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Genetic diversity of *Syagrus cearensis* Noblick in natural populations: implications for conservation of genetic resources

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ABSTRACT. The growing extractive activity in forest areas, the inefficient use of management practices combined with the intensification of socioeconomic activities, are the main factors in the loss of biodiversity. In order to reduce environmental impacts, genetic characterization of populations allows to infer about their real situation. Thus, the present study aimed to perform an analysis of the genetic diversity of populations of *Syagrus cearensis* using ISSR markers. Populations AQU (*Aquicultura*), MTB (*Mata do Bebo*) and MOD (*Mata Olho d´água*) were sampled, totaling 53 individuals. ISSR markers generated a total of 61 loci. The AQU population had the highest polymorphism index (71%), followed by MTB (57%) and MOD (53%). AQU showed the highest index of genetic diversity, compared to the MTB and MOD populations. There was a high and significant genetic differentiation between populations. Bayesian analysis identified the existence of two groups (K = 2). The genetic bottleneck test was significant for the AQU and MOD populations, according to the SMM model. Thus, the populations of genetic diversity index close to the averages found for tropical species with a similar life history. The presence of a genetic bottleneck was detected in populations. The AQU population presented low sharing of genotypes with the others and should be prioritized in conservation activities.

Keywords: variability; ISSR; conservation; catolé.

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Introduction

The family Arecaceae is distributed in several tropical regions. Currently, in Brazil, 39 genera and 119 species of palm trees have been identified, composing humid environments, such as the Amazon rainforest, to places with xerophilous vegetation, such as the Cerrado and Caatinga (Cappelatti & Schmitt, 2015). Several species that make up the clade of this family are subjectede to intense exploratory activities. As a result, species of the *Syagrus* genus have become a potential target for the exploration of the oil (Carvalho, Alves, & Ferreira 2015), artisanal (Elias & Santos, 2016), pharmaceutical (Sousa et al., 2017) industries, and the landscape (Pires, Melo, Oliveira, & Xavier-Santos, 2019).

The growing extractive activity in forest areas through inefficient use of management practices, coupled with the intensification of socioeconomic activities, are the leading players in the loss of biodiversity and degradation of these systems (Angelo, Pompermayer, Almeida, & Moreira, 2013). Due to such anthropic actions, thereduction impacts populations, causing an increase in self-fertilization rates and the crossing between related individuals (Dal Bem, Bittencourt, Moraes, & Sebbenn, 2015). The genetic structure in natural populations can be affected by the mode of reproduction and dispersal, population size and geographic distribution. To the detriment of this, several disorders shape genetic diversity (Davies, Cary, Landguth, Lindenmayer, & Banks, 2016). For example, effects associated with forest fragmentation, livestock production, use of fire (Smith, Bull, Gardner, & Driscoll, 2014) and disordered exploitation of non-wood forest products (Gaoue, Lemes, Ticktin, Sinsin, & Eyog-Matig, 2014) influence the adaptive process of species, which can lead to genetic changes in organisms.

Additionally, some evolutionary processes can be affected, altering the intra- and inter-population genetic diversity, such as gene flow and genetic drift. Therefore, studies on genetic diversity of populations can support strategies for adopting measures that aim at sustainable use, species conservation (Forest, Crandall, Chase, & Faith, 2015) and maintenance of genetic resources. Genetic characterization of populations provides inferences about the current condition of populations under given environmental impacts, enabling the development of management plans and implementation of the best ways to preserve genetic resources (Lima et al., 2015; Fajardo, Vieira, Felix, & Molina, 2017). Thus, the use of Inter Simple Sequence Repeat (ISSR) molecular markers has helped in a promising way, for analysis of the structure and genetic diversity in forest species (Vieira, Sousa, Silva, Fajardo, & Molina, 2015; Chagas, Freire, Pinheiro, Fajardo, & Vieira, 2019). It is a dominant molecular marker, which allows the amplification of several loci using the polymerase chain reaction (PCR), without previously requiring knowledge of the gene sequence, to generate the polymorphic models (Reddy, Sarla, & Siddiq, 2002; Turchetto-Zolet, Turchetto, Zanella, & Passaia, 2017).

