Original Article

GENETIC STRUCTURE OF MANGABA (*Hancornia speciosa* Gomes) POPULATIONS IN THE CERRADO REGION OF CENTRAL BRAZIL

ESTRUTURA GENÉTICA DE SUBPOPULAÇÕES DE MANGABEIRA (*Hancornia speciosa* Gomes) *NO CERRADO DO BRASIL CENTRAL*

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ABSTRACT: Mangaba tree (*Hancornia speciosa* Gomes) is a fruit species with great potential for commercial exploitation, found in most regions of Brazil and highly frequent in the Cerrado biome. Information on the genetic variability in this biome is scarce. This study aimed at characterizing the genetic structure of eight populations found in the states of Goiás, Bahia and Minas Gerais by using RAPD markers. Genetic variability was assessed by the analysis of variance on molecular data. A significant proportion of genetic variance was found among populations, corresponding to 19.6 % of the total variation. The correlation between the matrices of geographic and genetic distances was not significant, indicating that there is no spatial structure of the genetic variability among the populations, for the RAPD marker loci used here. The high variability suggests that a strategy for *in situ*, as well as *ex situ* conservation, must be based on sampling a large number of local populations.

KEYWORDS: Mangaba. Molecular marker. Population genetics. Native species.

INTRODUCTION

Mangaba (*Hancornia speciosa* Gomes) is a tropical tree of promising prospect for commercial exploitation. The species, found in most regions of Brazil, is frequent in the Cerrado biome. Mangaba fruits are consumed *in natura*, dried and used in preparations such as jelly or ice cream; the exquisite taste, makes mangaba highly appreciated by the population of the Center-West and Northeast regions.

Due to significant reduction of the original area of ecosystems where it occurs, mangaba is one of the fruit species most threatened by genetic erosion in Brazil. Knowledge on the genetic variability of the remaining populations in the Cerrado biome is scarce. The characterization of genetic variability is essential in support for domestication and breeding. In germoplasm sampling, it is important to take into account the population characteristics as well as the knowledge on the reproductive biology, the genetic structure and the effective size, since these can directly affect the viability of the populations in the long term (MOURA, 2003). Study on the reproductive behavior of *H. speciosa* var *speciosa* characterized this botanical variety as cross pollinating and self incompatible (DARRAULT; SCHLINDWEIN, 2006; PINTO et al., 2008). To our knowledge, there is no study on the reproductive biology of the botanical varieties occurring in the *core* area of Brazilian Cerrado, but the specialized floral morphology suggests that cross pollination can be assumed as the rule for the whole species.

Some studies have been carried out with native Cerrado fruit species, focusing on cultivation techniques (SOUZA et al., 2007; BERNARDES, et al. 2008; CAVALCANTE et al., 2008), nutritional aspects (VERA et al., 2007), and the biology and ecology (VERA et al., 2005). Little is known, however, about the genetic structure of natural populations. Some studies have shown the existence of high genetic variability, indicating the perspective for selection and breeding of these species (COLLEVATTI et al., 2001; COLLEVATTI, 2003; ZUCCHI et al., 2005; TRINDADE; CHAVES, 2005; MARTINS et al., 2006; SOARES et al., 2008a; SOARES et al. 2008b).

Study with morphological traits of fruits and trees from natural populations of *H. speciosa* from Cerrado region showed the existence of high variation among botanical varieties, among populations within botanical varieties and within populations (GANGA et al., 2010). High levels of genetic variation were also observed in a progeny study focusing on initial development traits both in nursery and field environment (GANGA et al., 2009).

A few studies were performed with *H. speciosa* populations from the Brazilian Cerrado based on molecular markers (SILVA ,2006;

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RODRIGUES, 2009). The results from these studies have shown high levels of genetic diversity among populations, suggesting that a great number of populations must be taken for genetic conservation of the species.

