GENETIC DIVERSITY ANALYSIS OF MANGABA (*Hancornia speciosa* Gomes), AN EXOTIC BRAZILIAN TROPICAL SPECIES

[ANÁLISIS DE LA DIVERSIDAD GENETICA DE MANGABA (*Hancornia speciosa* Gomes), UNA TROPICAL Y EXÓTICA ESPECIE BRASILEÑA]

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SUMMARY

Twenty genotypes from natural populations of mangaba located in Itaporanga D'Ajuda (Sergipe, Brazil) were analyzed using RAPD markers. Polymorphism, genetic diversity and structure parameters were determined to characterize the differences between individual plants. 60 DNA fragments were generated by 10 primers, 85% of which were polymorphic. Results show a quantitative genetic diversity value of 0.35 and a Shannon index of 0.46 in the population. The similarity among the specimens according to Jaccard's coefficient ranged from 0.36 to 0.87. Using cluster analysis, it was possible to indentify five groups. Three individuals also stand out because they presented significant divergence from the groups. The PCoA formed four groups, with three of the groups isolated from the others. This investigation showed that genetic diversity was relatively large among these individuals. In addition, the results demonstrated that RAPD markers are a useful tool for evaluating the genetic diversity and relationships among mangaba.

Key words: Apocynaceae; genetic variability; RAPD.

RESUMEN

Veinte genotipos pertenecientes a una población natural de mangaba localizada en la ciudad de Itaporanga D'Ajuda (Estado de Sergipe - Brasil) fueron sometidos a análisis mediante marcadores RAPD. Se determinaron parámetros de polimorfismo, estructura y diversidad genética para caracterización de las plantas. 60 fragmentos de ADN fueron generados mediante el uso de 10 primers, de los cuales 85% fueron polimórficos. Los resultados muestran un valor cuantitativo de la diversidad genética de 0,35 y un índice de Shannon de 0,46 en la población. La similitud entre los genotipos de acuerdo con el coeficiente de Jaccard varió desde 0,36 hasta 0,87. Utilizando el análisis UPGMA fue posible la identificación tres genotipos más distantes genéticamente. La agrupación PCoA ha formado cuatro grupos, con tres genotipos aislados de los demás. Hubo variación genética entre los individuos, y fue posible la identificación de los genotipos más divergentes en las muestras estudiadas. Además, los resultados demostraron que los marcadores RAPD son una herramienta útil para evaluar la diversidad genética y las relaciones entre las mangabas.

Palabras clave: variabilidad genética; Apocynaceae; RAPD.

INTRODUCTION

Hancornia speciosa Gomes (Apocynaceae), commonly known as mangaba, is a native Brazilian tree, distributed mainly in areas of open vegetation such as savannas, dunes, coastal tablelands and coastal lowlands. The fruit provides a rich source of protein (up to 3% on a fresh weight basis), has a highly desirable flavor for fresh consumption and is highly used in the production of juice and ice cream (Caldas *et al.*, 2009). Mangaba is also one of the most promising fruits for programs of sustainable harvesting. Additionally, the bark of this species is used as a traditional medicine to treat hypertension, gastric ulcers and inflammatory diseases (Almeida *et al.*, 1998; Mello Moraes et.al. 2008).

Because of these attractive features, the mangaba plant is under constant anthropogenic pressure in the agroecosystems where they occur, and is a species with the potential need for a conservation strategy. In response to a lack of published information concerning the agronomic characteristics of mangaba or genetic studies, several research projects have been developed to facilitate the collection and genetic characterization of mangaba specimens *in situ* and *ex situ*. The information provided by these studies will be extremely useful for the development of conservation

strategies and will help the process of domestication and breeding of the species (Ganga *et al.*, 2009).

Given the social, economic and cultural importance of mangaba, intense harvesting has put pressure on natural populations of this species, and the International Catalog call "Arca do Gosto" states that it is in danger of extinction unless a sustainable management plan is developed (Slow Food, 2011). Mangaba, like other native species in Brazil, are poorly understood and lack genetic studies. Research at the genetic level is essential to the development of effective conservation strategies and future domestication efforts.

