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Accessing the genetic diversity of natural populations of *Acrocomia aculeata* (Arecaceae) from the Cerrado biome through microsatellites

Acessando a diversidade genética de populações naturais de Acrocomia aculeata *(Arecaceae) através de microssatélites*

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ABSTRACT

Objctives: *Acrocomia aculeata* is a tropical arborescent species distributed throughout tropical and subtropical Americas, with a significant occurrence of natural populations in the Cerrado biome. Its broad distribution across various landscapes is supported by a mixed reproductive system, diverse pollination strategies, and resilience to environmental stressors. Despite its resilience in fragmented habitats, anthropogenic habitat fragmentation generally harms populations, affecting gene flow and genetic diversity. This study aimed to assess genetic diversity and structure of natural populations of *A. aculeata* from northern Minas Gerais using microsatellites. **Methods:** The five natural populations were Espinosa, Mirabela, Claro dos Poções, Grão Mogol, and Itacambira. **Results:** The results revealed that four SSR loci amplified 46 different alleles, totaling 1352 alleles. The

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loci showed significant deviations from Hardy-Weinberg Equilibrium, with a mean *f* of 0.288. Among the populations analyzed, ITA was the most genetically diverse, while ESP was the least diverse. As subpopulations, they displayed high genetic structure, with a mean F_{ST} of 0.329 and F_{IT} of 0.484. **Conclusion:** The study suggests the natural population of Macaw palm in northern Minas Gerais that can be used for germplasm banks, and the one that requires human intervention to restore genetic diversity.

Keywords: Macaw palm; SSR; Cerrado; AMOVA.

RESUMO

Objetivos: *Acrocomia aculeata* é uma espécie arbórea tropical distribuída por toda a América tropical e subtropical, com ocorrência significativa no bioma Cerrado. Sua ampla distribuição é decorrente do sistema reprodutivo misto, estratégias de polinização e resiliência a estressores ambientais. Apesar de sua resiliência, a fragmentação antropogênica dos habitats geralmente prejudica as populações, comprometendo o fluxo gênico e a diversidade genética. Este estudo avaliou a diversidade genética e a estrutura de populações naturais de *A. aculeata* do norte de Minas Gerais usando microssatélites. **Métodos:** As cinco populações naturais foram Espinosa, Mirabela, Claro dos Poções, Grão Mogol e Itacambira. **Resultados:** Foram identificados 46 alelos diferentes, totalizando 1352 alelos. Os quatro loci analisados apresentaram desvios significativos do Equilíbrio de Hardy-Weinberg, com um valor médio de *f* de 0,288. Entre as populações, ITA foi a mais geneticamente diversa, e ESP foi a menos diversa. Como subpopulações, apresentaram alta estrutura genética, com um valor médio de F_{ST} de 0,329 e F_{IT} de 0,484. **Conclusão:** O estudo sugere a população natural de Macaúba do norte de Minas Gerais para confecção de bancos de germoplasma, e destaca aquela que requer intervenção humana para restaurar a diversidade genética.

Palavras-chave: Macaúba; SSR; Cerrado; AMOVA.

INTRODUCTION

Macaw, *Acrocomia aculeata*, is a tropical arborescent palm [1,2], diploid (2n = 30), with a mixed reproductive system characterized by both self-fertilization and cross-fertilization [3,4]. The inflorescences are unisexual, with male flowers distributed at the top of the cluster and female flowers at the base [2]. These androgynous inflorescences with protogyny are pollinated by wind and Coleoptera, Nitidulidae, and Scarabaeidae insects [5,6]. The mixed reproductive system, pollination strategies, and morphology combined with phenotypic plasticity of the flowers, contributes to the reproductive success of the macaw palm and its wide geographic

distribution. Natural populations are found throughout tropical and subtropical Americas, with a significant occurrence in the Cerrado biome [7,8]

Among the palm species found in arid and semi-arid regions, the Macaw palm stands out as the second-largest producer of oleaginous fruits, following *Elaeis guineensis*. The oil produced in large quantities, present in the fruit and seed, has applications in the food and cosmetic industries. It also has diverse uses by indigenous peoples, such as using its leaves for animal feed and its fruits for producing charcoal, flour, and other desired co-products [9,10]. Holding significant cultural and economic importance and potential for exploitation as an agricultural crop [11,12].

While domestication and genetic improvement programs for this species are still in early stages, it shows emerging agro-industrial potential for biofuel production in dry tropical environments [1,5,13]. As a result of the Macaw palm's remarkable tolerance to environmental stressors, including high irradiance, elevated temperatures, fire, and drought. This tolerance enables it to colonize areas characterized by high fragmentation due to anthropogenic actions, as well as open vegetation and intense solar exposure, such as active or degraded pastures [1,12,14].