The purpose of this study was to characterize the genetic structure of eight mangaba populations from four regions of Brazilian Cerrado based on RAPD markers, in search of an applicable contribution for *in situ* and *ex situ* conservation of the genetic variability of the species.

MATERIAL AND METHODS

Eight local populations (or subpopulations) were sampled from August to December 2001 in four regions of the Brazilian Cerrado (Figure 1). Areas with little human influence and with 30 or more adult plants within a radius of 5 km were chosen (minimum distance of 20 km between populations). The altitude of each area was determined by an altimeter and the geographic coordinates determined using a GPS receptor (Global Positioning System, model Geo Explorer). A matrix of geographic distances among subpopulations was established based on the geographic coordinates. Each population was represented by 30 randomly sampled plants. Young leaves were collected from adult plants, placed in paper bags and stored on ice, until arrival at the laboratory, where they were deep-frozen, and prior to extraction.

The total genomic DNA was extracted by the CTAB method, described by Murray and Thompsom (1980) and Rogers and Bendich (1985), with modifications. DNA was extracted from a total of 217 plants, varying from 25 to 30 plants per subpopulation. The DNA concentration in the samples was estimated by comparison with a sample of known DNA concentration of the whole phage λ, on 0.8 % agarose gel. The DNA extracted from each plant was diluted at a concentration of 1 ng/μL and the stock solution maintained in the refrigerator until use.

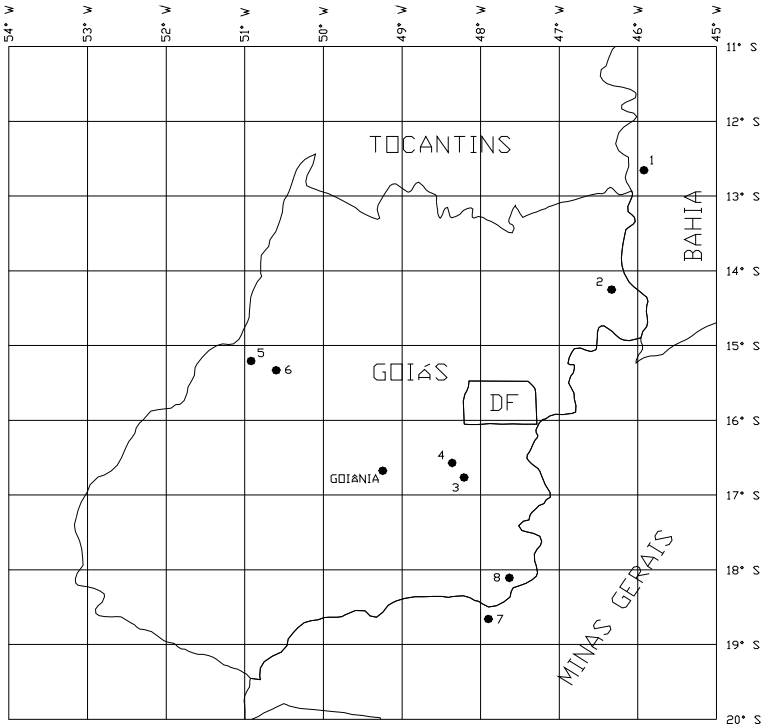

Figure 1. Spatial distribution of 8 populations of *Hancornia speciosa* Gomes from Brazilian Cerrado.

Each amplification reaction consisted of a volume of 25 μL containing: 2 ng DNA template; 50 mM KCl; 10 mM Tris-HCl pH 8.3; 2.0 mM MgCl; 250 μM dNTP; 250 nM primer and 2 units of Taq DNA polymerase. The reactions were subjected to 48 amplification cycles after initial denaturation at 94° C for five min. Each cycle consisted of 30 s at 92° C, 1 min and 30 s at 37° C and 1 min and 30 s at 72° C. After 48 cycles, a final extension step of 6 min at 72° C was performed. The amplified fragments were electrophoresed in horizontal 1.4 % agarose gels at 1 % TBE (Tris-borate 90 mM and EDTA 2 mM) buffer to detect polymorphisms. The gels were stained with

ethidium bromide and pictures taken under UV light (Kodak EDA camera). The gel images were interpreted and each plant was genotyped according to the presence (1) or absence (0) of each band for all primers analyzed; only the clearest and well-separated bands were selected. A binary data matrix was established based on this genotyping and the polymorphism level calculated for each locus.

