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Genetic diversity in populations of *Acrocomia aculeata* (Arecaceae) in the northern region of Minas Gerais, Brazil

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ABSTRACT. Macaúba (*Acrocomia aculeata*) is a palm of economic importance, widely distributed in natural forests from Mexico to Uruguay. We analyzed the genetic diversity of populations of macaúba (*A. aculeata)* in the northern region of the State of Minas Gerais, Brazil. Young leaves from 10 macaúba individuals encompassing 49 genotypes of macaúba were collected from Montes Claros, Itacambira, Brasília de Minas, Mirabela, and Grão Mogol. After extraction and amplification of samples, the amplified fragments were separated by electrophoresis. We found high levels of genetic diversity within the populations. Genetic diversity indices were high, except in the Itacambira and Mirabela

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populations. Results show that Mirabela and Itacambira populations can require conservation strategies because they present lower values of genetic diversity.

Key words: *Acrocomia aculeata*; North of Minas Gerais; Genetic diversity

INTRODUCTION

Acrocomia aculeata (Jacq.) Lodd., a member of the Arecaceae family popularly known as macaúba, macaibeira, and bocaiuva, is a palm tree that reaches more than 15 m in height and has wide geographic distribution (Scariot et al., 1995). Minas Gerais has an abundant macaúba population and is considered to be economically promising for pasture area. According to Lorenzi (1998), *A. aculeata* has pioneering characteristics and exhibits higher dispersion in secondary formations.

Macaúba dispersal is facilitated by a large production of fruits that are consumed by animals. The fruits of the species have great potential for oil production, with applications in industrial and energy sectors (Rolim, 1981). Vegetable oil production may reach 4000 $L \cdot ha^{-1} \cdot year^{-1}$. The use of macaúba oil for biodiesel production can help improve socioeconomic conditions in critical regions of the country. In cerrado areas, the macaúba is economically important and is used for ornamental, dietary, medicinal, and industrial purposes (Almeida et al., 1998).

Knowing the patterns of genetic variability among and within populations is a valuable tool that may help develop more efficient practices related to conservation, serve as a basis for suitable management techniques of fragments, and support *in situ* conservation measures (Frankel et al., 1995; Peakall et al., 2003; Renau-Morata et al., 2005). The basis of species conservation is the maintenance of genetic variability in populations (Yeh et al., 1996).

Characterization of genetic variability can be carried out from intra and interpopulation genetic diversity measures, which may be estimated from molecular marker data as the percentage of polymorphic loci, fixation index, expected heterozygosity in Hardy-Weinberg equilibrium, the observed heterozygosity (Berg and Hamrick, 1997; Cavallari-Neto, 2004).

Studies on the genetic variability of *A. aculeata* are scarce. Thus, knowing the natural genetic diversity of the species would be a crucial step toward the development of conservation strategies and further breeding programs. The aim of this paper was to study the genetic variability of macaúba in northern Minas Gerais, Brazil, using molecular markers.

MATERIAL AND METHODS

Material for determining genetic variability of *A. aculeata* was obtained from 5 sites in northern Minas Gerais. Young leaves from 10 macaúba individuals encompassing 49 genotypes were collected in Montes Claros, Itacambira, Brasília de Minas, Mirabela, and Grão Mogol (Figure 1). Genomic DNA was obtained from the leaves, which were transported to the Bioprospecting and Genetic Resources Laboratory at Universidade Estadual de Montes Claros and stored at -80°C. DNA extraction was performed using a cetyltrimethylammonium bromide method with modifications (Faleiro et al., 2003). Next, the DNA samples representative of each population were submitted to the polymerase chain reaction (PCR)-RAPD technique.