RAPD (Random Amplified Polymorphism DNA) has played a key role in identifying genotypes and understanding relationships among close individuals. The technique is also attractive because it does not depend on large quantities of genomic DNA for polymerase chain reaction (PCR) amplifications (Fritsch and Reiseberg 1996). It has been reported (Silveira *et al.*, 2009; Maina *et al.*, 2009) that RAPD analysis has successfully been used to characterize the genetic variability within and among populations of caroa (*Neoglaziovia variegate* Mez) and japanese apricot (*Prunus mume* Sieb. Et Zucc.) (Mayer *et al.*, 2008), as well as blueberry (*Vaccinium* spp.) (Silva *et al.*, 2008). Other examples using RAPD analysis in the population and conservation genetics of plants have also been reported (Rossetto *et al.*, 1995; Cardoso *et al.*, 1998; Lacerda *et al.*, 2001). Additionally, the genetic relationships of *Jatropha curcas* germplasm specimens (Kumar *et al.*, 2009) and mangaba genotypes (Moura et al., 2005) were successfully estimated using this technique.

Genetic diversity analysis of mangaba using RAPD markers is anticipated to increase the knowledge of genetic population structure and will contribute to the development of strategies for seed collection, which may be used to study future progenies and guide the allocation of conservation areas in a sustainable management program. Here, we report the molecular characterization of 20 genotypes of mangaba from a natural population located in the state of Sergipe in Brazil, using RAPD markers.

MATERIAL AND METHODS

Young leaves of 20 genotypes of mangaba were collected in the natural population from the Reserva do Caju located in the city of Itaporanga d'Ajuda (Sergipe, Brazil), in december of 2008 (Table 1). The DNA extractions were performed according to Doyle and Doyle (1991). The PCR reactions were performed according to Williams *et al.*, (1990) and modified to a final volume of 25 μL. To detect molecular polymorphisms among and within accessions, ten

primers were tested and used for the amplification of polymorphic loci, which were: IDT 2, IDT 3, IDT 4, IDT 6, IDT 8, IDT 9, IDT 10, IDT 11, IDT 14, and IDT 20 (IDT DNA Technologies, USA). The amplification products (bands) were separated in 1.5% agarose gel, stained with ethidium bromide and visualized under ultraviolet light using a Gel Doc L-Pix image system (Loccus Biotecnologia, Brazil).

Table 1. Location of mangaba (*Hancornia speciosa Gomes*) native from Reserva Ecológica do Caju, Itaporanga d'Ajuda (Sergipe, Brazil).

Genotype	Latitude	Longitude
RC 1	11º06'39.2''	37º11'13.3''
RC 2	11º06'39.3''	37º11'14.4''
RC 3	11º06'37.8''	37º11'15.0''
RC 4	11º06'38.1''	37º11'16.4''
RC 5	11º06'38.3''	37º11'18.4''
RC 6	11º06'39.0''	37º11'18.1''
RC 7	11º06'39.7''	37º11'18.6''
RC 8	11º06'40.6''	37º11'18.8''
RC 9	11º06'41.6''	37º11'18.6''
RC 10	11º06'41.3''	37º11'18.3''
RC 11	11º06'40.8''	37º11'16.9''
RC 12	11º06'40.8''	37º11'16.7''
RC 13	11º06'41.6''	37º11'16.2''
RC 14	11º06'41.7''	37º11'16.3''
RC 15	11º06'41.3''	37º11'15.4''
RC 16	11º06'40.6''	37º11'15.3''
RC 17	11º06'40.9''	37º11'14.1''
RC 18	11º06'41.5''	37º11'13.8''
RC 19	11º06'39''	37º11'13.1''
RC 20	11º06'37.6''	37º11'13.3''