The total variation was partitioned into components using the analysis of variance of molecular data (AMOVA) (EXCOFFIER et al., 1992) based on the binary data matrix, using software Arlequim (SCHNEIDER et al., 2000). AMOVA aims mainly at the estimation of parameter ϕ_{ST}, which measures the fraction of molecular genetic diversity (of RAPD) among subpopulations. This parameter was partitioned according to the structure of the subpopulations, into the following components of variability: ϕ_{ST} - fraction of the total variation that is due to the subpopulations altogether, ϕ_{RT} - fraction of the total variation that is due to the differences between regions and ϕ_{SR} - variation due to the subpopulations within regions. The components of variance of each hierarchical level were calculated based on the mean square expectations, using the method of moments.

Using the binary data, pair wise similarity measures (S_{ij}) were obtained from all plants using the Jaccard method. The respective dissimilarities or genetic distances were computed by the complement of each similarity measure relative to 1.0 ($D_{ij} = 1 - S_{ij}$). Based on the D_{ij} values the mean dissimilarity values within populations and among populations were calculated pairwise, resulting in a matrix of mean dissimilarity among subpopulations.

To investigate the spatial variation patterns the Pearson correlation coefficient (r) was estimated between the matrices of genetic dissimilarities and geographic distances, using software NTSYS 1.7 (*Numerical Taxonomy and Multivariate Analysis System*) (Rohlf, 1989). The significance of this correlation was verified by Mantel's Z statistic (1967), by using 9999 random permutations.

To evaluate the variation pattern among populations, a dendrogram was constructed based on the mean dissimilarities among populations, using the UPGMA grouping criterion (*Unweighted Pair-Group Method Using Arithmetic Averages*) (Sneath and Sokal, 1973). The cluster stability was also tested by the bootstrap resampling procedure, using 10,000 bootstraps.

RESULTS AND DISCUSSION

Of the 46 primers analyzed by Moura et al. (2005), 11 primers were pre-selected, totalizing 60 polymorphic loci. Subsequently the primers with a homogeneous profile and constant genomic DNA amplification for the populations were selected: OPB-12, OPC-04, OPH-18 and OPH-19.

The RAPD profiles detected in the primer selection trial (Figure 1) determined the requirements for the maintenance of the PCR conditions invariably. This procedure made possible the control of profile reproducibility in the amplification reactions.

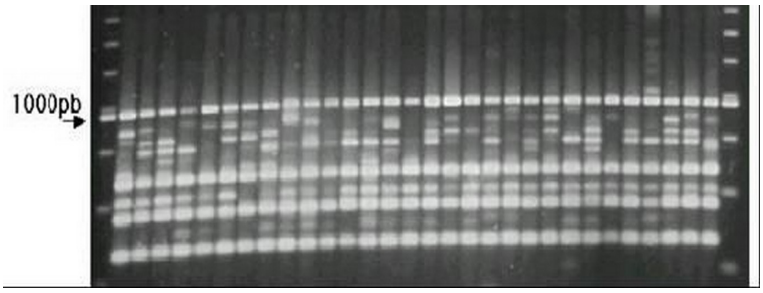

Figure 1. Profile of a RAPD gel using primer OPC04 of 30 mangaba trees, on the sides the DNA ladder (250 pb).

The results of AMOVA indicated a total variance of 4.45 (Table 1). The genetic variance among populations was significant (P<0,01) and corresponded to 19.67 % of the total variance. Of this, 18.73 % corresponded to the variation among populations within regions, a significant value at 1 % probability. The variation among regions represented only 0.94 %, which resulted non-significant. As expected, the major part of the variation (80.33 %) was concentrated within populations, a significant variation at 1 % probability.