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The amplification reactions were carried out in a final volume of 25 mL, with 11 primers selected. Each reaction consisted of 10 mM Tris-HCl, 50 mM KCl, 2 mM $MgCl_2$, 100 mM of each deoxinucleotide, 0.4 mM primer, 1 U Taq DNA polymerase, and 50 ng macaúba leaf DNA. The reactions were amplified in an Applied Biosystems thermal cycler model Veriti 96 Well. Amplifications were performed on a thermal cycler with 40-cycle programming (15 s at 94°C, 30 s at 35°C, and 60 s at 72°C) for the complete extension of amplified products. The temperature was then lowered to 4°C. Four microliters of bromophenol blue solution (0.25%) and sucrose (40%) was added at the end of the amplification reaction. Amplified products were separated via horizontal electrophoresis on agarose gels (1.2%), stained with ethidium bromide (1 mg/mL), and immersed in Tris-borate-ethylenediaminetetraacetic acid buffer (Tris-base, 0.1 M; boric acid, 1 M; ethylenediaminetetraacetic acid, 0.5 M). Electrophoretic separation was performed for approximately 2.5 h at 110 V. At the end of the run, the gels were photographed under ultraviolet light. RAPD markers were genotyped from the photographs as present (1) or absent (0) of fragments, and a binary matrix was constructed afterward. PopGene 1.32 was used to estimate genetic similarities among the studied populations (Yeh et al., 1997); the estimates obtained were used for dendrogram construction using the unweighted pair group method with arithmetic mean (UPGMA) method with the aid of NTSYS 2.11 (Rohlf, 2000).

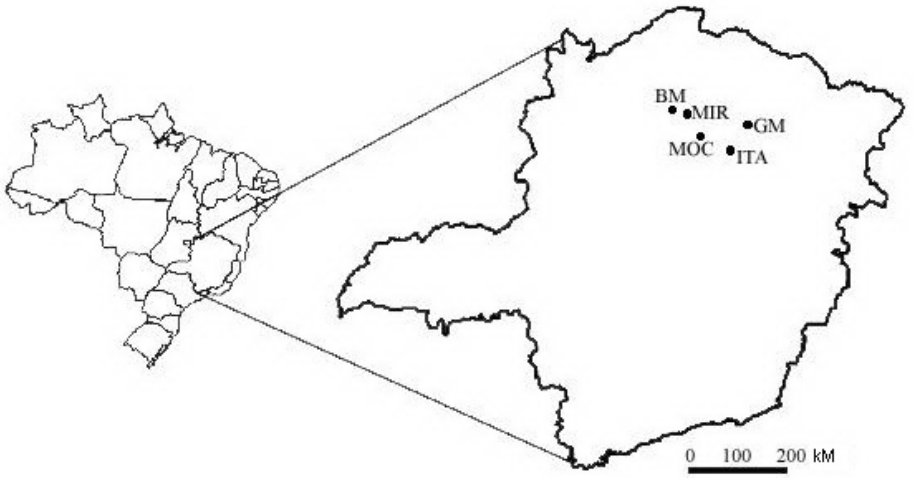

Figure 1. Five populations of *Acrocomia aculeata* (location) used in this study. BM = Brasília de Minas; MIR = Mirabela; MOC = Montes Claros; ITA = Itacambira; GM = Grão Mogol.

The analysis was complemented using Tools For Population Genetic Analyses version 1.3 (Miller, 1997) to verify the consistency of groupings from 1000 permutations. PopGene was used to analyze the intrapopulation genetic diversity, in which the number of observed alleles, number of effective alleles, Nei (1978) gene diversity ($\hat{H}_E$), and percentage of polymorphic loci were estimated. Total heterozygosity (H_T), mean heterozygosity within, coefficient of population differentiation (G_{ST}), and allelic flow (N_m) were also estimated. The analysis of variance F-test was used at 95% probability to compare values obtained among populations.

RESULTS

The primers generated 47 polymorphic loci ranging from 2 to 7 loci per primer (Figure 2). Table 1 displays the selected primers, their sequences, and the number of produced fragments. The average number of fragments obtained per primer was 4.2. Oliveira et al. (2008) obtained an average of 4.8 fragments per primer in populations of *Dimorphandra mollis* Benth in northern Minas Gerais.

