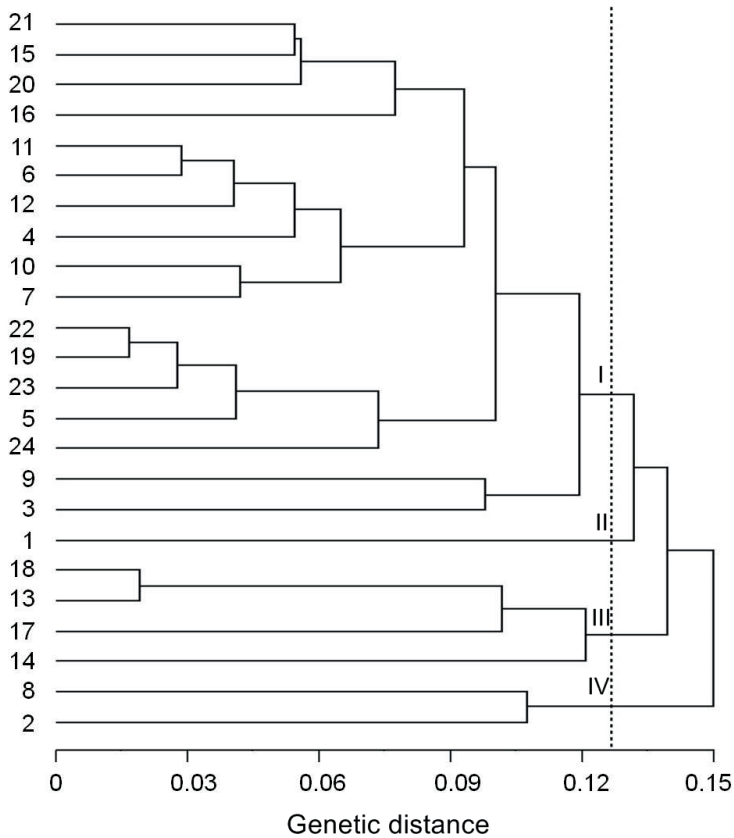
Primers	Sequence 5' → 3'	Ta (ºC)	TNB	PB	PPB (%)
UBC 807	AGA GAG AGA GAG AGA GT	49,8	5	3	60,0
UBC 809	AGA GAG AGA GAG AGA GG	51,5	8	6	75,0
UBC 810	GAG AGA GAG AGA GAG AT	50,0	7	3	42,8
UBC 812	GAG AGA GAG AGA GAG AA	50,0	2	1	50,0
UBC 818	CAC ACA CAC ACA CAC AG	52,5	3	2	66,7
UBC 827	ACA CAC ACA CAC ACA CG	53,0	6	4	66,7
UBC 834	AGA GAG AGA GAG AGA GYT	52,5	6	5	83,3
UBC 835	AGA GAG AGA GAG AGA GYC	50,2	8	2	25,0
UBC 841	GAG AGA GAG AGA GAG AYC	50,0	6	3	50,0
UBC 891	HVH TGT GTG TGT GTG TG	50,4	6	4	66,7
Total			57	33	

Based on the grouping by UPGMA, there was differentiation of mother trees from a dissimilarity of 0.02 (Figure 2), indicating a low genetic divergence among some mangabeira mother trees. Using mean genetic dissimilarity (0.13), four similarity groups were established: G-1: 17 mother trees; G-2: one mother tree (1); G-3: four mother trees (18, 13, 17 and 14); and G-4: two mother trees (8 and 2).

The cophenetic correlation coefficient showed an association of 68% between the distances obtained by the Jaccard coefficient (dissimilarity matrix) and those represented in the dendrogram (cophenetic

matrix). The cophenetic value observed was r = 0.68 ($p < 0.001$), which is adequate as $r \geq 0.56$ values are considered ideal, thus reflecting a consistency with genetic similarity values (VAZ PATTO et al., 2004).

Figure 2. Dendrogram of 24 mangabeira mother trees, obtained by UPGMA, using the complement of the Jaccard similarity index from 10 ISSR primers. The value of the cophenetic correlation coefficient (r) is 0.68.



