ORIGINAL ARTICLE – Conservation of Nature

Genetic Diversity Between and Within *Astrocaryum acaule* Mart. (Arecaceae) Populations

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Abstract

This article sought to assess the genetic diversity within and between natural populations of *Astrocaryum acaule* by means of AFLP markers. Ninety individuals were sampled in three populations located in Manaus (state of Amazonas) across regions with different intensities of anthropogenic activity. Two clusters were differentiated by means of dendrograms and Bayesian cluster analysis, the first consisting of individuals from the Tarumã-Açu population in an intensely agricultural region, and the second, of populations from UFAM and Tupé conservation areas. The physical isolation of *A. acaule* populations has resulted in good conservation of its genetic identity, since anthropogenic activity is an important modifier of the genetic diversity of natural populations. Genetic diversity was higher within populations (74.58%) than between them (25.42%), which means that we have to collect representative samples from each of the genetically distinct groups for genetic conservation of the species. Therefore, *in situ* conservation is recommended.

Keywords: Amazonian palm, genetic resource, AFLP, population structure.

1. INTRODUCTION AND OBJECTIVE

The Amazon region is rich in animal and plant diversity. Among the plant groups, palm trees of the species from the Arecaceae family stand out. Palm trees have different purposes, with usage in/as food and in construction, and also generate by-products that are sources of income for the survival of Amazonian populations (Wallace, 2014). Although having great potential for development in the region, the native palms of the Amazon are most present in the wild or semi-wild (Gomes et al., 2011), such as the *Astrocaryum acaule* Mart. (tucumanzinho) palm tree, which is still a neglected species in studies that would enable its better use.

Astrocaryum acaule has edible fruits with reduced size compared to *Astrocaryum aculeatum* (tucumã-do-Amazonas); moreover, its seeds are used for manufacturing biojewelery, being one of the species most frequently used for ring production in the Amazon region. The leaves are used for fiber extraction and these have suitable physical, chemical and organoleptic properties for producing natural tissues (Pacheco et al., 2011). The species has very interesting characteristics, such as its subterranean stem, which facilitates collection of fruits; its leaves, which sprout directly from the soil; and the peduncle of the spadix, which grows between the leaves with the fruits (Wallace, 2014). As *A. acaule* is very small, it can be considered for interspecific improvement with *A. aculeatum* to reduce the size of this important Amazon fruit tree. There are already indications of the existence of natural hybrids between these species; however, the studies that verify the viability of this crossing are still in the initial phase of experimentation.

Deforestation in the Amazon, either by extraction or for agricultural use, has been reducing the genetic variability of many

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tree species, including palm trees. This problem is believed to aggravate in populations present in urban areas, being necessary studies that evaluate the impacts on the genetic variability according to the intensity of the anthropic action in these populations. According to Viegas et al. (2011), when the forest becomes fragmented, the size of the populations decreases, and, consequently, their genetic diversity, making them isolated and vulnerable to environmental, demographic and genetic events.

The maintenance of genetic variability has become a key factor for species to maintain their ability to react to environmental stresses, especially in the face of global climate change (Cruz et al., 2011). Thus, it is necessary to know the quantification and distribution of genetic diversity among and within populations to outline usage and conservation strategies, aiming to overcome the challenges of environmental changes and the inadequate exploitation of native forests.

Studies of genetic variability in natural populations of palm trees in tropical regions show that they preserve great variability within the populations when compared with those existing in other environments such as the *Euterpe edulis* (juçara – *palmito-juçara*) (Cardoso et al., 2000), *Butia capitata* (jelly palm – *butiá*) (Buttow et al., 2010), *Mauritia flexuosa* (moriche palm – *buriti*) (Gomes et al., 2011), *Astrocaryum aculeatum* (*tucumã*) (Ramos et al., 2012) and *Astrocaryum jauari* (*jauari*) (Oliveira et al., 2014) palm trees. The distribution of natural genetic variability is influenced by factors such as breeding mode, crossing system, population size, geographic distribution and gene flow, as well as anthropic influence, which has been an agent that modifies the genetic diversity of many natural populations of many species (Viegas et al., 2011).