In this context, the following hypotheses were tested: 1. Human pressure indices in populations of *Syagrus cearensis* imply low genetic diversity; 2. There are indications of the presence of genetic bottlenecks in populations with a higher level of anthropization; 3. The geographical distribution of *S. cearensis* populations is correlated with their levels of genetic diversity; 4. Populations with the best conservation statuses have more significant genetic similarities. Therefore, the objective of this study was to carry out an analysis of the genetic diversity of populations of *S. cearensis* using ISSR markers.

Material and methods

Target species

Syagrus cearensis is a species native to the Brazilian Northeast and can be found in the states of Rio Grande do Norte, Ceará, Pernambuco, Paraíba and Alagoas (Lorenzi, 2010). The species is predominant in phytophysiognomies with seasonal vegetation of hills and mountains, with distribution along the Atlantic coast, as well as in Caatinga. It raises agroindustrial interest because of the potential use in the food industry (almonds) and pharmaceuticals (treatment of diseases, such as erysipela), in the production of biodiesel and cosmetics, in addition to wide ornamental use (Sousa et al., 2017; Edelman & Richards, 2019; Pascoal, Oliveira, Figueiredo, & Assunção, 2020). *Syagrus cearensis* can measure up to 10 m in height. Its fruits have a typical characteristic, with endosperm formed by a central cavity, in addition to the formation of multiple stems (Noblick, 2010). When studying Syagrus oleracea, Lorenzi (2010) found that it is a hybrid of licuri (S. coronata) and catolé (*S. cearensis*). Also, they share strict relationships of phylogenetic characteristics (Meerow et al., 2009). Thus, this may indicate a similarity between pollinators and dispersers of the mentioned species. The most common floral visitors belong to the class Insecta, corresponding to Hymenoptera and Coleoptera (Manso, 2009). In the field, the arapuá bees were observed in individuals of *S. cearensis*, through phenological studies (unpublished data, personal observation).

Study area

The study was carried out with three populations, called *Aquicultura* (AQU), *Mata do Bebo* (MTB) and *Olho d´água* (MOD) comprising phytogeographic domains of Atlantic Forest and Ecotone between the Atlantic Forest and Caatinga (Figure 1). The local climate is characterized as a transition area between As 'and BSh' according to the Köppen classification, with high temperatures throughout the year and a rainy season from fall to winter (Cestaro & Soares, 2004).

A scale was developed to assess human pressure (Table 1). According to Santos and Vieira (2005), parameters include agricultural activity, erosion, ruminant animals and selective cutting, with scores from 1 to 10 determined for characteristics around and within the environments. As a result, scores varied between 1 (most conserved) and 5 (most degraded), according to the equation: $\frac{(\sum QN)}{VM}$, where Σ is the sum of the scores per item evaluated; QN is the number of levels; VM is the maximum available value resulting from the total score of the items (Santos & Vieira, 2005).