The presence or absence of RAPD band was scored as "1" or "0", respectively. The minimum number of fragments to conduct a study of genetic diversity was based on Oliveira *et al*. (2006), and these were analyzed by the bootstrap method (Manly, 1997) with a resampling of 1000, as recommended by Moura *et al*. (2005), using the software Genes (Cruz, 2006). The number of polymorphic fragments were considered optimal when the assumed stress value was less than 0.05 (Kruskal, 1964).The data matrix of the RAPD scores was generated and similarity coefficients were calculated using Jaccard's arithmetic complement index (1908). The dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster algorithm. In order to determine the robustness of the dendrogram, the data was bootstrapped with 10,000 replications using FreeTree software (http://web.natur.cuni.cz/flegr/programs/freetree.htm) and for visualization of the cluster we used the TreeView package

(http://web.natur.cuni.cz/flegr/programs/freetree/Tree View.exe). Principal Coordinates Analysis (PCoA) was performed using the software XLSTAT (http://www.xlstat.com/) based on similarity matrix of Jaccard. Polymorphic information content (PIC) is a parameter that provides an estimate of the discriminatory power of molecular marker per primer and was calculated according to Ghislain *et al.*, (1999). The marker index (MI) was determined as a product of PIC and the number of polymorphic bands per assay unit as described in Zhao *et al.*, (2007). To measure the population genetic structure, we used Genalex v.6.3 (www.anu.edu.au/BoZo/GenAlEx/) and calculated the Shannon Index (I) (Brown and Weir 1983) as well as genetic diversity (H) as described by Lynch and Milligan (1994) and Maguire *et al.*, (2002) for dominant markers.

RESULTS AND DISCUSSION

The primers used resulted in a banding pattern that was distinct, as seen in Figure 1 which shows the RAPD profiles of the primer IDT 3.

A total of 60 DNA fragments were generated by the 10 primers, 85% of which were polymorphic. Results of the bootstrap analyses of were plotted (Figure 2). We observed that there was a direct relationship between the number of fragments analyzed and the magnitude of correlation values, and was consistency from 48 polymorphic fragments, where the correlation (r) was 0.97 and 0.047 to stress value (SV) (Kruskal, 1964). The genetic diversity estimates from 55 polymorphic fragments among the mangaba genotypes showed excellent precision in our study.
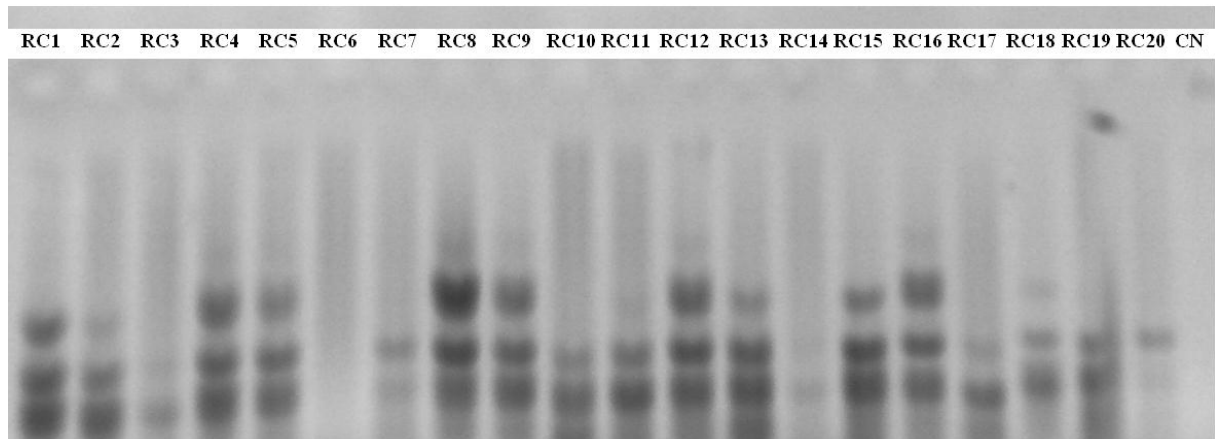