Based on the knowledge of genetic diversity among and within natural populations, it is possible to understand how selection is acting as a function of adaptability and to establish conservation strategies, since the greater the genetic variability in the population, the greater the chance of species perpetuation (Almeida et al., 2015; Rossi et al., 2014). Several techniques are used in studies of genetic diversity; among these, molecular markers of DNA enable identification of polymorphic loci in the genome and are not influenced by environmental effects. Amplified Fragment Length Polymorph (AFLP) markers are distinguished from other markers because they detect a large number of loci with wide genome coverage, and can be used for species with little or no previous molecular knowledge (Lopes et al., 2003).

This study sought to evaluate the genetic diversity among and within natural populations of the species by means of the AFLP molecular marker, since knowledge of the genetic variability of *A. acaule* populations is essential for the use, conservation and breeding of this species.

2. MATERIALS AND METHODS

The *Astrocaryum acaule* populations were studied in three distinct regions of the city of Manaus: the campus of the Universidade Federal do Amazonas (UFAM), the Julião Community located in the Tupé Sustainable Development Reserve (RDS) and Tarumã-Açu Community (study registered in SisGen under No. A96D1EA) (Figure 1).

This study consisted of 90 plants (30 of each population) randomly sampled, properly identified and with their geographical positions registered by GPS (Global Positioning System) (Table 1). The three collection sites had similar characteristics such as being sandy soils, floodable, and near water courses. In general, the *A. acaule* populations were composed of several individuals close to each other; however, a minimum distance of 100 m between individuals was observed at the time of collection.

Young and healthy leaves were harvested from each plant, packed in plastic bags containing silica gel, duly identified and stored at −20 °C for subsequent extraction of genomic DNA and analysis with AFLP markers in the Laboratory of Plant Genetic Improvement of the Faculdade de Ciências of UFAM. Genomic DNA was extracted using the 2% CTAB method (cetyltrimethylammonium bromide) described by Murray & Thompson (1980). For each sample, approximately 80 mg of leaf tissue was macerated without the main vein. Analysis of the quality of the extracted DNA was performed by electrophoresis in 0.7% agarose gel, and the DNA was quantified by absorbance measurements under UV light (260 and 280 nm) in a Vilber Lourmat spectrophotometer (Quantum model).

Analysis of AFLP markers was performed according to the procedures originally proposed by Vos et al. (1995), with modifications by Lopes et al. (2003). The combination of EcoRI/MseI restriction enzymes in 30 ng of genomic DNA was used for the DNA digestion reactions. Specific EcoRI and MseI adapters were subsequently prepared, which had complementary ends to the extremities resulting from digestion by the restriction enzymes. In the selective amplification stage, three of the four combinations of tested primers (E+AAC/M+CAC, E+ACA/M+CGC and E+AAC/M+CGA) were selected due to the presence of a higher number of polymorphic loci and a better amplification quality of the bands. Therefore, they were used in all the samples. After the selective amplification reactions, each sample was applied to the Bio-Rad vertical electrophoresis cell (SEQUI Gen GT model) in 6% polyacrylamide gel (acrylamide/bis-acrylamide (19:1 v/v) under constant potency of 80 W for four hours. The silver nitrate staining method was used for development of the gel, according to the protocol proposed by Creste et al. (2001).
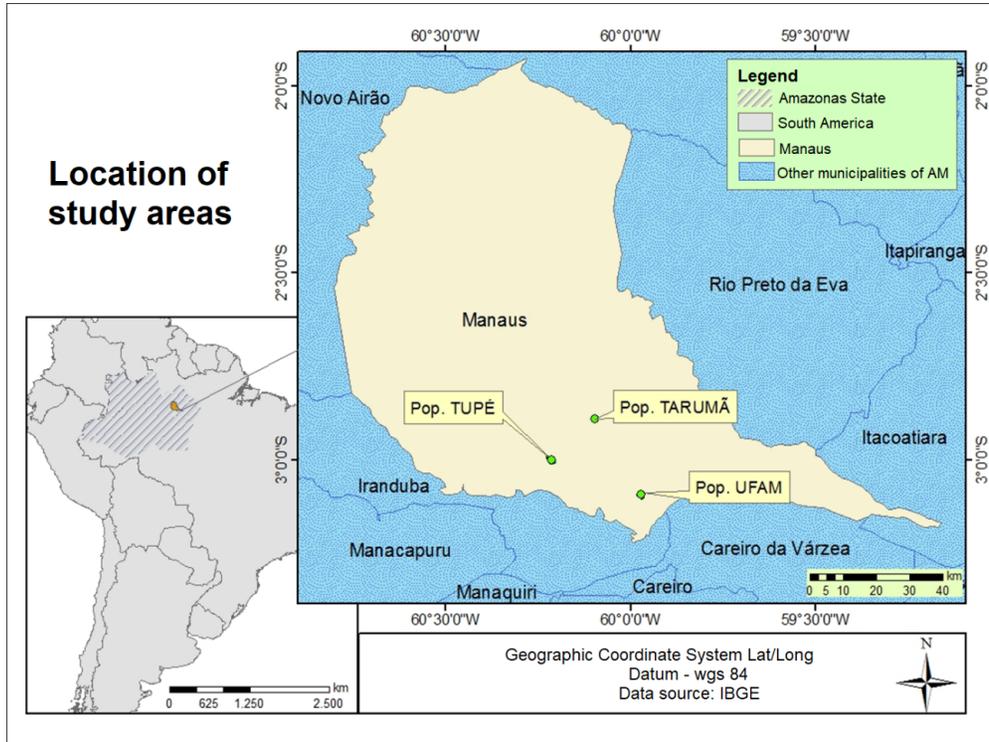
Figure 1. Location map of the *Astrocaryum acaule* study populations.