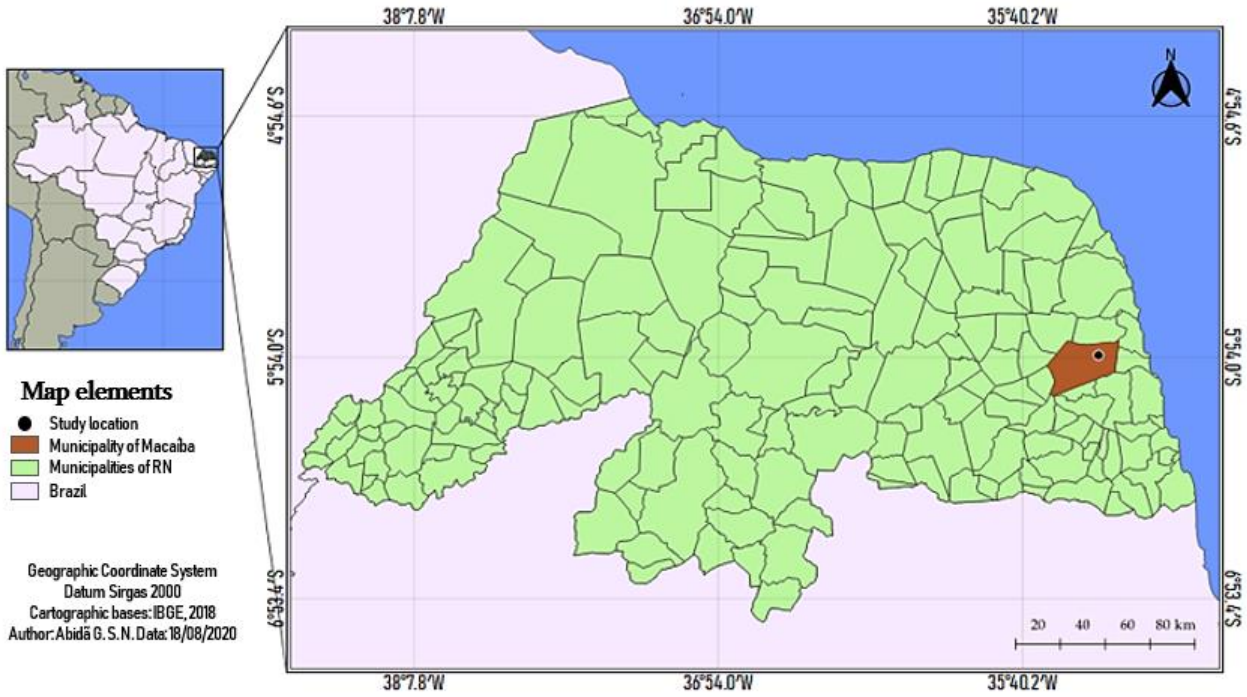

Figure 1. Geographic location of the sampling region for *Syagrus cearensis* populations.

Table 1. Codes and characterization of populations, geographical coordinates, number of samples (n) and human pressure scale for *Syagrus cearensis* populations.

Population	Lat./Long.	n	Vegetation	Soil use	Ground	Human pressure
AQU (*Aquicultura*)	5°53'16.96" S / 35°21'43.34" W	20	Open forest with palm trees	Agricultural, presence of buildings and roads	High humidity	4
MTB (*Mata do Bebo*)	5°53'30" S / 35°21'07" W	20	Lowland Semideciduous Seasonal Forest	Agricultural and trails	High humidity and organic matter content	3
MOD (*Mata Olho d'água*)	5°52'41" S / 32°22'12" W	13	Lowland Deciduous Seasonal Forest	Trails	Sloping and river courses	2

Sampling, DNA extraction and ISSR reactions

Leaf material was collected with pruning shears, previously sterilized, from 53 individuals of *S. cearensis*. Samples were stored in plastic tubes, containing 2 mL CTAB 2X (cetyltrimethylammonium bromide), and stored in a freezer at -20°C until DNA extraction.

Genomic DNA was extracted following a pre-established protocol by Doyle and Doyle (1987) with some modifications. It was used: 100 mM Tris pH 8.0; 1.4 M NaCl; 20 mM EDTA pH 8.0; 2% (w v^{-1}) CTAB; 1% (w v^{-1}) PVP-40 and 0.2% (v v^{-1}) β-mercaptoethanol preheated in a water bath at 60°C. The extracted DNA was quantified using a spectrophotometer (Epoch™, BioTek Instruments Inc Winooks, USA) and diluted in TE (10 mM TRis-HCl; EDTA mM pH 8.0) to a final concentration of 50 ng μL^{-1}.

Polymerase chain reactions (PCRs) were performed from seven ISSR primers (Table 2) (UBC primer set # 9, University of British Columbia, Vancouver, Canada). The PCR mix consisted of Buffer (10 X), BSA (1.0 mg mL^{-1}), $MgCl_2$ (50 mM), dNTP (2.5 mM), primer (2 µM), Taq polymerase (U μl^{-1}), DNA (1:50 concentration) and ultra-pure water, in a final volume of 12 µL per sample. PCRs were performed on a Biocycler automatic thermocycler. The amplification procedure was as follows, with an initial denaturation process starting at 94°C for 2 min. (1 cycle), followed by starting at 15 s at 94°C (37 cycles); after that 47°C for 30 s and 72°C for 1 min.; finally, 7 min. at 72°C and cooled to 4°C.