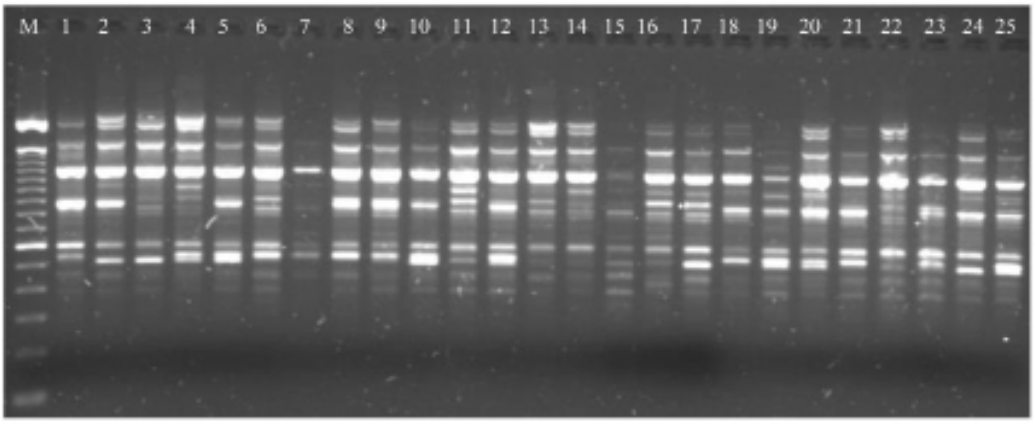

Figure 2. Pattern of amplified bands in macaúba individuals obtained in Montes Claros, using RAPD technique. *Lane M* = molecular marker. *Lanes 1-25* = macaúba individuals.

Table 1. List of primers, sequences and number of bands.

Primer	Sequence (5'-3')	Number of bands
P1	CCGCATCTAC	6
P2	TGTCATCCCC	3
P3	CCCGCCTTCC	7
P4	TCACACGTGC	4
P5	ACAGGGCTCA	4
P6	TTAACCGGGG	2
P9	TTCGGGCCGT	4
P10	GAAGCGCGAT	5
P11	GTGACATGCC	2
P20	GGCTCATGTG	4
P26	GGGACCGTGT	6
Total		47

$\hat{H}_E$ was relatively high (Table 2) in Grão Mogol (0.42), Montes Claros (0.34), and Brasília de Minas (0.34), demonstrating reasonable genetic variability in these populations. However, the population from Mirabela displayed lower genetic diversity (0.25). Our examination of the distribution of genetic variability among and within populations (Table 3) showed that 17.2% of genetic variability occurred among populations (G_{ST} = 0.172) and 82.8% within populations.

The UPGMA dendrogram (Figure 3) showed a similarity range from 87.5 to 95% with Nei's (1978) genetic identity matrix among sampled populations. Above 88% similarity, the

formation of 3 groups is observed: the first group comprises the Montes Claros and Brasília de Minas populations, the second group comprises Grão Mogol and Itacambira populations, and the third group comprises the Mirabela population.

Table 2. Diversity estimates from five populations of *Acrocomia aculeata*.

Populations	N_A	N_E	$\hat{H}_E$	P (%)
GM	1.93 (0.24)	1.80 (0.25)	0.42 (0.12)	93.62
ITA	1.72 (0.45)	1.50 (0.37)	0.28 (0.19)	72.34
MOC	1.85 (0.35)	1.61 (0.34)	0.34 (0.17)	85.11
BM	1.82 (0.37)	1.61 (0.37)	0.34 (0.18)	82.98
MIR	1.61 (0.49)	1.46 (0.43)	0.25 (0.22)	61.70
F_{ANOVA}	4.49*	6.13*	6.05*	-

Data are reported as means (standard deviation). N_A = number of observed alleles; N_E = effective number of alleles; $\hat{H}_E$ = Nei's genetic diversity; P = percent of polymorphic loci. *P < 0.05. For population abbreviations, see Figure 1.