Table 1. Location of the 90 *Astrocaryum acaule* subsamples used in the genetic diversity study.

ID	Geographic Coordinates					
	UFAM (UF)		RDS Tupé (TU)		Tarumã-Açu (TA)	
	Latitude	Longitude	Latitude	Longitude	Latitude	Longitude
1	–03°05'44.72"	–59°58'31.72"	–02°59'05.36"	–60°11'52.19"	–02°53'24.20"	–60°05'53.40"
2	–03°05'42.19"	–59°58'34.77"	–02°59'05.49"	–60°11'50.48"	–02°53'25.40"	–60°05'53.60"
3	–03°05'41.88"	–59°58'33.80"	–02°58'43.24"	–60°11'30.05"	–02°53'23.70"	–60°05'54.40"
4	–03°05'42.20"	–59°58'33.30"	–02°58'43.64"	–60°11'31.36"	–02°53'23.60"	–60°05'55.60"
5	–03°05'42.64"	–59°58'32.87"	–02°59'05.52"	–60°11'52.17"	–02°53'23.00"	–60°05'56.70"
6	–03°05'42.02"	–59°58'36.33"	–02°59'53.90"	–60°13'13.14"	–02°53'23.70"	–60°05'57.30"
7	–03°05'42.19"	–59°58'35.22"	–02°59'26.40"	–60°13'28.24"	–02°53'23.90"	–60°05'53.80"
8	–03°05'41.74"	–59°58'38.19"	–02°59'26.08"	–60°13'27.94"	–02°53'23.50"	–60°05'58.70"
9	–03°05'42.07"	–59°58'35.88"	–02°59'26.42"	–60°13'28.40"	–02°53'23.40"	–60°05'59.20"
10	–03°05'43.27"	–59°58'31.58"	–03°00'25.13"	–60°12'54.22"	–02°53'23.10"	–60°05'59.50"
11	–03°05'43.02"	–59°58'31.91"	–03°00'24.82"	–60°12'53.82"	–02°53'24.80"	–60°05'58.60"
12	–03°05'44.03"	–59°58'31.33"	–03°00'18.22"	–60°12'54.02"	–02°53'25.20"	–60°05'58.70"
13	–03°05'43.56"	–59°58'31.56"	–03°00'20.68"	–60°12'19.64"	–02°53'24.20"	–60°05'58.50"
14	–03°05'42.67"	–59°58'32.83"	–03°00'26.21"	–60°12'38.53"	–02°53'26.30"	–60°05'57.60"
15	–03°05'42.94"	–59°58'32.16"	–03°00'24.68"	–60°12'46.43"	–02°53'26.90"	–60°05'56.90"
16	–03°05'42.18"	–59°58'33.30"	–03°00'23.87"	–60°12'51.36"	–02°53'27.70"	–60°05'57.00"
17	–03°05'56.17"	–59°58'12.98"	–03°00'02.47"	–60°13'06.60"	–02°53'28.20"	–60°05'56.50"
18	–03°05'56.79"	–59°58'11.51"	–02°59'53.90"	–60°13'13.14"	–02°53'28.90"	–60°05'56.20"
19	–03°05'41.77"	–59°58'37.10"	–02°59'41.62"	–60°13'22.68"	–02°53'29.60"	–60°05'54.80"
20	–03°05'56.16"	–59°58'11.41"	–02°59'30.59"	–60°13'30.91"	–02°53'30.10"	–60°05'51.10"
21	–03°05'43.79"	–59°58'32.18"	–02°59'26.58"	–60°13'28.11"	–02°53'30.30"	–60°05'53.90"
22	–03°05'55.92"	–59°58'15.21"	–02°59'26.40"	–60°13'28.24"	–02°53'30.80"	–60°05'53.40"

Table 1. Continued...