Products resulting from amplification were subjected to electrophoresis in 1.5% agarose gel (w v^{-1}), with TAE 1X buffer (Tris-Acetate EDTA), with a voltage of 100 V, for three hours. Gel was stained with GelRed™ (Biotium, USA) and ABF (Bromophenol Blue). The sizes of amplified fragments were estimated by comparison

with the 1,000 base pair DNA marker (ladder). After electrophoresis was completed, gels were photographed under ultraviolet light and with the aid of a photo documentation system (E-box™ VX2, Vilber Lourmat, Marne la Valle, France).

Table 2. Nucleotide sequence of the ISSR primers used, number of locis (NL) and Polymorphism Information Content (PIC).

ISSR primers	Sequence (5' – 3')	NL	PIC
M1 CAA (GA)5	CAAGAGAGAGAGA	9	0.41
UBC 808 (AG)8-C	AGAGAGAGAGAGAGAGC	8	0.46
UBC 840 (GA)8-YT	GAGAGAGAGAGAGAGAYT	6	0.33
UBC 852 (TG)8-RC	TGTGTGTGTGTGTGTGRC	10	0.47
UBC 857 (AC)8-YG	ACACACACACACACACYG	10	0.42
UBC 860 (TG)8-RA	TGTGTGTGTGTGTGTGRA	6	0.48
UBC 880 (GGAGA)3	GGAGAGGAGAGGAGA	12	0.49
Average		9	0.44

R = purine (A or G) and Y = pyrimidine (C or T)

Statistical analysis

Genetical diversity

ISSR fragments were encoded in binary matrices, based on the absence (0) and presence (1). The PopGene 1.32 sofwtware (Yeh, Boyle, & Yang, 1999) was used to estimate genetic diversity, obtaining the percentage of polymorphic loci (% P), number of observed alleles (Na), the effective number of alleles (Ne), Nei's genetic diversity (h) and Shannon index (I). The diversity indices I and h were tested by analysis of variance (ANOVA), using the statistical software BioEstat v.5.0 (Ayres, Aires, Ayres, & Santos, 2007).

PIC Value

Estimation of polymorphism information content (PIC), which evaluates the discriminatory power of a loci was obtained by the equation: $PICj = 1 - \sum_j Pij^2$, where P_{ij} is the frequency of alleles 'j' in marker 'I' (Anderson, Churchill, Autrique, Tanksley, & Sorrells, 1993). Values obtained at the end of the calculation classified the markers in three parameters, the value being highly informative (PIC > 0.50), moderately informative (0.50 > PIC > 0.25) and little informative (PIC < 0.25) (Botstein, White, Skolnick, & Davis, 1980).

Genetic structure

The analysis of molecular variance (AMOVA) was generated using the Arlequin 3.5 software (Excoffier & Licher, 2010). A dendrogram was constructed based on the genetic identity of Nei (1978) between populations, using the hierarchical clustering method and the unweighted pair group method with arithmetic mean (UPGMA) in the NTSYS software (Rohlf, 1993).

To determine the number of genetic groups (K) in the population, Bayesian analysis was used in software Structure v.2.3.4 (Pritchard, Stephens, & Donnelly, 2000). The *K* value varied from 1 to 3, and obtained using the ancestral mixture model and the correlated allele frequency model. Ten independent interactions were used for each K group, and 250.000 Monte Carlo permutations in the Monte Carlo and Markov chain (MCMC), with 500,000 repetitions. The final value of K (number of assumed populations) was verified in the Structure Harvest software (Evanno, Regnaut, & Goudet, 2005; Earl, 2012). Evanno et al. (2005) denote that variation in K values is from 1 to (P+3) since P is the number of real populations.

Genetic bottleneck

The occurrence of reductions in the effective population size was evaluated using software Bottleneck v.1.2.02 (Piry, Luikart, Cornuet, 1999). This analysis is based on the identification of significant deviations from the balance between mutation and genetic drift (Nei & Roychoudhury, 1974). To identify genetic bottlenecks, the infinite allele (IAM) and the mutation step (SMM) models were used according to Kimura and Crow (1964), and Kimura and Otha (1978). The significance test was checked by means of the sign test (α = 0.05), to assess the effect of allelic reduction.