According to Bayesian analysis performed using the software Structure and in accordance with the Δ*K* method described by Evanno et al. (2005), the most likely number of clusters was *K* = 2 (Figure 3-A). The graphic display of the population structure allowed the separation of mother trees into two clusters: Cluster I, which had 15 mother trees and Cluster II, which had nine mother trees (Figure 3-B). These results were consistent with the UPGMA grouping method, assuming that based on the genetic distance of 0.11 (70% similarity), the population is divided into two subpopulations.

A joint analysis of the information between the UPGMA grouping and the Bayesian analysis revealed a consistency in groupings using both methods. Structure Cluster I had the highest number of mother trees and was similar to the G1 in the dendrogram. The remaining mangabeira mother trees, except mother trees 9 and 3, were allocated to the Structure Cluster II, corresponding to the G-2, G-3 and G-4 groups of UPGMA. Therefore, it appears that there is a genetic diversity among the mangabeira population evaluated, because mother trees were not allocated to the same cluster by any of the grouping methods.

The Structure groupings did not reflect a geographical structure in relation to genetic diversity (Figures 1 and 3). It is also observed that more similar

mangabeira mother trees (22 and 19, 18 and 13) were not the closest geographically (Figures 1 and 2). This similarity between geographically distant pairs can be explained by activities of humans and seed dispersers (OLIVEIRA et al., 2010).

The Nei's genetic distance (*He*) for the two clusters formed by the software Structure was 0.18. The observed number of alleles was 1.58 and the effective number of alleles was 1.29. The Shannon index (*I*) was 0.27 (Table 2). The values observed for *He* and *I* indicate that there is a genetic diversity within the population. In their research with mangabeira, Silva et al. (2012), using molecular markers, found a value of 0.35 for *He* and a value of 0.46 for *I*. They concluded that the species showed a high genetic diversity. Costa et al. (2015), assessing the genetic diversity among mangabeira genotypes, found average values of 0.17 and 0.25 for *He* and *I*, respectively. This was similar to the values found in this study. Jimenez et al. (2015) observed absolute values for *He* ranging from 0.197 to 0.229.

Figure 3. Distribution of Δ*K* probability. A. Estimated structure of the mangabeira population. B. Representation of 24 mangabeira mother trees divided into clusters according to molecular data using ISSR by the software Structure. Mother trees are represented by vertical bars with a coloring related to the group to which they belong (two clusters, *K* = 2).