ID	UFAM (UF)		RDS Tupé (TU)		Tarumã-Açu (TA)	
	Latitude	Longitude	Latitude	Longitude	Latitude	Longitude
23	−03°05'55.75"	−59°58'13.25"	−03°00'25.10"	−60°12'54.20"	−02°53'29.00"	−60°05'53.30"
24	−03°05'42.65"	−59°58'34.18"	−03°00'25.14"	−60°12'54.20"	−02°53'28.80"	−60°05'52.70"
25	−03°05'43.45"	−59°58'32.12"	−03°00'25.05"	−60°12'54.20"	−02°53'30.20"	−60°05'52.60"
26	−03°05'41.74"	−59°58'38.19"	−03°00'25.28"	−60°12'54.49"	−02°53'30.70"	−60°05'52.70"
27	−03°05'41.97"	−59°58'39.01"	−03°00'25.24"	−60°12'54.67"	−02°53'30.60"	−60°05'54.50"
28	−03°05'56.58"	−59°58'11.88"	−03°00'24.82"	−60°12'53.82"	−02°53'30.80"	−60°05'54.10"
29	−03°05'55.80"	−59°58'14.12"	−03°00'18.17"	−60°12'53.56"	−02°53'32.00"	−60°05'52.50"
30	−03°05'56.20"	−59°58'12.68"	−03°00'20.68"	−60°12'19.64"	−02°53'27.70"	−60°05'52.90"

A binary matrix was constructed from the obtained fragments, in which zero and one indicated absence and presence of fragments, respectively. The data were used in the construction of a matrix of genetic distances between individuals, based on the arithmetic complement of the Jaccard coefficient, and between populations from the genetic distance of Nei (1978).

A dendrogram was constructed by UPGMA analysis (Unweighted Pair Group Method using Arithmetical Averages) with the aid of the R statistical program (R Development Core Team, 2017) to visualize the genetic differentiation among the *A. acaule* individuals. The adequacy of the cluster analysis to the original data was evaluated by the co-optic correlation coefficient. Non-metric multidimensional scaling analysis (NMDS) was subsequently applied, in which greater dispersion of the units was identified as having greater genetic dissimilarity. The vegan package of the R program was used (Oksanen et al., 2013) to obtain this analysis.

The Bayesian approach implemented in Structure v.2.4 software (Pritchard et al., 2000) was used to reveal the structures of the populations. The mixed model was considered for the analyses with frequencies correlated with a priori incorporation of the information about the sampling sites. Grouping values (K) ranged from 1 to 8, with five replicates for each tested K value. The most probable K value was determined by the ΔK criterion described by Evanno et al. (2005).

Assuming populations are in Hardy-Weinberg equilibrium, the Popgene program (Yeh et al., 1999) was used to estimate genetic variation within the three populations. Estimates of genetic diversity included expected heterozygosity (He) (Nei, 1978), Shannon-Weaver (I) genetic diversity index (Shannon & Weaver, 1949) and percentage of polymorphic loci. In an additional analysis, the gene flow value among populations was estimated.

The Mantel test (Mantel, 1967) was used to investigate the correlation between geographic distance and genetic diversity of populations. The distribution of genetic variation among and within populations was quantified by the molecular variance analysis (AMOVA), performed by the GenALEX v.6.5 program (Peakall & Smouse, 2012). The significance test for AMOVA estimation was based on bootstrap, considering 10,000 re-samples.

3. RESULTS AND DISCUSSION

The oligonucleotide combinations analyzed in the three *A. acaule* populations showed high levels of polymorphism (Table 2). A total of 200 loci were detected from three pairs of primers, of which 198 (99%) were polymorphic. There was a variation of 59 to 72 polymorphic loci by oligonucleotide combination. Although the other combinations showed similar results, the E+AAC/M+CAC combination obtained better amplification. The percentage of polymorphic loci was higher than that obtained by Cardoso et al. (2000) working with *Euterpe edulis*. Using five combinations of AFLP primers, these authors obtained 429 loci, of which 395 (92%) were polymorphic.