Results and discussion

ISSR polymorphism and PIC value

Analyses with the eight ISSR markers generated a total of 61 loci, which ranged from 6 to 12 per primer, with an average value of 9 (Table 2). The PIC value ranged from 0.33 to 0.49, with an average value of 0.44,

and thus classified as moderately informative (Botstein et al., 1980). The UBC 880 primer revealed the highest number of polymorphic locis (12) and PIC value (0.49), while UBC 840 was the least informative primer, presenting only six loci, with a PIC value equivalent to 0.33.

Obtaining an ideal number of loci, associated with minimizing the number of primers, are essential as optimize work and reduce activity costs. Through this, studies by Laxmikanta and Pranta (2010) and Oliveira, Venturini, Rossi, and Hastenreiter (2010) used a lower number of primers concerning the number used in the present study.

Genetical diversity

Of the 61 loci observed in the study, 59 (96%) were polymorphic, since the AQU population had the highest polymorphism index (71%), followed by MTB (57%) and MOD (53%) (Table 3). Thus, the technique based on ISSR markers proved to be an efficient method in the evaluation of genetic diversity in populations of *S. cearensis*. Vieira et al. (2015) obtained a high level of polymorphism (93%) when analyzed *Copernicia prunifera* populations using ISSR markers.

Table 3. Averages of genetic diversity for populations of *Syagrus cearensis*.

Populations	L / %P	Na	Ne	h	I
AQU	46 / 71% a	1.70 ± 0.10 a	1.45 ± 0.08 a	0.26 ± 0.04 a	0.38 ± 0.06 a
MTB	35 / 57% a	1.57 ± 0.11 a	1.39 ± 0.08 a	0.22 ± 0.04 a	0.33 ± 0.06 a
MOD	32 / 53% a	1.52 ± 0.13 a	1.41 ± 0.11 a	0.22 ± 0.06 a	0.32 ± 0.08 a
Average	38 / 60%	1.60	1.42	0.23	0.34
Total	59 / 96%	1.97 ± 0.02	1.58 ± 0.04	0.34 ± 0.02	0.51 ± 0.02

Polymorphic locis, percentage of polymorphic locis (%), Number of observed alleles (Na), Effective number of alleles (Ne), Nei's index (h), Shannon index (I) and standard error (±). Means followed by different letters in the same column, h and I, are significantly different by Kruskal-Wallis test at 5% probability.

The average number of alleles observed was 1.60, and the number of effective alleles was 1.42. Averages for the Nei's index and the Shannon index were 0.23 and 0.34, respectively. The three populations did not statistically differ for the indices of genetic diversity (h and I), according to ANOVA (Kruskal-Wallis), presenting expected means for plant species with a life history similar to *S. cearensis* (endemic distribution and barochoric dispersion), as reviewed by Nybom (2004).

Numerically, the AQU population showed the highest genetic diversity index, compared to the MTB and MOD populations. Statistically, their variability indices did not differ, suggesting that the populations presented a similar gene flow. Despite not being statistically evidenced, agricultural activities, roads, trails, erosive processes, should be taken into consideration to maintain the genetic diversity, since they act directly in the perpetuation of ecological cycles of the species. The constant fragmentary events resulted in a reduced genetic diversity, as reported by Fajardo, Costa, Chagas, and Vieira (2018).

Genetic structure of the population

The results of AMOVA revealed that most of the genetic diversity is found at the intra-population level (53.7%) than at the inter-population level (46.3%). There was a significant and high genetic differentiation between populations (Table 4).

Table 4. Analysis of molecular variance (AMOVA) between and within populations

Source of variation	df	SS	Variation component	Percentage of variation	p
Between populations	2	131.83	4.55	46.30%	< 0.00*
Within populations	50	206.39	4.12	53.70%	
Total	52	338.22	7.68	100%	
Genetic differentiation Fst	0.46				

df: Degrees of freedom, SS: Sum of squares of the deviations, Fst: Genetic differentiation, p: probability.