Table 1. Analysis of variance of molecular data (AMOVA) for eight subpopulations of *H. speciosa* Gomes in the Cerrado of Central Brazil, based on thirty one RAPD bands.

Source of variation	D. F	S. S.	Variance components	Variation (%)	p-value
Regions	3	85,360	0,0419	0,94	0.400
Population/Regions	4	104,540	0,8337	18,73	0,001
Within populations	209	747,428	3,5762	80,33	0,001
Population 1	26	100,296	3,9761	13,81	
Population 2	29	100,833	3,5400	12,30	
Population 3	27	95,964	3,6199	12,58	
Population 4	26	101,370	3,8858	13,50	
Population 5	28	105,793	3,8781	13,47	
Population 6	29	112,033	3,8752	13,46	
Population 7	20	57,857	2,9220	10,15	
Population 8	24	73,280	3,0876	10,73	
Total	216	937,327	4,4518		

If the over all population is panmitic, supposing any restriction to gene flow, it is expected that the variation among randomly sampled populations would be zero and, therefore, all genetic variation would be found within them. Actual significant difference among populations however, demonstrates that these have been influenced by microevolution factors, differentiating them.

The estimated value of the ϕ_{ST} statistic was 0.197, significant at 1% probability. This parameter derived from AMOVA is analogous to Wright's F_{ST} statistic and is a measure of the differentiation among populations caused by genetic drift. In the case of gene flow among geographically close populations, these will tend to become similar than distant ones, according to a microevolution isolation-by-distance model or stepping-stones model (FUTUYMA, 1992). In this case the genetic variability found among regions was not significant, indicating that the dissimilarities among populations within regions tend to be similar to the ones among different regions. This can be explained by a possible restriction of gene flow even at smaller distances, among populations, by possible genetic drift, generating random variation pattern, which would correspond to the Wright's island model (FUTUYMA, 1992).

The genetic variability in a population is the result of the balanced joint action in microevolution processes (selection, mutation, migration and genetic drift) responsible for the genetic and genotypic changes. Since the RAPD markers are considered selectively neutral, the differentiation among populations found here must be result of genetic drift, not counterbalanced by gene flow. Although being a cross pollinated species, *H. speciosa* presents mass-flowering and pollination is mainly by geitonogamy (PINTO et al., 2008). So the fertilization can occurs mainly between neighbor plants. Seed dispersion is another factor responsible for gene flow in plant populations. The strong structure found in the mangaba populations suggests that seed dispersion is insufficient to maintain the genetic connection among the sampled populations. The fragmentation of the Cerrado vegetation can be contributing to this, restricting the movement of pollen and seed dispersants among remaining populations.

The apparent gene flow (Nm) for a set of subpopulations can be estimated indirectly via the F_{ST} statistic. In this method Wright's island model (1951) is admitted and the equilibrium of migration and genetic drift is assumed. Thus, when the Nm values are higher or equal to 1, i.e., when the apparent gene flow is equivalent to one or more individuals migrated per generation, the gene flow effects are sufficient to counterbalance genetic drift effects and impairs the divergence of populations. For $Nm \geq 1$, F_{ST} must therefore be ≤ 0.20 (WRIGHT, 1951).

The ϕ_{ST} parameter, based on dominant markers, is analog to F_{ST}, since it measures the differentiation among subpopulations caused by genetic drift. However, it may not be used indiscriminately to determine the number of migrants per generation, since ϕ_{ST} and F_{ST} estimates are frequently discrepant for the same set of subpopulations. With these restrictions, a ϕ_{ST} value of 0.1956, as found in the present case, could suggest that the subpopulations under study are in a condition close to equilibrium between gene drift and gene flow.