Table 2. Polymorphism in *Astrocaryum acaule* subsamples detected by three oligonucleotide combinations using AFLP markers.

Oligonucleotide combinations	Total loci	Polymorphic loci	Polymorphism (%)	Size range (Pb)
E+ACA/M+CGC	60	59	98.33	
E+AAC/M+CAC	72	72	100.00	50 – 500
E+AAC/M+CGA	68	67	98.53	
Total	200	198	99.00	-

Based on the 198 AFLP polymorphic fragments, the genetic similarity relationships among *A. acaule* individuals were characterized in the form of a dendrogram, enabling separation of the 90 subsamples into several clusters (Figure 2). In this case, the individuals of the Tarumã-Açu population stand out, being able to be gathered in a single group. Together with some individuals from the Tupé population (TU19, TU18 and TU11), the Tarumã-Açu population were genetically divergent from the other analyzed individuals. It is noteworthy that the co-behavior correlation coefficient (r = 0.9011) showed good fit of the dendrogram in relation to the dissimilarity matrix.

Although the subsamples of the UFAM and Tupé populations form smaller groups separately, they are generally shown to be genetically close. Although they are located in an urban environment, the individuals collected in the UFAM population belong to a conservation area, and the Tupé population is included in the Tupé Sustainable Development Reserve, with both regions having restricted access to the human population.

Since they keep much of the naturally observed biodiversity intact, these sites are the subject of morphology and reproduction studies of several plant species; however, with less anthropic interference. Unlike the aforementioned populations, the Tarumã-Açu population is located in an area with great human interference, where local residents have free access to the seed collection areas. Thus, this population possibly lost genetic variability due to genetic drift shortly after forest fragmentation, and humans, as an important seed dispersing agent of the species, has been intensifying the gene flow, thus causing greater genetic distance when compared with the other populations from Tarumã-Açu.
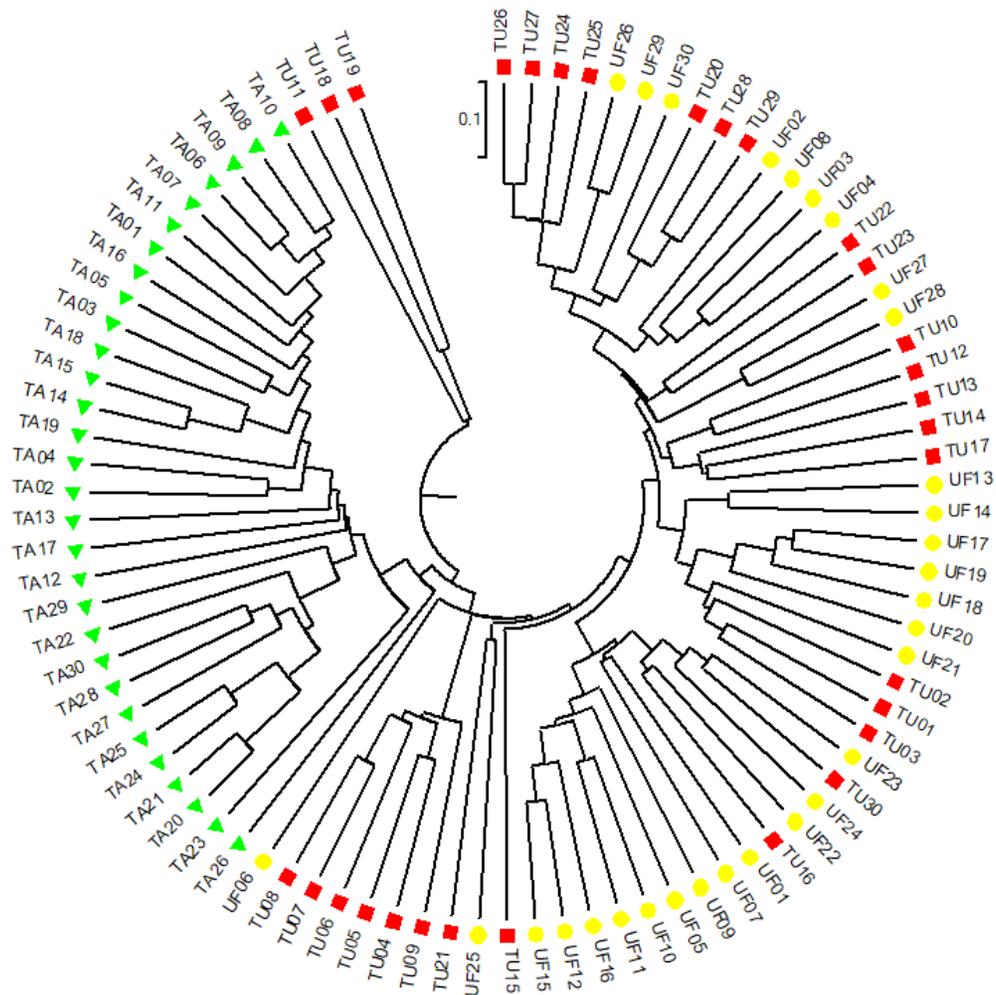