Figure 1. RAPD profiles generated by IDT 3 primer and negative controls (CN) between 20 genotypes of mangaba (*Hancornia speciosa* G.) from Reserva Ecológica do Caju, Itaporanga d'Ajuda (Sergipe – Brazil).
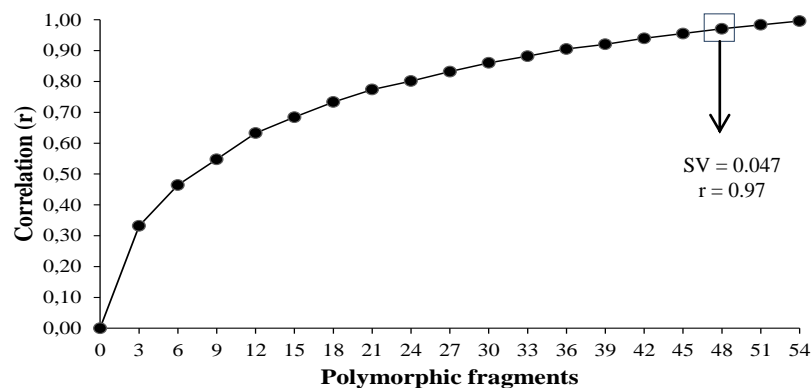

Figure 2. Correlation estimate (r), polymorphic fragments, and the stress value (SV) between the similarity obtained by bootstraps to increasing numbers of polymorphic RAPD fragments to get the minimum fragments number to study of genetic diversity of mangaba genotypes (*Hancornia speciosa* G.) from Reserva Ecológica do Caju, Itaporanga d'Ajuda (Sergipe, Brazil).

IDT20 presented the greatest number of fragments (11), resulting in a polymorphism of 100%, as well as the sequences IDT 6 and IDT 14. The PIC value was 0.321 (0.116 - 0.48), and the global value MI was 1.31 (0.66 - 3.53). For the genetic structure, the Shannon's Index (I) was 0.46 (±0.041) and the genetic diversity (H) was 0.35 (±0.026) (Table 2).

High polymorphism (85%) was identified in mangaba from Sergipe, Brazil, which corroborates previous studies by Silveira *et al.*, (2009), who found similarly high polymorphism (93%) in *Neoglaziovia variegate* in 501 DNA fragments generated by 36 primers. Additionally, a study of *Butia capitata* from Nunes *et al.*, (2008) obtained 136 fragments obtained from 21 primers, where 77 were polymorphic. Mayer *et al.*, (2008), however, evaluated three japanese apricot genotypes and found that only 23.6% were polymorphic using 42 primers generating a total of 432 profiles. According to Nei (1987), the proportion of polymorphic loci is not a good measure of genetic variation, and the analyses more appropriate to measure is by gene diversity (H). In the present study, genetic diversity observed (H - 0.35 ±0.026) in the population indicated that it does not fit the criteria of the Hardy-Weinberg equilibrium, suggesting an excess of homozygotes or heterozygotes among individuals (Kageyama *et al.*, 2003). This is expected, considering that individuals are likely to incorporate or losing alleles by genetic drift in natural conditions.

Shannon's Index was calculated to provide a relative estimate of the degree of variation in the genotypes. The values of Shannon's Index (I) found in mangaba (0.46 ±0.041) were similar to values in previously published studies: 0.31 in *Taxus baccata* using 120 genotypes (Zarek, 2009), and 0.36 in *Jatropha curcas* species using 13 genotypes (Gupta *et al.*, 2008).

Others studies reported higher values in other plant species (Jogait *et al.*, 2006), e.g., *Vaccinium oxycoccus* (0.19) using 56 samples (Žukauskien *et al.*, 2009). The Shannon index provides information applicable to other fields in ecology, is relatively insensitive to the effects of distortion caused by the inability to detect heterozygous loci (Dawson *et al.*, 1995), and is a good tool in the study of populations. Additionally, estimates of diversity using the Shannon index do not rely on Hardy-Weinberg equilibrium (Bussell, 1999) and can be compared across species with different breeding systems.

