



ORIGINAL ARTICLE

Genetic diversity within and among populations of Cajazeira

Diversidade genética entre e dentro de populações de Cajazeira

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ABSTRACT: *Spondias mombin* is a fruit species belonging in the Anacardiaceae family with a large geographical distribution. It is of economical and social importance due to its fruit being intensively used for the industrial purposes of making juice, popsicle, jelly, and liquors. The objective of this work was the characterization of the genetic diversity both intra and inter populations of this species. This analysis was made by means of molecular markers of the type AFLP (amplified fragment length polymorphism) in three populations in a region close to Manaus city, state of Amazonas: Amazonas National University (UFAM), Buriti village (VLB) and the Executive Commission for the Cultivation of Cacao (Ceplac). Ten oligonucleotide combinations were tested and four of them were picked for this analysis: E + AAC/M + CAT, E + AGT/M + CTC, E + AAC/M + CTC, E + AGT/M + CAT. The oligonucleotides revealed 283 polymorphic loci with a variation of 63 to 97.2% inter populations. The calculated *F*_{st} (genetic differentiation between populations) was of 0.52, this meaning a high level of genetic differentiation inter populations. According to the molecular analysis of variance, the variations inter and intra populations were of 52.8 and 47.1%, respectively. The dendrogram based on the AFLP markers showed the existence of two groups, one being that of the natural populations (UFAM and VLB) and another group formed by a population developed under cropping practices (Ceplac). The AFLP markers are efficient to detect genetic diversity in *S. mombin* and thus differentiate between populations of distinct constitutions. Most of the genetic variability is found to occur inter populations.

RESUMO: *Cajazeira* (*Spondias mombin* L.) é uma fruteira pertencente à família Anacardiaceae e tem uma distribuição geográfica ampla. Apresenta importância econômica e social, e tem seus frutos bastante utilizados pela indústria para confecção de sucos, picolés, sorvetes, geleias e licores. Objetivou-se caracterizar a diversidade genética entre e dentro de populações de *S. mombin* L. A análise foi realizada por meio de marcadores moleculares AFLP (Amplified Fragment Length Polymorphism) em três populações localizadas na cidade de Manaus, Estado do Amazonas: Universidade Federal do Amazonas (UFAM), Vila Buriti (VLB) e Comissão Executiva do Plano da Lavoura Cacaueira (Ceplac). Foram testadas dez combinações de oligonucleotídeos e selecionadas quatro para esta análise: E+AAC/M+CAT; E+AGT/M+CTC; E+AAC/M+CTC; E+AGT/M+CAT. Os oligonucleotídeos revelaram 283 locos polimórficos, variando de 63% a 97,2% entre as populações. O *F*_{st} (diferenciação genética entre as populações) calculado foi de 0,52, revelando um alto nível de diferenciação genética entre as populações. De acordo com a Análise Molecular de Variância, a variação entre e dentro das populações foi de 52,8 e 47,1%, respectivamente. O dendrograma construído com base nos marcadores AFLP revelou a formação de dois grupos: o grupo das populações naturais (UFAM e VLB) e o grupo da população enriquecida com plantio (Ceplac). Os marcadores moleculares AFLP são eficientes para detectar diversidade genética em *S. mombin* e diferenciar populações de constituições distintas. A maior parte da variabilidade genética ocorre entre as populações.

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1 Introduction

Spondias mombin L., a member of the Anacardiaceae family, is a fruit tree with origin in Tropical America and an ample geographical distribution. It may be found everywhere in Latin America, in most of the Caribbean islands, in some Asian and African countries, in islands of the Pacific and in India (JUSTINIANO et al., 2001; DUVAL, 2006; WISHNIE et al., 2007). In Brazil, it is found in the Amazon region, in the Atlantic Forest, and in the more humid areas of the northeastern states, mainly in the littoral zone and in the hilly areas (PINTO et al., 2003). In those regions the species has received several names, such as ‘taperebá’, ‘caja’, ‘caja-mirim’, ‘caja-pequeno’, ‘yellow mombin’, ‘jobo’ (SACRAMENTO; SOUZA, 2000; PINTO et al., 2003). It is also found in house backyards, domestic orchards, and alongside city streets.

Although the species is not mentioned in official Brazilian statistics, it is of considerable social and economical importance specially in the North and Northeast regions of the country due to the increasing “in natura” use of its fruits as well as in industrialized forms. In both forms, the fruits are amply commercialized in markets, super markets, and restaurants. The fruit pulp has a very high nutritional value – it is rich in vitamins A, B1, B2, and C, proteins, lipids, calcium, phosphorus, and iron (SACRAMENTO; SOUZA, 2000). Although displaying a rapidly growing market, the species is not until today cultivated as a cash crop in Brazil; all the commercialized *S. mombin* fruits are originated from extractive exploitation.

Spondias mombin is classified as an allogamous species and certain facts which favor cross fecundation such as protandric dichogamy and self incompatibility have been reported (SACRAMENTO; SOUZA, 2000). Studies concerned with genetic variability in natural plant populations in tropical regions show that these populations preserve large amounts of genetic variability mainly in allogamous species. The distribution of the natural genetic variability is influenced by factors such as the reproduction mode, crossing system, the population effective size, the geographical distribution of the plants, and gene flux (PAIVA, 1998).