Figure 2. Dendrogram obtained from the arithmetic complement of the Jaccard genetic distance and *UPGMA* cluster method on 90 *Astrocaryum acaule* subsamples prospected in UFAM area (yellow circle), Tupé (red square) and Tarumã-Açu (green triangle). Cophenetic correlation coefficient r = 0.9011.

The representation of the similarity relation between the subsamples from two axes was performed by non-metric multidimensional scaling (NMDS) (Figure 3). The components of the Tarumã-Açu population were closer to each other, forming a group of individuals from the Tupé and UFAM populations, confirming the results of the previous analyses.

Bayesian clustering analysis was performed by the Structure software to determine the genetic patterns of population differentiation and substructuration, and according to the ΔK method described by Evanno et al. (2005), the most likely number of clusters was K = 2 (Figure 4). Graphical

visualization of the population structure enabled easy identification of two groups; one formed by the Tarumã-Açu subsamples, and the other formed by most of the subsamples of the UFAM and Tupé populations.

It is possible to understand the relationship between populations through an increasing alignment of the population structure obtained according to the tested K value (Viana et al., 2013). Thus, UFAM and Tupé individuals were still allocated in the same group, even considering K = 3, and the Tarumã-Açu population tended to divide into two groups. This fact emphasizes the high genetic similarity obtained between the first two populations.
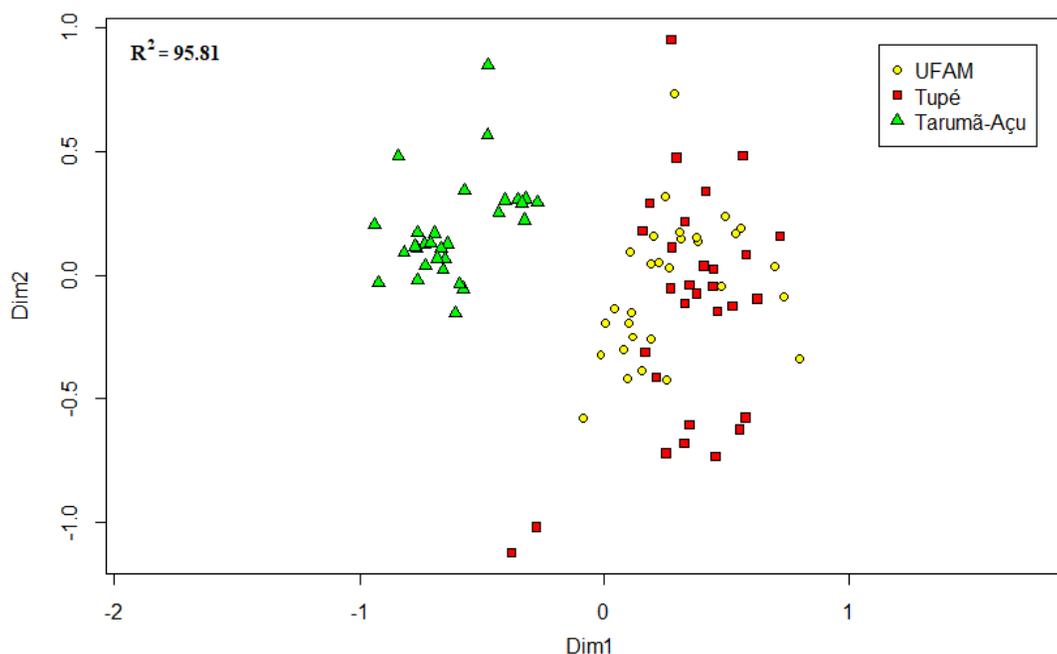
Figure 3. Analysis of non-metric multidimensional scaling of genetic distances between 90 *Astrocaryum acaule* subsamples prospected from three populations.
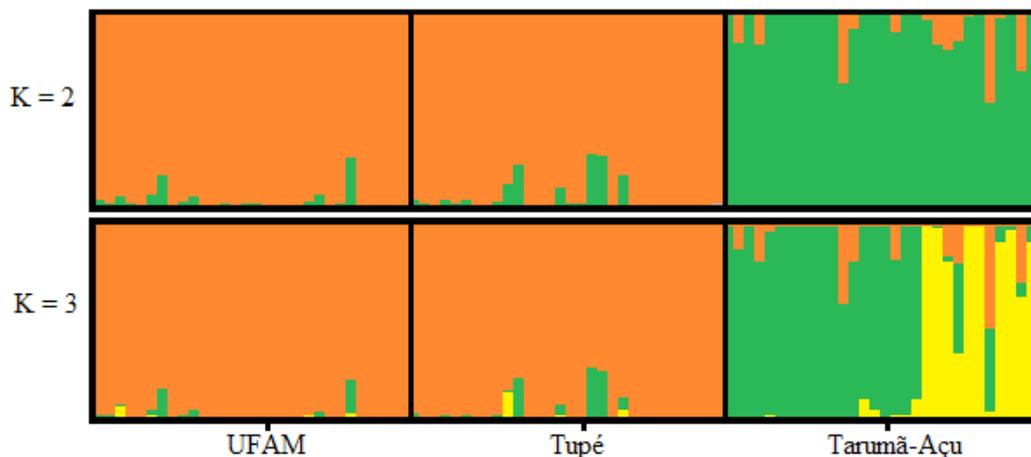
Figure 4. Population structure obtained by *Structure* software from 90 subsamples prospected from three *Astrocaryum acaule* populations for different values of clusters (K).