The uses of genetic parameters, such as e.g. genetic diversity and Shannon's index, are convenient in the study of populations, (Zhao *et al.*, 2006). Regarding the ecological factors which determine the genetic structure in plant populations (Loveless and Hamrick, 1984), the population of mangaba presented high values, regarding I (0.43 ±0.041) and H (0.35 ±0.026), which might be related to presence of important ecological characteristics of mangaba due to the conversation of genotypes and allelic movement.

The values from the genetic similarity obtained by the Jaccard coefficient ranged from 0.36 (RC13 - RC19) to 0.87 (RC16 - RC17). The average genetic similarity was equal to 0.59. After UPGMA analyses, it was possible to visualize the formation of five sub-clusters, I (RC12), II (RC1, RC13, RC3, RC2, RC4, RC5, RC11, RC14, and RC15), III (RC10, RC6, RC7, RC8, and RC9), IV (RC20) and V (RC19, RC18, RC16 and RC17). The most significant distance was RC12 (0.57), RC1 (0.62), RC19 (0.62) and RC20 (0.59). The natural population sampled here had a wide genetic variability, and these results may be used to assist breeding programs and propagation studies and/or cloning mangaba species (Figure 3).

Table 2. Primers, totals (TF) and polymorphic fragments (PF%), polymorphic information content (PIC), marker index (MI), Shannon index (I) and genetic diversity (H) between 20 genotypes of mangaba (*Hancornia speciosa* G.) from Reserva Ecológica do Caju, Itaporanga d'Ajuda (Sergipe, Brazil).