Fruit species from the Amazon region, such as *S. mombin*, which are extractively exploited, may undergo significant modifications due to the domestication process implemented by man (RAMOS et al., 2011) such as loss of variability. In the Amazon state, naturally occurring populations and populations enriched by planting are found. No information about the genetic pattern of those enriched populations is known in comparison with naturally occurring populations and how much these populations were modified by the action of man.

The knowledge of the genetic diversity of a species is important for the management or to start new plantings so as to adequately proceed extractivism as well as for conservation and genetic improvement (ESFAHANI; SHIRAN; BALALI, 2009; SETOTAW; DIAS; MISSIO, 2010). Information on genetic diversity inter and intra natural populations allow to understand how a selection is acting as a function of adaptability (ESTOPA, 2006).

Molecular markers reveal DNA polymorphic sequences and have been largely used to access genetic diversity of plant

species. Among the markers, the AFLP (Amplified Fragment Length Polymorphism) technique is highlighted as a technique which can be applied to any plant species, detecting a large number of polymorphic loci, making possible an ample view of the genome with a low cost per locus (FERREIRA; GRATTAPAGLIA, 1998; LOPES et al., 2003). The AFLP marker has been used in studies of several fruit tree species, such as *Mauritia flexuosa* (‘Buriti’) in a study by Gomes et al. (2011), *Eugenia uniflora* L. (‘Pitangueira’), by Franzon, Castro and Raseira (2010), *Mangifera indica* L. (Mango), by Santos, Rodrigues and Zucchi (2008), *Spondias tuberosa* Arruda (‘Umbuzeiro’), by Santos and Oliveira (2008).

Studies of the genetic diversity of *Spondias mombin* are seldomly found in the literature. The knowledge and organization of the genetic variability of any plant species is an important tool for the extractive exploitation of that species, its genetic conservation and for its genetic improvement.

The objective of this work was to characterize the inter and intra genetic diversity of *S. mombin* natural populations as well as of a population enriched by planting by means of AFLP molecular markers.

2 Materials and Methods

Three *Spondias mombin* populations were studied, all of them localized at Manaus, state of Amazonas, Brazil. One was a population localized at the National University of Amazon (UFAM) whose geographic coordinates are of 59° 58’ 33” of West longitude and 3° 5’ 50.9” of South latitude. The second population was that of the Vila Buriti (VLB), at 59° 56’ 46.4” W and 3° 08’ 16” S. The third population was that being grown by the Executive Commission for the Cultivation of Cacao (Ceplac) at 60° 02’ 16.3” W and 02° 33’ 08.1” S. The UFAM and the VLB are naturally occurring populations whereas that of the Ceplac was an enriched population due to the planting of plants which were selected for fruit quality. Thirty individuals were taken from each population for the genetic diversity study. Each individual was identified with a metal tag and reference framed. Leaf samples were taken, placed inside plastic bags containing silica gel and transported to the Genetic Improvement Laboratory, a unit of UFAM. In the laboratory, the samples were stored in a freezer. The leaves, after being dried in silica gel, were submitted to DNA extraction and analyzed with the help of AFLP markers. Dry leaves were used for the extraction of genomic DNA. The CTAB 2% (DOYLE; DOYLE, 1987) method with modifications was used. A quantity of 0.6 g of the plant tissue of each individual was used. Maceration was accomplished in a tissue homogenizer. The quantification of genomic DNA was carried out in agarose gel 0.8% ($p v^{-1}$) by means of visual comparisons of its fluorescence with those of patterns of DNA of known molecular mass of the lambda phage of 50 to 100 ng. The gels were colored with ethyl bromide (10 mg mL^{-1}) and photographed under UV light. After the quantification, all the DNA samples were diluted in ultrapure water to a concentration of $20 \text{ ng } \mu\text{L}^{-1}$. DNA was extracted from young leaves, in a state of development between intermediate and advanced. The purest and of higher integrity DNA was extracted from leaves of intermediate degree of development. The very young leaves, when dried, became very

brittle, forming a dust during the maceration process and thus did not result in a quantity of DNA enough to be seen in the quantification gel. The older leaves showed a higher resistance to maceration so that it was not possible to extract DNA of good quality in the needed quantity.

The analysis with AFLP markers was carried out in accordance with procedures initially proposed by Vos et al. (1995) and with modifications proposed by Lopes et al. (2003). The digestion reactions were made using 300 ng of genomic DNA, 5.0 μL of the buffer "One for All" 10X (OPA, Amersham), 0.5 μL of bovine serum albumin (10 $\mu\text{g } \mu\text{L}^{-1}$), 0.5 μL of the MseI enzyme (10 U μL^{-1}), and 0.5 μL of the enzyme EcoRJ (10 U μL^{-1}), both provided by the New England Biolabs, in a final volume of 50 μL . The reactions were carried out under a temperature of 37 °C for 3 h after what the enzymes were inactivated at a temperature of 70 °C for 15 min)

The resulting fragments of the digestion process were linked to specific adaptors for the restriction loci Eco RJ and MseI. At this phase, 10 μL of the digested DNA and 9.6 μL of the adaptors linking solution and 0.4 μL of the enzyme T4 DNA ligase (Invitrogen) were added. The incubation period of the material was of two hours at 20 °C.