Table 3. Genetic structure of *Acrocomia aculeata*.

	H_T	H_S	G_{ST}	N_m
Mean	0.399	0.330	0.17	2.4
Standard deviation	0.012	0.011	-	-

H_T = total genetic heterozygosity; H_S = mean heterozygosity within population; G_{ST} = coefficient of population differentiation; N_m = allelic flow.

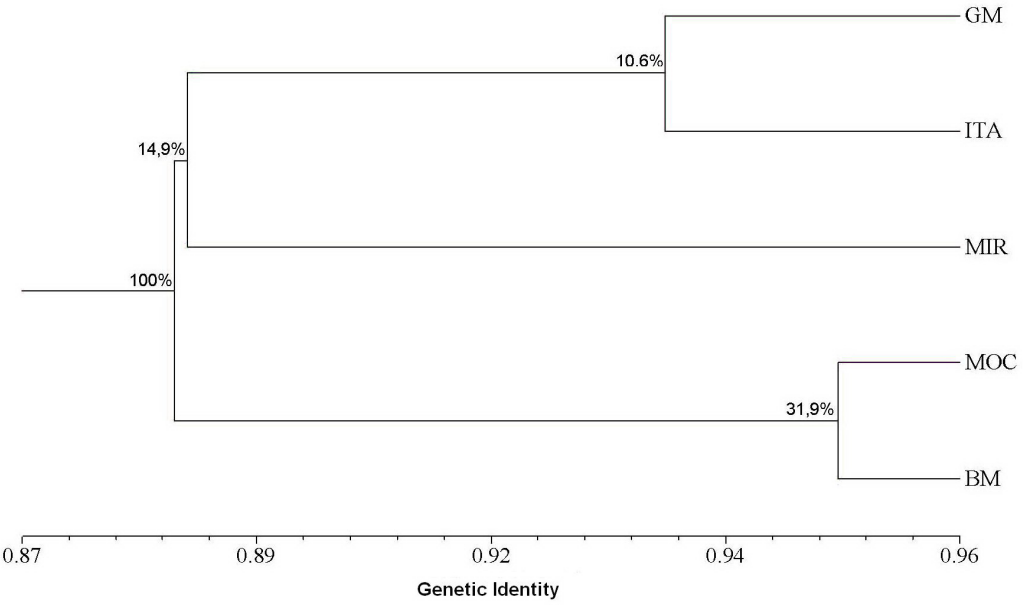

Figure 3. UPGMA dendrogram of *Acrocomia aculeata* sampled from five populations, estimated according to Nei's genetic identity (1978). For population abbreviations, see Figure 1.

DISCUSSION

The results obtained through variance analysis revealed significant differences in genetic diversity indices among populations (see Table 2). The percentage of polymorphic loci has been used as a genetic diversity measure in studies with natural populations through the use of dominant markers (Xia et al., 2007). The lowest values of polymorphic loci were observed in Mirabela (61.7%) and Itacambira (72.3%). The highest values were observed in Grão Mogol (93.6%) and Montes Claros (85.1%). The low values observed in Mirabela may be indicative of the greater isolation of local populations. Oliveira et al. (2008) researched 5 distinct populations of *D. mollis* Benth from northern Minas Gerais. The percentage of observed polymorphic loci varied from 44.29% in Jequitaí to 72.86% in Mirabela populations. The Patis population displayed a lower percentage (58.57%) of polymorphic loci. A higher percentage of polymorphic loci was obtained by Zimback et al. (2004), who reported estimates of polymorphism from 90.3 to 97.3% in populations of *Trichilia pallida* Swartz.