Some studies with fruit species of the Brazilian Cerrado have shown considerable genetic variability among populations which is, in general, spatially structured (TELLES et al., 2003; ZUCCHI

et al., 2003; TRINDADE; CHAVES, 2005; SOARES et al., 2008a; SOARES et al., 2008b), this fact requires broad sampling for conservation purposes.

Studies with *H. speciosa* based on molecular markers are scarce. In a study based on polymorphism of two non-coding regions of chloroplast DNA, significant variation was found among populations, corresponding to 7 to 9% of the total genetic variation (SILVA, 2006). Using SSR markers to analyze populations of four botanical varieties of the Cerrado region, Rodrigues (2009) found a F_{ST} estimate of 0.19, indicating high level of genetic differentiation among populations. This result is similar to the Φ_{ST} estimates found here.

Variation among subpopulations is also possible at a larger geographic scale, in other words, the more distant they are from each other, the more they differ in allele frequencies and in the value of phenotypic traits, although the correlation is often not high. The study of spatial distribution of genetic variability is a strong support for evolution studies of the species.

The results of AMOVA did not detect significant variation among regions, indicating that the spatial structure could not be detected with the molecular marker used here. This indicates that the effect of genetic drift superposes that of gene flow, since genetic drift causes a random structure of variability among populations. Results suggest that the gene flow did not counterbalance this differentiation, even at relatively small distances. A possible explanation for this variation pattern could be a restricted seed and pollen dispersion. In fact, mangaba subpopulations tend to form plant aggregates in isolated demes. Mangaba is predominantly pollinated by night-active Lepidoptera (DARRAULT; SCHLINDWEIN, 2006). The spatial pattern of plant distribution, together with the short reach of the pollinating insects favors preferential pollination of nearby plants, which in turn favors the differentiation of populations. The actual fragmentation of the Cerrado areas may lead to a greater isolation among subpopulations which could affect gene flow even more between them.

The variance found within subpopulations corresponded to 80.33% of the total variance and, when this source of variation was partitioned, the variability within each subpopulation varied only slightly, in other words, the contribution of each population to the total variation was similar.

The distribution of intrapopulation genetic variation reflects, at least partly, the action of genetic processes from the population point of view, i.e., of selection and genetic drift (LOVELESS; HAMRICK, 1984). When a large discrepancy between genetic variability within populations is observed, the action of these genetic processes differs for the subpopulations. Our results therefore suggest that the genetic erosion is not threatening the variability among plants within populations. In the sampling process the variability in any one of the subpopulations studied is practically equal to the others. This is important for the maintenance of the genetic variability, since the conservation strategy is facilitated.

The mean dissimilarity among plants within populations varied from 0.26 to 0.39 (Table 2). The AMOVA-based variances and the dissimilarities therefore indicate that the maintenance of internal variability in the populations is relatively similar.

Table 2. Mean similarity (Jaccard) and the respective mean dissimilarity of plants within subpopulations of *H. speciosa*, based on thirty one RAPD bands.

Populations	Similarities	Dissimilarities
01	0,6749	0,3251
02	0,7207	0,2793
03	0,6525	0,3475
04	0,6066	0,3934
05	0,6880	0,3120
06	0,6239	0,3761
07	0,7412	0,2588
08	0,6631	0,3369

The mean genetic similarity among subpopulations varied from 0.54 to 0.68. No grouping according to regions was observed in the dendrogram (Figure 2). The association of dendrogram nodes was generally weak (highest association 53.56 %) among populations and their grouping pattern was not clear for the analyzed amplified fragments. This result had been expected,

since the non-significance of the variability found among regions indicates random distribution for the amplified fragments analyzed.

The matrix correlation between the genetic dissimilarities and geographic distance matrices was not significant. The Pearson correlation coefficient (r) was 0.005, non-significant by the Mantel test. This result reinforces the assumption that the genetic variability among subpopulations, detected with the molecular markers used, is randomly distributed in space.