In the pre-amplifying reactions, initiators complementing the sequences of the restriction enzyme sites with one selective nucleotide and the combination of initiators EcoRJ – MseI + C were used. In the preparing of the reactions, 2.5 μL of the DNA sample (digested – linked), 0.5 μL of the initiator of the rare cut enzyme (EcoRI + A) (5' – GAC TGC GTA CCA ATT CA – 21) (25 ng μL^{-1}), 0.5 μL of the frequent cut enzyme initiator (MseI + C) (5' – GAT GAG TCC TGA GTA AC – 3' (25 ng μL^{-1}), 0.4 μL of dNTP 2.5 mM (Promega), 2.0 μL of the buffer 10X Buffer B (Fermentas), 1.2 μL of MgCl₂ 25 mM (Fermentas), 0.3 μL of Taq DNA polymerase, and 3.6 μL of ultrapure water. The PCR program, during the pre-amplification procedure, was composed of 26 amplification cycles after the initial denaturation at 94 °C for 2 min. Each cycle was constituted of one minute at 94 °C (denaturation), one minute at 56 °C (curling), and one minute at 72 °C (extension). The final cycle was followed by an extension for the action of Taq polymerase for five minutes at 72 °C. The pre-amplification products were diluted by adding 40 μL of ultrapure water and stored at –20 °C.

For the selective amplified reactions, 2.5 μL of the diluted amplification product, 1.0 μL of the rare cut enzyme with three selective nucleotides (E + ANN), 1.2 μL of the frequent cut enzyme with three selective nucleotides (M + CNN), 0.4 μL of the mix between dNTP 2.5 mM (Promega), 2.0 μL of the Taq DNA polymerase buffer 10X (Fermentas). 1.2 μL of MgCl₂ 25 mM, 0.2 μL of the Taq DNA polymerase 5 U μL^{-1} (Fermentas), and 11.5 μL of ultrapure water. The PCR program for the selective amplification consisted in a initial denaturation at 94 °C, 12 cycles composed of 30 s at 94 °C, 30 s at 65 °C, and one minute at 72 °C, followed by 23 30 second cycles at 94 °C, 30 s at 56 °C, and one minute at 72 °C. The last cycle was of 2 min at 72 °C.

Ten AFLP oligonucleotide combinations were tested: E + AAC/M + CTC, E + AGC/M + CGC, E + AAC/M + CGC, E + ATC/M + CTC, E + ACA/M + CTC, E + ATC/M + CGC, E + ACA/M + CGC, E + AGT/M + CTC, E + AGT/M + CTC,

E + AGT/M + CGC in a random sample of ten individuals. Four combinations were selected due to their larger number of polymorphic loci and better amplification quality: E + AAC/M + CAT, E + AGT/M + CTC, E + AAC/M + CTC, and E + AGT/M + CAT.

The samples were submitted to polyacrylamide gel electrophoresis (acrylamide/bisacrylamide). The sequencing gel system ("Sequi-Gen GT") was used (Biorad), with dimensions of 38 × 50 cm and a source of 3,000 V. The oligonucleotide combinations used for the selective amplification were used. For the preparing of the matrix solution of the polyacrylamide gels 252 g of urea, 250 mL of TEB (tris-base, boric acid, EDTA, and distilled water) 1X, 1.8 g of bis-acrylamide, 36 g of acrylamide were used with the final volume adjusted to 600 mL with distilled water.

For the preparing of a gel, 120 mL of the matrix solution was used, 120 μL of Tetramethylethylenediamine (Temed) and 800 μL of ammonium persulfate. Temed and ammonium persulfate were added immediately before the application of the matrix between the plaques. To the selective amplification product (20 μL), 8 μL of the Loading buffer (formamide 98%, EDTA 10 mM pH 8.0, bromidephenol 0.002%, p/v, and xylene cyanol 0.002%, pv⁻¹) were added. For the denaturation process, the samples were submitted to the temperature of 95 °C for 5 min in a thermocycler and then applied to the polyacrylamide gel. 20 μL of this solution were applied to a sequencing gel (polyacrylamide 6% pv⁻¹, urea 7M) with a Bio-Rad (Sequi Gen GT model) electrophoresis box.

For the revealing of the gels, the coloration with the silver nitrate method (according to protocol proposed by Creste, Tulmann Neto and Figueira (2001)) was applied. The size of alleles was estimated by visual comparison with the 25 pb (InvitroGen Life Technologies, Carlsbad, Calif., USA) DNA molecular weight marker.

Only the visible bands were considered - each band was considered as a genetic locus in which the presence was registered as "1" and the absence as "0". After the reading of the plaques, a binary matrix was made. The data provided by the molecular markers were used to make a dissimilarity matrix by means of the Jaccard arithmetic coefficient. To have the dendrogram, the dissimilarity matrix was applied to unweight pair-group method of arithmetic averages (UPGMA). The adjustment between the similarity matrix and the dendrogram was estimated by the cophenetic correlation coefficient - r (SOKAL; ROHLF, 1962). The distance between groups was determined by the mean distance between pairs of individuals belonging in the different groups.