| Primers	Sequence 5' – 3'	TF	PF%	PIC	MI	I		H	
						Value	Variance	Value	Variance
IDT 2	TGC CGA GCT G	4	50	0,48	0,96	0,42	0,045	0,25	0,031
IDT 3	GTT TCG CTC C	3	67	0,43	0,86	0,65	0,048	0,46	0,029
IDT 4	TGA TCC CTG G	5	60	0,25	0,74	0,40	0,020	0,25	0,012
IDT 6	GTG AGG CGT C	6	100	0,35	2,08	0,53	0,000	0,35	0,000
IDT 8	GGA CCC AAC C	5	80	0,16	0,66	0,40	0,091	0,25	0,048
IDT 9	CCC AAG GTC C	4	75	0,38	1,14	0,58	0,047	0,39	0,028
IDT 10	GGT GCG GGA A	7	86	0,25	1,48	0,27	0,073	0,14	0,034
IDT 11	ACG GAT CCT G	7	86	0,30	1,85	0,67	0,000	0,48	0,000
IDT 14	GGC ACT GAG G	8	100	0,29	2,30	0,62	0,000	0,43	0,000
IDT 20	GGA GGA GAG G	11	100	0,32	3,53	0,53	0,036	0,35	0,023
Total		60	85%	0,32	1,31	0,46	0,041	0,35	0,026
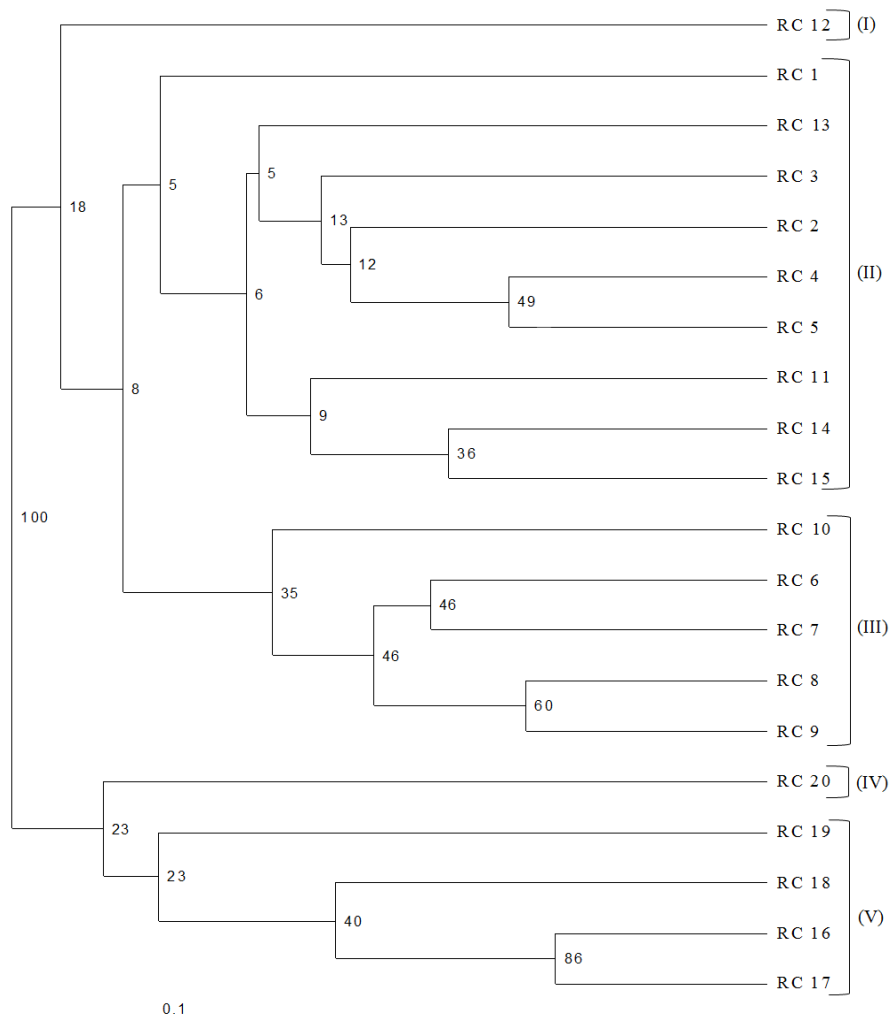
Figure 3. Cluster generated from the Jaccard similarity coefficient and UPGMA clustering using 10 RAPD primers between 20 genotypes of mangaba (*Hancornia speciosa* G.) from Reserva Ecológica do Caju, Itaporanga d'Ajuda (Sergipe, Brazil).

The wide variation in genetic distance among the different mangaba species reflected a high level of polymorphism at the DNA level. The similarity coefficients (0.59) found in our study were to previous studies using RAPD markers. Santana *et al.*, (2008) (studying *Enterolobium contortisiliquum*) found a similarity of 0.67 and Caixeta *et al.*, (2003) (evaluating *Eucalyptus* genotype) found values greater or equal to 0.70.

Principal coordinate analysis (PCoA) was performed in order to determine the genetic relationships among the accessions with minimum distortion. The average distance among samples was plotted in a two-dimensional plot, and explains 40% of the total variation (Figure 4).

The PCoA resulted in a clear separation of groups, corroborating the distribution obtained from the cluster analysis. The individuals were separated into four distinct groups, according to genetic similarity: I (RC1, RC2), II (RC14, RC4, RC13, RC5, RC15, RC13), III (RC7, RC6, RC8, RC9, RC16) and IV (RC17, RC 18, RC19). Three individuals were not grouped (RC12, RC10, RC11), and corresponded to the most genetically divergent specimens.