The $\hat{H}_E$ values in the present study were similar to those in other studies. Zimback et al. (2004), studying species of *T. pallida*, found $\hat{H}_E$ values varying from 0.27 to 0.33. Torezan et al. (2005) obtained a mean $\hat{H}_E$ of 0.287 in *Aspidosperma polyneuron* Muell. Arg. During a study of natural populations of *Eremanthus erythropappus*, Estopa et al. (2006) obtained an $\hat{H}_E$ with variation between 0.299 and 0.333. Oliveira et al. (2008) obtained values of $\hat{H}_E$ ranging from 0.17 to 0.29 in 5 distinct populations of *D. mollis* Benth in northern Minas Gerais.

According to Aagaard et al. (1998), tree species, in general, show higher genetic variation within populations. Reports of lower values of genetic variability among populations have also been reported in the literature; however, Sales et al. (2001) found genetic divergence (28.58%) among populations of *Digitalis minor*. Wadt (2001) observed genetic diversity of 28.1% among populations of *Piper hispidinervum.* Zimback et al. (2004) worked with populations of *T. pallida* and found a genetic divergence of 12.5% among populations. Oliveira et al. (2008) obtained a genetic variability of 28.83% among and 71.17% within populations of *D. mollis* Benth in northern Minas Gerais.

The G_{ST} values obtained were close to the expected average for species with mixed-mating systems (G_{ST} = 0.2; see Table 3; Nybom, 2004). N_m obtained among populations was low (2.4). According to Hartl and Clark (1997), when gene flow among populations exceeds four migrants per generation, homogenization of alleles occurs between them. Wright (1931) has stated that N_m values lower than 1 show genetic isolation. Wright (1949) has also stated that an Nm value higher than 1 is sufficient to prevent random loss of alleles within populations (drift effect). Zimback et al. (2004), studying populations of *T. pallida*, observed an N_m of 0.78. Oliveira et al. (2008) obtained an N_m of 1.23 in populations of *D. mollis* Benth in northern Minas Gerais. The low N_m value obtained in this study may indicate genetic isolation and be associated with the geographic distance among studied populations. The mean genetic distance among macaúba populations was 0.108 (Table 4). The smallest genetic distance (0.054) was among the Montes Claros and Brasília de Minas populations. The greatest distance (0.153) was among the Grão Mogol and Mirabela populations.

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Table 4. Nei's (1978) genetic identity (above diagonal) and genetic distance (below diagonal) among populations of *Acrocomia aculeata*.

Populations	GM	ITA	MOC	BM	MIR
GM	****	0.931	0.923	0.874	0.857
ITA	0.070	****	0.888	0.871	0.904
MOC	0.079	0.118	****	0.947	0.883
BM	0.134	0.137	0.054	****	0.898
MIR	0.153	0.100	0.123	0.107	****

For abbreviations, see Figure 1.

In general, geographically close populations were genetically similar. The Mirabela population, in addition to presenting low values of genetic identity with the other populations (see Table 4), displayed low indices of genetic diversity (see Table 2). The low indices observed in that population may indicate genetic isolation caused by the drift effect, which indicates the need for species conservation strategies in Mirabela. *Acrocomia aculeata* presents high levels of genetic diversity within populations analyzed in northern Minas Gerais. Genetic diversity indices were high, except in Itacambira and Mirabela populations. Results also show that Mirabela and Itacambira populations can require conservation strategies because they present lower values of genetic diversity.

Heterozygosity or genetic diversity is the most used measure for estimating genetic variability. The estimated H_T obtained was 0.399 (see Table 3). Lower values of H_T (0.308) were found by Oliveira et al. (2008) in studies with *D. mollis* Benth in northern Minas Gerais. Estopa et al. (2006) observed higher H_T values (0.404) in studies with *E. erythropappus*.

In cerrado areas, the macaúba is economically important and exhibits great ornamental, dietary, medicinal, and industrial purposes (Almeida et al., 1998). Thus, it must be protected. Data obtained in this paper are important for monitoring the current status of the species in northern Minas Gerais, Brazil. More studies must be carried out, including those using microsatellite markers, to obtain information that can be used to establish conservation strategies and further breeding programs for the species.

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