With basis on the developed original matrix the inter and intra genetic variability of the populations were estimated by the analysis of molecular variance (AMOVA) and after that the Fst value, which is equivalent to the proportion of the total genetic fractioned variation inter populations (EXCOFFIER; SMOUSE; QUATTO, 1992). All the described analyses were made by means of the Genes program (version 2013).

3 Results and Discussion

The primer combinations in the three populations totaled 853 bands, with a variation of 180 to 246 loci

per primer combination (Table 1). The mean percentage of polymorphic loci found in each population per primer combination varied from 64.7% in the VLB population to 77.65% in the UFAM population. In a study with *Euterpe edulis*, Cardoso et al. (2000) reported that, for five pairs of AFLP primers, 429 fragments were found and that 395 (92%) were polymorphic. Clement et al. (2002), studying three races of *Bactris gasipaes* (peach palm), used six primer combinations and got 245 fragments of which 135 (55.1%) were polymorphic.

The AFLP markers used in the present work also presented high levels of polymorphism when compared to the RAPD (random amplified polymorphic DNA) marker of dominant nature. Lima et al. (2011), in a study of the genetic diversity in “cajazeira” (*S. mombin*), making use of RAPD markers, analyzing 21 primers, found a total of 145 fragments, 115 (79.3%) of which were found to be polymorphic.

With basis on the mean genetic similarity calculated for the 90 individuals of *S. mombin* it was possible to verify in the dendrogram the formation of two groups, the first formed by the populations UFAM and VLB and the second by the Ceplac population (Figure 1). This result is supported by a high bootstrap value (2,000 re-samplings). The cophenetic correlation coefficient was of 0.9792, this being an evidence of a good adjustment between the graphic representation of the distances in the dendrogram and its original matrix, thus being reliable data.

In the dendrogram, for the individuals of the UFAM population, a four group formation was observed, one of them formed by isolated individual (Figure 2). The similarity coefficients showed that the most divergent individuals were UFAM02 and UFAM025 with an r value of 0.77586 and the

most divergent ones were UFAM16 and UFAM17, with an r value of 0.0303.

In the dendrogram for the VBL population, a four-group formation was found to occur (Figure 3). The genetic similarity coefficients showed that the most divergent individuals were VLB45 and VLB59, with an r value of 0.82178 and the less divergent were VLB36 and VLB37 with an r value of 0.06796.

In the dendrogram for the Ceplac population, three groups were verified to occur (Figure 4). The similarity coefficient pointed the most genetically divergent individuals as Ceplac74 and Ceplac76, with an r value of 0.79412 and the least divergent as Ceplac72 and Ceplac73, with an r value of 0.05455.

The genetic inter populations variability was of 52.8% and among individuals in the analyzed populations was of 47.2% (Table 2). The Fst value found for the three populations (0.52) showed a large genetic differentiation between them. But there is no fixation of different alleles; this would happen only if the test showed a value equal to or larger than one (1). The results herein reported are different from those in the literature for natural populations in which the largest part of the genetic diversity is found in the populations (PAIVA, 1998; GOMES et al., 2011). It is though necessary to emphasize that in the present study just two of the populations are natural (UFAM and VLB) and that the fact that an enriched population (Ceplac) was included in this study supports the obtained data. The introduction of a planting enriched population (Ceplac) in this study is the factor responsible for the differentiation of this population of the others. The Ceplac people responsible for the project informed us that the introduced plants were selected for their fruit quality and that they proceeded from several regions of the state of Amazonas, although their exact point of origin had not been possible to determine.

The results of this experiment suggest that although most of the genetic variation of the species is found inter populations, the variation verified intra populations is also considerable. Management and conservation plans of *S. mombin* should consider the genetic variability found in those populations. In order to guarantee that most of that variability is preserved, individual samples of each one of the populations must be conserved.

Considering the pattern of genetic variability found in *S. mombin* and that it is an allogamous species, this meaning that the individuals intercross in a random fashion, for its preservation a representative sample of individuals of all populations is necessary, since the largest diversity is found

Table 1. Number of loci resulting from primer combinations and polymorphism percentage found in each *S. mombin* population.

Primer combination	Loci number	Polymorphic loci (%)		
		UFAM	VLB	Ceplac
E+AAC /M+CAT	210	97.22	63.49	78.66
E+AGT /M+CTC	217	72	73.23	76.05
E+AAC /M+CTC	180	70.96	59.01	70.17
E+AGT/M+CAT	246	70.45	66.25	71.79
Mean		77.65	64.74	75.79
Total	853			