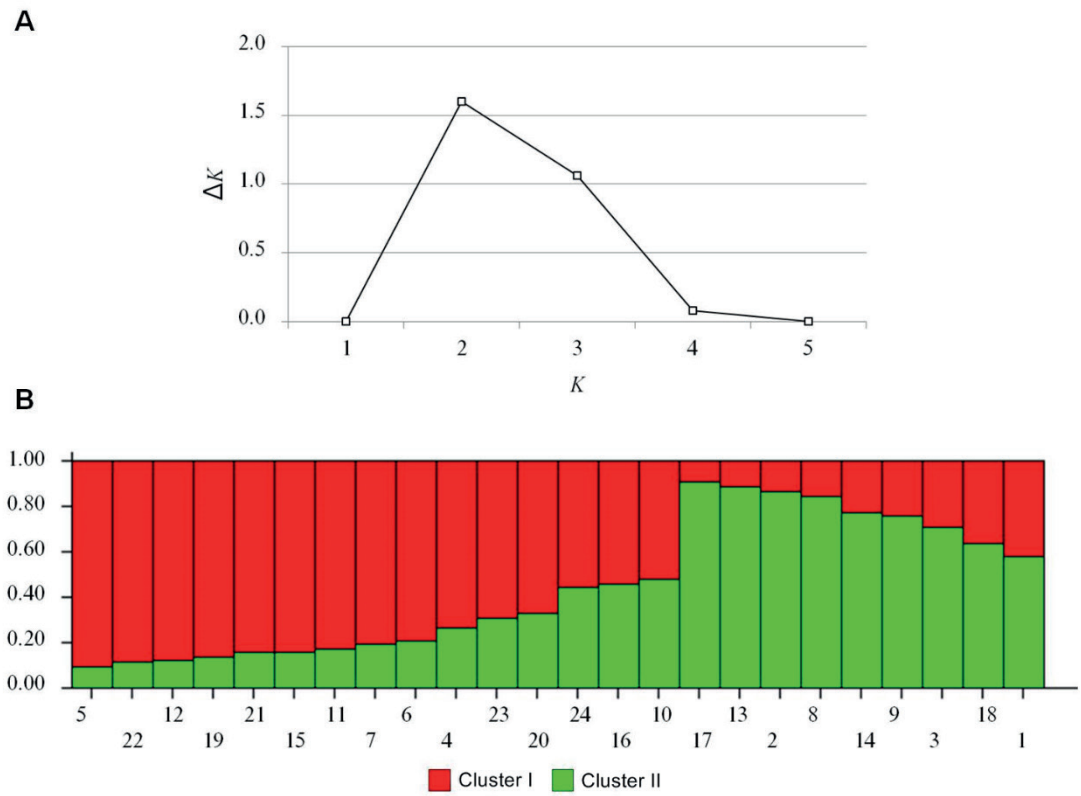


Table 2. Number of mother trees (N) and genetic parameters for the two clusters (K = 2), as determined by the software Structure for 24 mangabeira mother trees.

Parameters	Cluster I	Cluster II	Total
N	15	9	24
Na	1,35 (0,48)	1,49 (0,50)	1,58 (0,50)
Ne	1,21 (0,33)	1,32 (0,39)	1,29 (0,33)
He	0,12 (0,19)	0,18 (0,21)	0,18 (0,18)
I	0,19 (0,27)	0,27 (0,30)	0,27 (0,27)
%P	35,09	49,12	57,89

N: Number of mother trees, Na: Number of observed alleles, Ne: Number of effective alleles, *He:* Nei's genetic distance, *I*: Shannon diversity index, and %P: Percentage of polymorphic loci. The numbers in parentheses represent the standard deviation.

Higher Nei's (*He*) and Shannon (*I*) genetic diversity indices were found for Cluster II (*He* = 0.18 and *I* = 0.27) (Table 2). Despite having the lowest number of mother trees, this cluster also showed higher Na (1.49) and Ne (1.32) values than those of Cluster I. Therefore, the effects of genetic drift will be lower in this cluster. In small and isolated populations, the genetic drift may lead to a short-term distancing of gene frequencies of the original population, resulting in losing alleles. As the time progresses, there may be an increase of inbreeding between related plants to avoid a greater probability of crossbreeding (KAGEYAMA et al., 1998).

The analysis of molecular variance (AMOVA) showed that the genetic variation happens more within (83.39%) than among clusters (16.61%), indicating that mangabeira is allogamous. Levels of genetic variation of allogamous species are theoretically high within populations and relatively low among populations (HAMRICK et al., 1992). These results corroborate some studies conducted with Cerrado tree species (SOARES et al., 2008; ZUCCHI et al., 2005). Darrault and Schlindwein (2005), studying the reproductive biology of mangabeira, identified a self-incompatibility mechanism that favors cross-pollination.

Mangabeira is a highly-threatened fruit tree species owing to a significant decrease in the ecosystem areas occupied by it in coastal zones (JIMENEZ et al., 2015) or in central Brazil (MOURA et al., 2011). In this study, mother trees were found in grasslands and within road zones under a strong anthropic pressure, forming aggregates of individual plants isolated 2.1 km from each other. Such characteristics may restrict the movement of pollen and short-distance seed dispersers, facilitating crossbreeding between neighboring plants and causing a further isolation between subpopulations (MOURA et al., 2011). *In situ* and *ex situ* conservation strategies are needed to promote the preservation of the remaining genetic variability.

Conclusion

ISSR primers identified a molecular polymorphism among mangabeira mother trees and may be used for genetic analysis to obtain information for development of conservation strategies and breeding programs, and for commercial use. Most of the genetic exchange happens within groups, providing evidence for the need of conserving various mother trees to preserve the genetic variability of the population.

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