This situation of random genetic spatial distribution, though atypical, was also found in same instances (e.g. SAENZ-ROMERO et al. 2001). Among Cerrado species, a similar situation was verified in 11 natural populations of *Annona crassiflora* in the state of Goiás (BLANCO et al., 2007). Study with 32 *H. speciosa* populations based on SSR markers, also showed no significant correlation between genetic and geographical distances (Rodrigues, 2009), corroborating the random pattern of the genetic variation among populations and the occurrence of restricted gene flow just between neighbor populations.

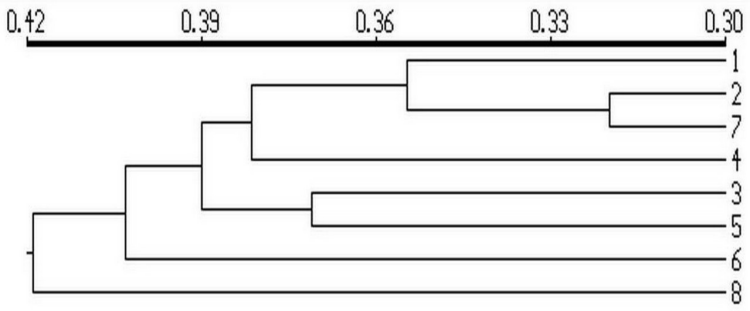

Figure 2. Grouping pattern of eight mangaba (*H. speciosa*) subpopulations defined by the UPGMA method on the mean similarity index of Jaccard (cophenetic correlation = 0.771**) and thirty one RAPS bands.

Vencovsky and Crossa (2003) showed that the higher the value of F_{ST}, the larger must be the number of subpopulations sampled to ensure an adequate effective size. Although some studies show that ϕ_{ST} estimates tend to be higher than those of F_{ST} (ZUCCHI et al., 2005), it is admitted that a high ϕ_{ST} value corresponds also to significant, F_{ST} values. The high variability found in the subpopulations therefore suggests that a strategy for *in situ*, as well as *ex situ* conservation for the species must be based on sampling a large number of subpopulations.

The fact that no spatial structure of genetic variability was detected indicates that subpopulations may be sampled randomly. It must however be emphasized that aside from the quantification and distribution of genetic variability detected by molecular markers, other variability descriptors, such as phenotypic variation of morphological traits, must also be considered; this includes variation in leaf pilosity and color, color pattern, fruit size, flower morphology and others. Pronounced differences between the subpopulations studied were observed but not quantified, with regard to some of these traits. This indicates that the sampling of subpopulations should cover different regions of occurrence of the species.

CONCLUSIONS

The natural populations of *H. speciosa* present highly significant interpopulation genetic variability, suggesting the need of sampling a large number of areas for collecting germplasm or *in situ* conservation.

The natural populations of *H. speciosa* have similar within population genetic variability, showing no spatial genetic structure among populations, for the RAPD loci used here.

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RESUMO: A mangabeira (*Hancornia speciosa* Gomes) é uma espécie frutífera com grande potencial para exploração econômica, encontrada em várias regiões do Brasil, sendo freqüente no bioma Cerrado. Informações sobre a variabilidade genética das populações existentes neste bioma são escassas. Este estudo teve como objetivo caracterizar a estrutura genética de oito populações situadas nos Estados de Goiás, Bahia e Minas Gerais por meio de marcadores RAPD. A estrutura da variabilidade genética foi avaliada utilizando-se a análise de variância de dados moleculares. Uma proporção significativa da variância genética foi encontrada entre populações, correspondendo a 19,6 % da variação total. A correlação entre as matrizes de distâncias geográficas e genéticas não foi significativa, indicando que não existe estruturação espacial da variabilidade genética entre populações, para os locos marcadores RAPD utilizados. A alta variabilidade sugere que estratégias para conservação *in situ*, bem como *ex situ*, devem ser baseadas em amostragem de um grande número de populações locais.

PALAVRAS-CHAVE: Mangaba. Marcador molecular. Genética de população. Espécie nativa.

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