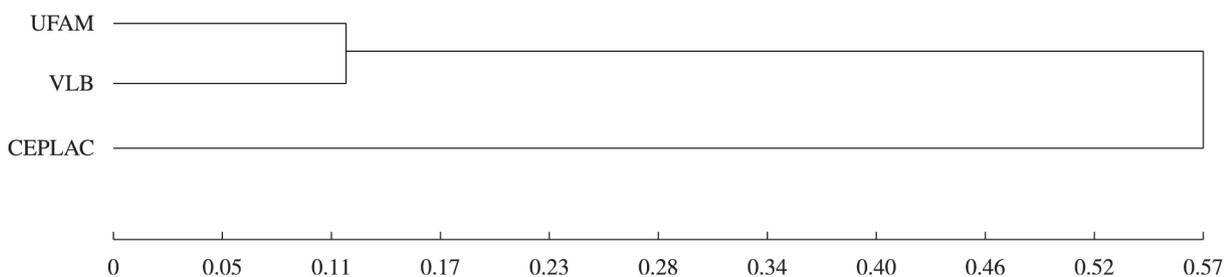


Figure 1. UPGMA dendrogram among the three *Spondias mombin* populations with basis on four AFLP marker primer combinations (EcoRI/Msel).

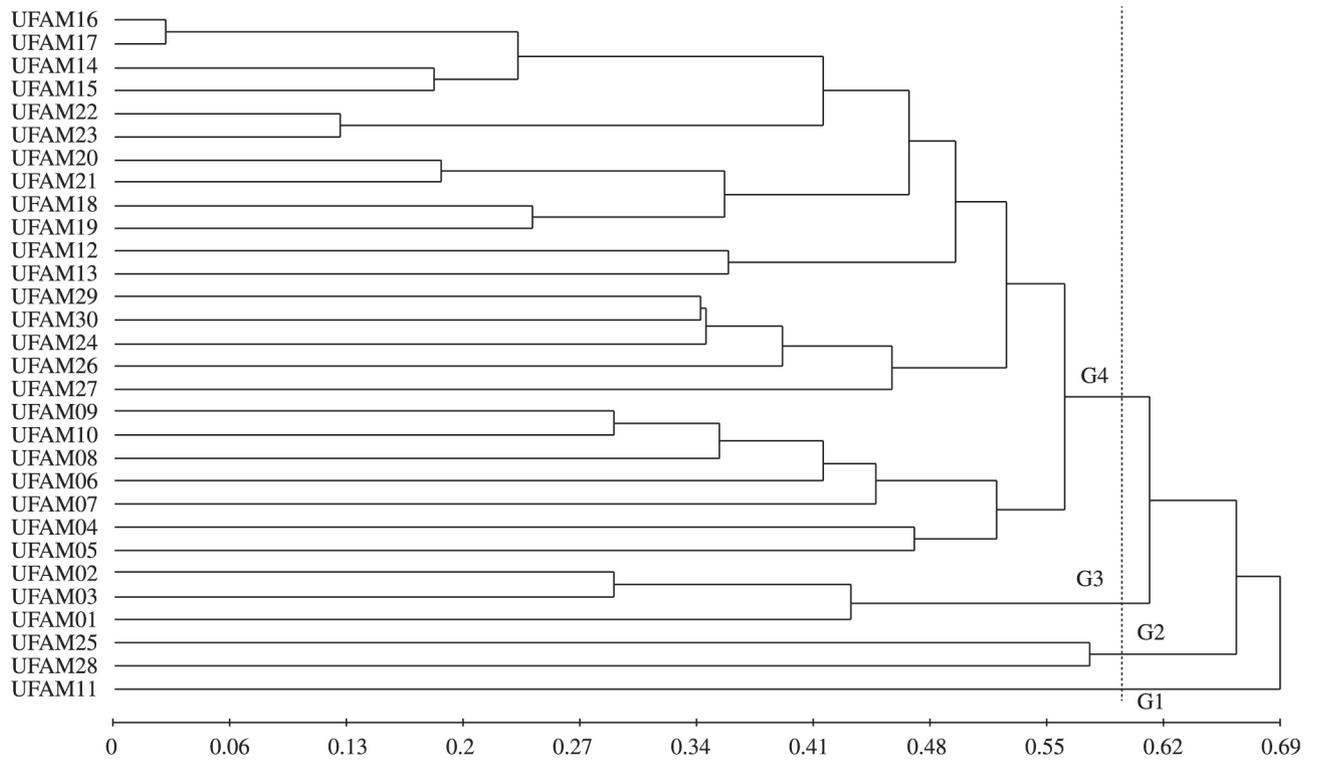


Figure 2. Dendrogram of a *Spondias mombin* population made with basis on the unweighted pair-group method of arithmetic averages (UPGMA) of 30 individuals belonging in the UFAM population. The dotted vertical line represents the estimated cut by the Mojema (1977) method.