The level of genetic differentiation for populations is determined by the balance of events occurring in gene flow and processes that promote differentiation, such as local adaptations (environmental factors) and effects of genetic drift (Bradburd, Ralph, & Coop, 2013). Thus, gene flow is one of the characteristics of transmission of genetic information between populations. However, if it hindered, there will be a tendency

for more significant genetic differentiation, which will lead to independent development of populations (Slatkin, 1987).

From the dendrogram (Figure 2), it was possible to identify more significant genetic similarities between MTB and MOD populations, and the AQU population was the most divergent. The constant human activities around the AQU population may have caused this distancing, since they act as barriers for dispersers and make pollination systems fail, thus resulting in population isolation and consequent genetic dissimilarity.

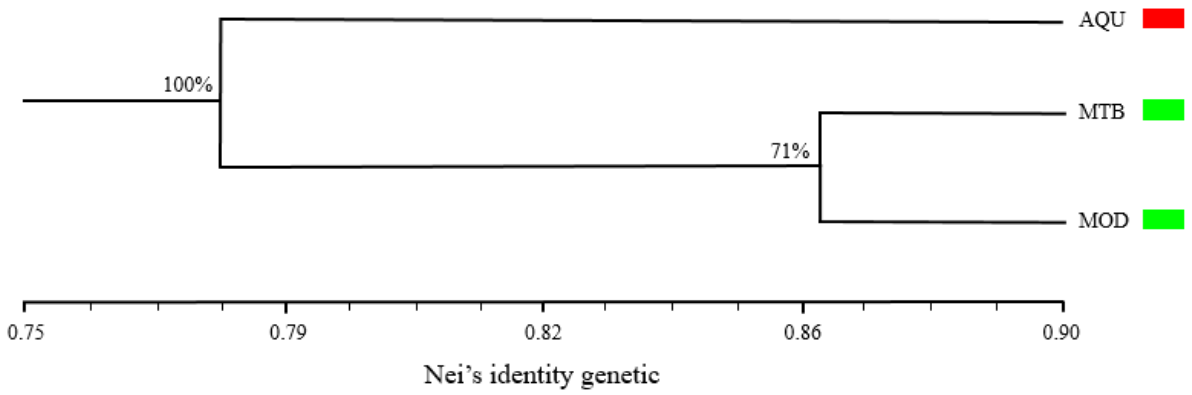

Figure 2. UPGMA dendrogram based on Nei's genetic identity among *Syagrus cearensis* populations.

Bayesian analysis

The Bayesian analysis identified two distinct groups (K=2) (Figure 3). The two groups indicate that genotypes of MTB and MOD populations were more similar (Figure 4). Both populations probably exchanged alleles throughout history and thus established homologous genes in areas with higher conservation rates (lower human pressure, see Table 1). The diverse floristic composition, species richness, forest size, and the environment are factors that maintain ecological services in populations (Corsini, Scolforo, Oliveira, Mello, & Machado, 2014) Despite the geographical proximity of populations, there is a high genetic differentiation, since factors such as human pressure, which include the presence of monocrops, civil construction and roads, mark the scenario that represents the AQU population.