The genetic distance of Nei (1978) among the pairs of populations was lower between UFAM and Tupé (0.016), and higher between UFAM and Tarumã-Açu (0.098) and Tupé and Tarumã-Açu (0.093). As previously reported, the UFAM and Tupé populations are present in places considered as conservation areas, while the Tarumã-Açu population is in an area of free access which has greater possibility of anthropic interference for the exchange of genes with populations of other regions, and/or a loss of genes caused by deforestation for urban growth and agriculture. These factors may have altered the genetic variability within this population, leading to a greater distance from the UFAM and Tupé populations.

In this study, another important factor that can be correlated with genetic distance must be considered, namely that of physical isolation (Silva & Souza, 2014). This hinders or inhibits human access to different areas, and can be considered a fundamental factor for the observed distance between isolated populations with restricted access to those populations with free human access for seed exchange.

The hypothesis that the three populations could initially be part of a single population resulting from the same seed bank can be raised. After urban fragmentation and greater physical isolation, UFAM and Tupé populations were genetically distanced from the Tarumã-Açu population.

The Mantel statistic indicated a positive (r = 0.4076) and significant (p = 0.001) correlation between the geographic distance and genetic distance of moderate magnitude. Thus, geographical distance influences the genetic divergence of the populations under study; however, this may not be the determining factor. In this case, the physical isolation of the UFAM and Tupé populations also explains that, despite the largest geographic distance between the analyzed populations and the separation by watercourses, they have a higher genetic similarity. In a study of diversity of the *Euterpe edulis* palm tree, Cardoso et al. (2000) obtained results indicating that genetic distance does not solely depend on geographical distance, although clustering based on genetic distances reflects geographic relationships in most cases. Differing results were observed by Oliveira et al. (2014), indicating that the relationship between populations of *Astrocaryum jauari* is independent of geographic distortions.

The coefficient of population differentiation (Gst) was equal to 0.1473. Assuming that the gene flow (Nm) can be estimated based on Gst (Nm = 0.5 (1 – Gst)/Gst) and recognizing that all the assumptions required for the calculation are often not met (Whitlock & McCauley, 1999), the estimated mean gene flow was Nm = 2.89 when all populations were considered as a group of populations. This estimate was similar to that obtained by Cardoso et al. (2000) in a study with *E. edulis*

(Nm = 2.99), and well below that obtained by Adin et al. (2004) for peach palm (Nm = 4.8) from AFLP markers and in river interconnected populations. It is important to emphasize that Nm values greater than 1.0 indicate that gene flow will be high enough to prevent differentiation between populations due to drift (Slatkin & Barton, 1989).