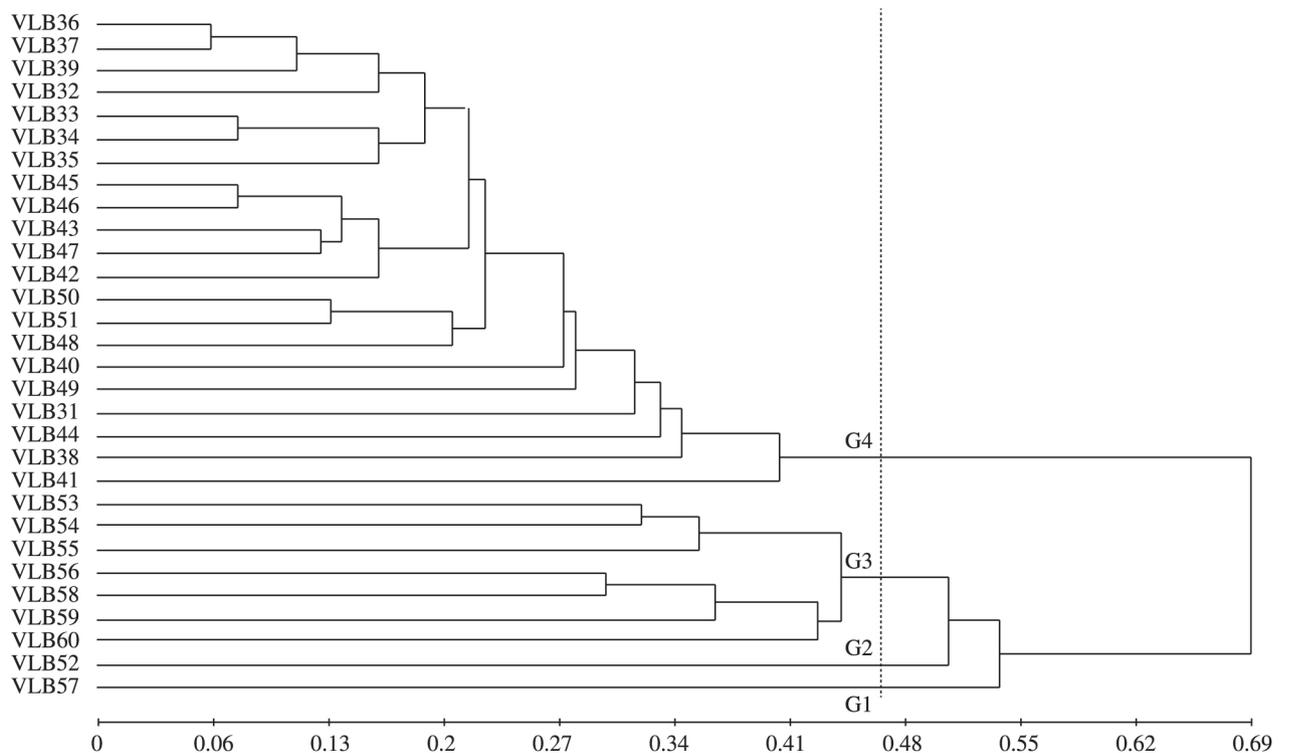


Figure 3. Dendrogram of a *Spondias mombin* population made with basis on the unweighted pair-group method of arithmetic averages (UPGMA) of 30 individuals belonging in the VLB population. The dotted vertical line represents the estimated cut by the Mojema (1977) method.