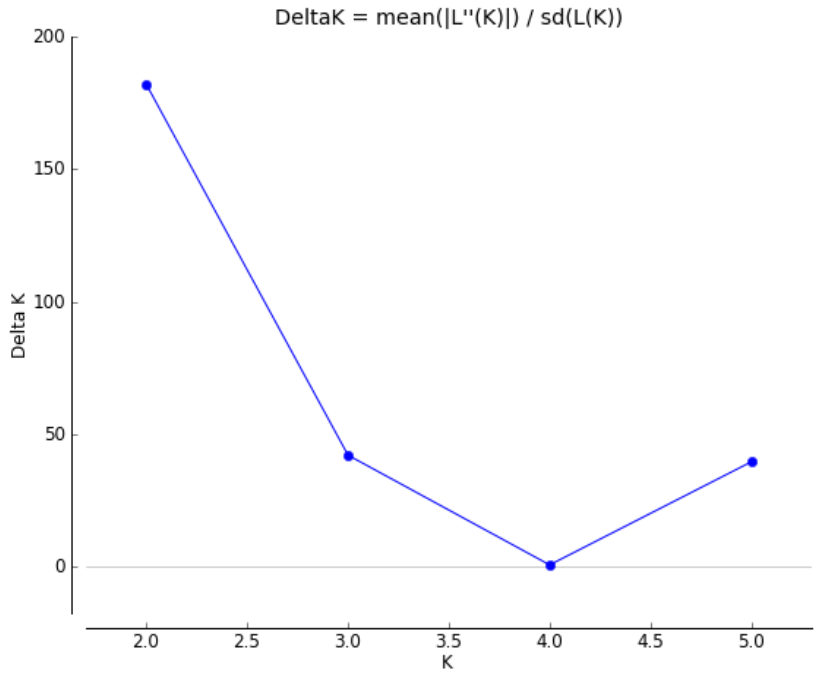

Figure 3. Bayesian analysis of *Syagrus cearensis* with genotype proportion among the three sampled populations presenting K=2. Populations are bounded by the darkened vertical bar. Different colors indicate the different groups found.

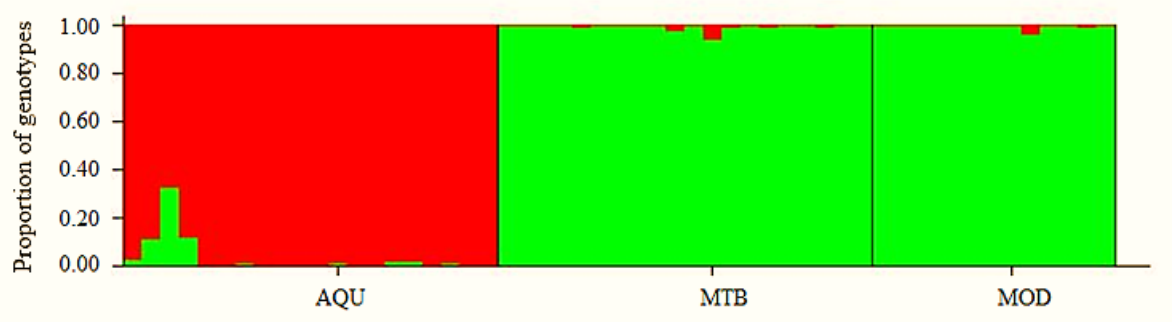

Figure 4. Bayesian analysis indicating K = 2, representing the most likely number of groups formed.

Genetic bottleneck

The genetic bottleneck test was significant for populations AQU and MOD, according to the SMM model, indicating that the populations has suffered a reduction in its population genetic size, over time. There was no significance of the IAM model for populations (Table 5). In line with the studies carried out in similar areas by Chagas et al. (2019), human disturbance can interfere with genetic variation of the population, reducing its population size. Sirakov et al. (2019) analyzed hierarchical stages of *Attalea speciosa* in an anthropized open area. They found that monoculture activities, livestock farming impact and interspecific competition between seedlings limited the number of adult individuals and, consequently, confirmed the existence of a genetic bottleneck.

An aspect associated with population reduction is the epigenetic effect by which, due to environmental oscillations, species undergo adaptive processes, which cause changes in their genetic expression (Vieira, 2017). As a corrective measure, aimed at the maintenance of genetic characteristics (both ancestors and most related organisms), studies focused on ecological activities of species are essential, since they provide the development of strategies to maintain the reproductive cycles in their natural environment.

Table 5. Tests of balance between mutation and genetic drift for populations of *Syagrus cearensis* using IAM and SMM models.

Population	IAM			SMM		
	n	He	P	n	He	P
AQU	26.35	39/22	0.15	32.12	40/21	0.00*
MTB	28.37	28/33	0.14	31.52	31/30	0.39
MOD	28.24	33/28	0.52	35.10	33/28	0.04*

n = expected number of locis with excess heterozygosity for the respective model; He = number of locis with excess heterozygosity; P = probability; * = significant at 5% probability of error.

Conclusion

The sampled populations showed genetic diversity rates close to the averages found for tropical species with a similar life history. Also, genetic bottlenecks were detected in the populations, warning that fragmented landscapes require urgent actions to conserve the genetic diversity remaining there. The AQU population indicated low genetic sharing with the others. This should be prioritized in conservation activities since the intensification of human pressure in the surroundings makes their ecological system more vulnerable.

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