The values of expected heterozygosity (He) and Shannon & Weaver (I) genetic diversity index did not show large variations among the three populations (Table 3). However, the highest values of He (0.2169) and I (0.3413) were found in the Tarumã-Açu population, indicating greater genetic diversity within this population.

These results were already expected, since this population is more likely to receive genetic material from other regions, resulting in greater genetic variability between the individuals of which this population is composed. However, the values obtained for the studied populations were lower than those estimated by Oliveira et al. (2014) when analyzing *A. jauari* populations based on microsatellite molecular markers, in which the average genetic diversity for a population was 0.56 and for other two 0.63 and 0.58.

The polymorphism percentage detected within the populations (84.85%, 83.33% and 85.86%) indicated that the combinations of primers selected for this study were sufficient to analyze the intraspecific genetic diversity, since a high percentage of polymorphic loci was detected even when the number of individuals analyzed was reduced from 90 to 30 individuals.

The amount of genetic variation obtained by the Molecular Variance Analysis – AMOVA (Table 4) showed that 74.58% of the genetic variability is found among individuals within the populations and 25.42% among the analyzed populations. These results show that similar sampling and/or conservation strategies may not be equally effective in all analyzed populations.

Table 3. Genetic diversity within *Astrocaryum acaule* populations and in the species.

Population	N	P(%)	He	I
UFAM	30	84.85%	0.2012 (± 0.0117)	0.3222 (± 0.0162)
Tupé	30	83.33%	0.1864 (± 0.0112)	0.3034 (± 0.0156)
Tarumã-Açu	30	85.86%	0.2169 (± 0.0123)	0.3413 (± 0.0169)
Species	90	100.00%	0.2363 (± 0.1385)	0.3819 (± 0.1819)

N: sample size; P: polymorphism percentage; He: expected heterozygosity of Nei (1978) (assuming the Hardy-Weinberg equilibrium); I: Shannon & Weaver (1949) genetic diversity index. The standard deviations of the considered parameters are within parentheses.

Table 4. Distribution of genetic variation among and within natural *Astrocaryum acaule* populations.

Source of Variation	GL	Mean Square	Component of Variance	% of Variation
Among Populations	2	296.3778	8.9991**	25.42
Within de Populations	87	26.4034	26.4034**	74.58
Total	89	32.4703	35.4025	100.00
Fst = 0.2542				

** $P < 0.01$

The results found agree with those reported in the literature for natural populations of tropical tree species, which generally show that most of the genetic diversity is found within populations (Almeida et al., 2015; Rossi et al., 2014). Studies with other palm species have found similar results, as in a study by Gomes et al. (2011), in which the authors reported that the variability between and within *M. flexuosa* populations was 22.82 and 77.18%, respectively. Buttow et al. (2010) obtained 83.68% within *B. capitata* populations and 13.67% among them, and Cardoso et al. (2000) obtained 57.40% within and 42.60% among *E. edulis* populations.

The results found in this study suggest that a large part of the genetic variation of the species is intrapopulational. Thus, usage and conservation plans of the species must observe the genetic variability found in these populations to guarantee the preservation of their genetic resources.

The genetic variability pattern found in *A. acaule* should be considered for conservation, and a representative sample of the group of genes contained in the individuals of each group should be sampled with the sampling of individuals from both groups (1 – Tarumã-Açu and 2 – UFAM and Tupé), since the greatest diversity is within the populations. To perform genetic conservation, this sampling may lead to a high number of individuals, composing an *ex situ* collection, since the collections established in the field require permanent resources for maintenance. In this way, the best strategy would be to promote participatory *in situ* conservation in the natural occurring areas of *A. acaule*.

4. CONCLUSIONS

Physical isolation of *A. acaule* populations enables greater maintenance of their genetic identity, as anthropic action is an important agent that has been modifying the genetic diversity in natural populations of the species.

The distribution of genetic diversity in the studied *A. acaule* populations was greater within the populations than among them, being necessary to obtain representative samples of each of the genetically distinct groups for the genetic conservation of the species. Therefore, *in situ* conservation is more recommended.

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