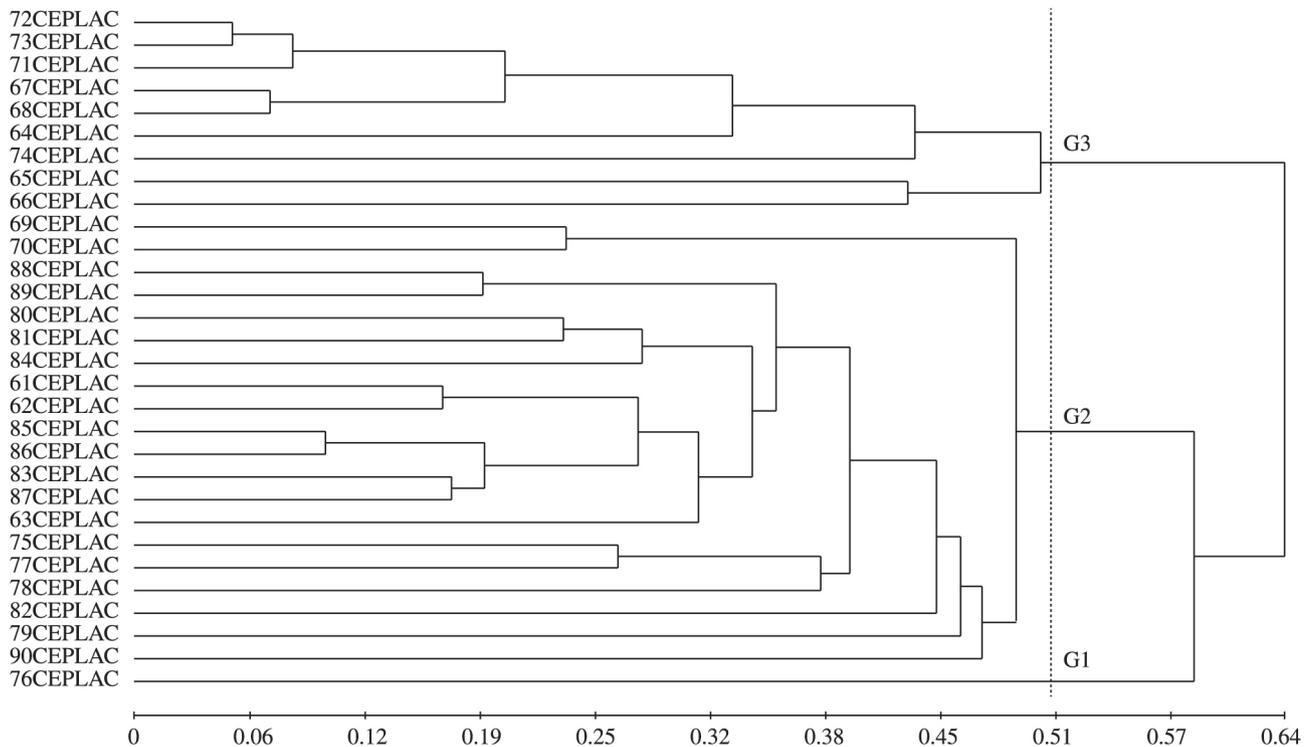


Figure 4. Dendrogram of a *Spondias mombin* population made with basis on the unweighted pair-group method of arithmetic averages (UPGMA) of 30 individuals belonging in the Ceplac population. The dotted vertical line represents the estimated cut by the Mojema (1977) method.

Table 2. Genetic variation as indicated by the molecular variance analysis (AMOVA).

Variation	Variance components	Variation (%)
Inter	31.7464	52.8131
Intra	28.3644	47.1869
Total	60.1107	100.000

$F_{st} = 0,52.$

inter populations, this causing a large number of individuals to compose an *ex situ* collection. The collections established in the field demand permanent resources for their keeping. The experience resulting from the conservation in the field of perennial species natives to the Amazon region has shown that the participative *in situ* conservation in the areas where the species is exploited by extractive procedures, should be stimulated. For the conservation *in situ*, efforts and resources may be concentrated in collections of small proportion works and include only the genotypes of higher potential, selected *in situ* which could be capable of, even in the first selection cycle, become the first seed production fields which would be used for future sowings (GOMES et al., 2011).

4 Conclusions

The molecular markers of the type AFLP reveal a high content of genetic information in *S. mombin* and may be used for genetic analyses having in mind to get information concerning the genetic sustainability and the forest management of the species.

The distribution of the genetic diversity in the studied populations of *S. mombin* is larger inter than intra populations so that it is necessary to unite individuals of all the populations for the genetic conservation of the species.

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References

- CARDOSO, S. R. S.; ELOY, N. B.; PROVAN, J.; CARDOSO, M. A.; FERREIRA, P. C. G. Genetic differentiation of *Euterpe edulis* Mart. populations estimated by AFLP analysis. *Molecular Ecology*, v. 9, p. 1753-1760, 2000. PMID:11091311. <http://dx.doi.org/10.1046/j.1365-294x.2000.01056.x>
- CLEMENT, C. R.; SOUZA, N. R.; RODRIGUES, D. P.; ASTOLFI-FILHO, S.; MORENO, Y. N.; PASCUAL, V. T.; RODRÍGUEZ, F. J. G. Use of AFLPs to distinguish landraces of Pajibaye (*Bactris gasipaes*) in Brazilian Amazonia. *Scientia Agricola*, v. 59, n. 4, p. 749-753, 2002. <http://dx.doi.org/10.1590/S0103-90162002000400019>
- CRESTE, S.; TULMANN NETO, A.; FIGUEIRA, A. Detection of single sequence repeat polymorphisms in denaturing polyacrylamide sequencing gels by silver staining. *Plant Molecular Biology Reporter*, v. 19, n. 4, p. 299-306, 2001. <http://dx.doi.org/10.1007/BF02772828>