Grouping of individuals within a natural population may be expected due to many factors, for example, anthropic factors and selection inside of the population. According to UPGMA, some individuals were close (e.g., RC16 - RC17), and also in the PCoA (RC4 - RC13, as well as RC15 - RC3).
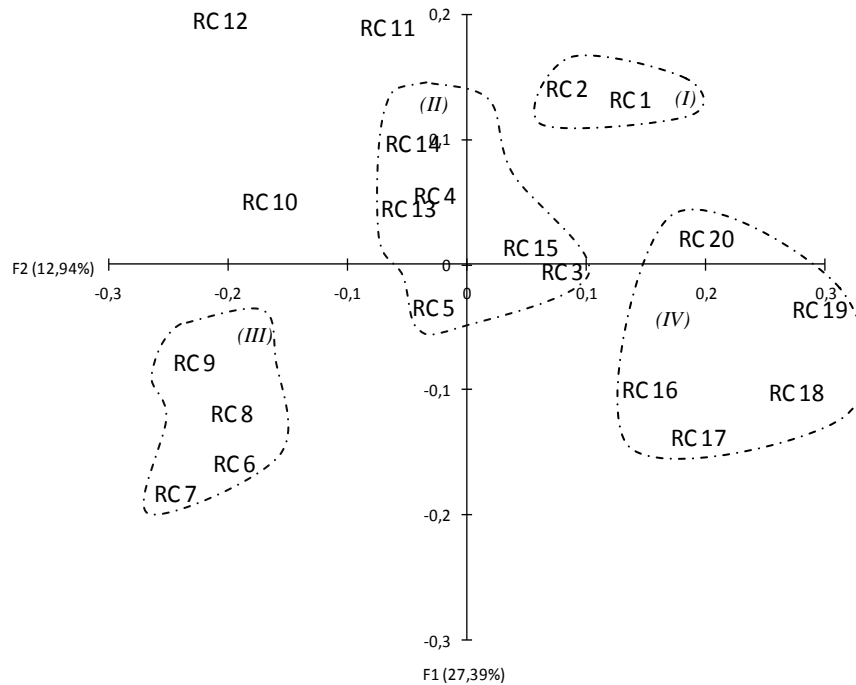
Figure 4. Principal Coordinates Analysis (PCoA) obtained from the Jaccard similarity coefficient using 10 RAPD primers between 20 genotypes of mangaba (*Hancornia speciosa* G.) from Reserva Ecológica do Caju, Itaporanga d'Ajuda (Sergipe, Brazil).

The high genetic variability in this population may be a consequence of adaptation to changes of habitat. Theoretically, variations caused by selection pressures can maintain genetic polymorphism (Sheng *et al.*, 2004). An alternative hypothesis is that, although has many factors that influence the population, the genotypes of mangaba are very active genetically, which provides the development of the variation observed. According to Darrault and Schlindwein (2005) the pollination mechanism in mangaba increases pollination efficiency because it reduces pollen loss and prevents autogamy, thereby favoring cross pollination and contributing to the genetic dynamics. The other hypothesis is that various anthropogenic selection pressures provided this alteration. In the future, more research with the species is needed to support our hypothesis, and to further explore this variation and how it influences the mangaba population.

In the plant breeding field, a key step to obtain genetic variability among individuals of a specific species or among groups is interspecies crosses. The genetic structure of plant populations reflects the interactions of many different processes, such as the long-term evolutionary history of the species (e.g., shifts in distribution, habitat fragmentation, and/or population isolation), mutation, geographical, ecological, reproductive isolation, genetic drift, mating system,

gene flow, and selection (Schaal *et al.*, 1998; Tripathi *et al.*, 2007; Thendral Hepsibha *et al.*, 2010). The genetic diversity greatly contributes to the sustainability of plant populations (Wang *et al.*, 2007) and its measurement is important to understand the events that occur in the population.

CONCLUSIONS

This is the first report on the mangaba species from northeast of Brazil, and our study is a contribution to the characterization of native genotypes. The genetic variation and the genetic relationships among wild mangaba were efficiently determined using RAPD markers. The discrimination of mangaba from Sergipe (Brazil) and identification of genotypes more genetically divergent may contribute to development of strategies for the implementation of conservation, breeding programs efforts and commercial exploitation.

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