- DOYLE, J. J.; DOYLE, J. L. Isolation of plant DNA from fresh tissue. *Focus*, v. 12, p. 13-15, 1987.
- DUVAL, C. S. On the origin of the tree *Spondias mombin* in Africa. *Journal of Historical Geography*, v. 32, p. 249-266, 2006. <http://dx.doi.org/10.1016/j.jhg.2005.02.001>
- ESFAHANI, S. T.; SHIRAN, B.; BALALI, G. AFLP markers for the assessment of genetic diversity in european and North American potato varieties cultivated in Iran. *Crop Breeding and Applied Biotechnology*, v. 9, n. 1, p. 75-86, 2009.
- ESTOPA, R. A.; SOUZA, A. M.; MOURA, C. O. M.; BOTREL, M. C. G.; MENDONÇA, E. G.; CARVALHO, D. Diversidade genética em populações naturais de candeia (*Eremanthus erythropappus* (DC.) MacLeish). *Scientia Forestalis*, v. 70, p. 97-106, 2006.
- EXCOFFIER, L.; SMOUSE, P. E.; QUATTO, J. M. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, v. 131, p. 479-491, 1992. PMID:1644282.
- FERREIRA, M. E.; GRATTAPAGLIA, D. *Introdução ao uso de marcadores moleculares em análise genética*. Brasília: Embrapa-CENARGEN, 1998. 222 p.
- FRANZON, R. C. F.; CASTRO, M. C.; RASEIRA, M. C.B. Variabilidade genética em populações de Pitangueira oriundas de autopolinização e polinização livre, acessada por AFLP. *Revista Brasileira de Fruticultura*, v. 32, n. 1, p. 240-250, 2010. <http://dx.doi.org/10.1590/S0100-29452010005000016>
- GOMES, L. R. P.; LOPES, M. T. G.; BENTES, J. L. S.; BARROS, W. S.; COSTA NETO, P. Q.; CONTIM, L. A. S. Genetic diversity in natural populations of Buriti (*Mauritia flexuosa* L. f.). *Crop Breeding and Applied Biotechnology*, v. 11, n. 3, p. 216-222, 2011. <http://dx.doi.org/10.1590/S1984-70332011000300003>
- JUSTINIANO, M. J.; FREDERICKSEN, T. S.; NASH, D. *Ecologia y Silvicultura de Espécies menos Conocidas: Azucaró Spondias mombin* L., Anacardiaceae. Santa Cruz: Bolfor, 2001. 43 p.
- LOPES, R.; LOPES, M. G.; OLIVEIRA, FIGUEIRA, A. V. O.; CAMARGO, L. E. A.; FUNGARO, M. H. P.; CARNEIRO, M. S.; VIEIRA, M. L. C. Marcadores moleculares dominantes (RAPD e AFLP): aspectos técnicos e interpretação genética. *Biociência e Desenvolvimento*, v. 29, p. 64-68, 2003.
- LIMA, A. T. B.; SOUZA, V. A. B.; GOMES, R. L. F.; LIMAS, P. S. C. Molecular characterization of cajá, *Spondias mombin* (Anacardiaceae), by RAPD markers. *Genetics and Molecular Research*, v. 10, n. 4, p. 2893-2904, 2011. PMID:22179961. <http://dx.doi.org/10.4238/2011.November.25.1>
- MOJEMA, R. Hierarchical grouping methods and stopping rules: An evaluation. *Computer Journal*, v. 20, p. 359-363, 1977.
- PAIVA, J. R. *Melhoramento genético de espécies agroindustriais na Amazônia: estratégias e novas abordagens*. Brasília: Embrapa-SPI; Fortaleza: Embrapa-CNPAT, 1998. 135 p.
- PINTO, W. S.; DANTAS, A. C. V. L.; FONSECA, A. A. O.; LEDO, C. A. S.; JESUS, S. C.; CALAFANGE, P. L. P.; ANDRADE, E. M. Caracterização física, físico-química e química de frutos de genótipos de cajazeiras. *Pesquisa Agropecuária Brasileira*, v. 38, n. 9, p. 1059-1066, 2003. <http://dx.doi.org/10.1590/S0100-204X2003000900006>
- RAMOS, S. L. F.; MACÊDO, J. L. V.; MARTINS, C. C.; LOPES, R.; LOPES, M. T. G. Tratamentos pré-germinativos e procedência de sementes do tucumã-do-amazonas para a produção de mudas. *Revista Brasileira Fruticultura*, v. 33, n. 1, p. 962-969, 2011. <http://dx.doi.org/10.1590/S0100-29452011000300033>
- SACRAMENTO, C. K.; SOUZA, F. X. *Cajá (Spondias mombin L.)*. Jaboticabal: Funep, 2000. 42 p.
- SANTOS, C. A. F.; RODRIGUES, M. A.; ZUCCHI, M. I. Variabilidade genética do umbuzeiro no Semiárido brasileiro por meio de marcadores AFLP. *Pesquisa Agropecuária Brasileira*, v. 43, n. 8, p. 1037-1043, 2008.
- SANTOS, C. A. F.; OLIVEIRA, V. R. Inter-relações genéticas entre espécies do gênero *Spondias* com base em marcadores AFLP (Polimorfismo de Comprimento de Fragmento Amplificado). *Sociedade Brasileira de Fruticultura*. Jaboticabal, v. 30, n. 3, 2008.
- SETOTAW, T. A.; DIAS, L. A. S.; MISSIO, R. F. Genetic divergence among barley accessions from Ethiopia. *Crop Breeding and Applied Biotechnology*, v. 10, n. 4, p. 116-123, 2010.
- SOKAL, R. R.; ROHLF, F. J. The comparison of dendrograms by objective methods. *Taxon*, v. 11, n. 2, p. 33-40, 1962. <http://dx.doi.org/10.2307/1217208>
- SOUZA, F. X. Efeito do porta-enxerto e do método de enxertia na formação de mudas de cajazeira (*Spondias mombin* L.). *Revista Brasileira de Fruticultura*, v. 22, n. 2, p. 286-290, 2000.
- VOS, P.; HOGERS, R.; BLEEKER, M.; REIJANS, M.; LEE, T.; HORNES, M.; FRIJTERS, A.; POT, J.; PELEMAN, J.; KUIPER, M.; ZABEAU, M. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, v. 23, n. 21, p. 4.407-4.414, 1995.
- WISHNIE, M. H.; DENTA, D. H.; MARISCALA, E.; DEAGOA, J.; CEDEÑO, N.; IBARRA, D.; CONDITA, R.; ASHTON, P. M. S. Initial performance and reforestation potential of 24 tropical tree species planted across a precipitation gradient in the Republic of Panama. *Forest Ecology and Management*, v. 243, n. 1, p. 39-44, 2007. <http://dx.doi.org/10.1016/j.foreco.2007.02.001>