Although the macaw palm demonstrates resilience to environmental and anthropogenic stresses [1,9], these factors generally have a detrimental impact on populations [15]. Considering the ongoing climate change, along with the rapid expansion of human populations, stress conditions are expected to increase, affecting not only threatened species. Given that the importance of the macaw palm lies not only in its ecological roles but also in its potential economic value, it is crucial to conserve the genetic diversity of this species.

Therefore, the objective of this study was to analyze the genetic diversity of five natural populations of *A. aculeata* from northern Minas Gerais using microsatellites (SSR). The aim was to identify the most genetically diverse sites for collecting genetic resources to establish a germplasm collection. Additionally, the study provides data to support the implementation of assisted management strategies for populations at greater risk of losing valuable genetic information.

MATERIAL AND METHODS

Sampling and genomic DNA extraction

Sampling was conducted randomly across five natural populations of *A. aculeata* in the Northern region of Minas Gerais State, demonstrated in Figure 1, encompassing the municipalities of Espinosa (ESP), Mirabela (MIR), Claro dos Poções (CLP), Grão Mogol (GRM), and Itacambira (ITA). Populations were named according to the municipality where they were collected. Each individual was tagged and identified using a georeferencing system, and were assigned according to the municipality in which they were collected.

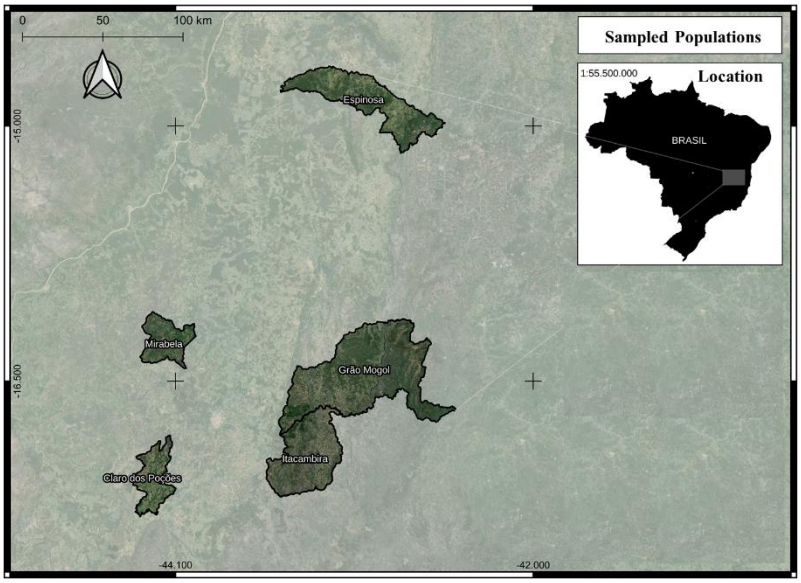

Figure 1. Municipalities where *A. aculeata* populations were sampled for this study.

Chart produced in QGis 3.34.4[16]; Spatial reference: Sirgas 2000; Cartographic base: Malha municipal[17]. Leaf samples were collected from 30 adult individuals within each of the five populations, totaling 150 individuals, and stored on silica at –20 °C until genomic DNA (gDNA) extraction [18,19]. The gDNA was extracted following the protocol developed by Doyle (1991) with modifications defined by Faleiro et al (2004) and its integrity was determined by a 1% agarose gel electrophoresis stained with ethidium bromide.

SSR and genotyping

From the seven tested SSR developed for *A. aculeata* [4], four pairs of oligonucleotides featuring a dinucleotide repeat motif, Aacu07 $((GA)_{13})$ (BV703504), Aacu10 $((AG)_{16})$ (BV703505), Aacu12 $((TC)_{20})$ (BV703506) and Aacu26 $((AC)_{13} / (AG)_{14})$ (BV703508) were

polymorphic for the genetic analyses. PCR reactions were performed with 1 X buffer (10 mM Tris-HCl, pH 8.4, 50 mM KCl), 1.34 μM of each oligonucleotide, 250 μM of each dNTP, 1 U of Taq DNA polymerase (Invitrogen), 0.25 mg of Bovine Serum Albumin, 1 mM of $MgCl_2$, and 3 ng of DNA.

Amplifications were executed using Veriti™ 96 wells thermal cycler (Applied Biosystems) following the conditions: 94°C for 1 min, 35 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 30 min. Amplicon weight was determined by comparison with known-size DNA Liz 600 (Applied Biosystems) on an ABI 3500 automated DNA sequencer (Applied Biosystems). Chromatograms were analyzed using GeneMapper® v. 4.1 software (Applied Biosystems).

Data Analysis

The data integrity was assessed using Micro-checker 2.2.3 software [20]. To estimate the genetic diversity of the codominant data, population genetic parameters were calculated using GenAlEx 6.5 [21] after correcting for null alleles using FreeNa software with the Excluding Null Alleles (ENA) correction [22,23]. The parameters included were Expected Heterozygosity (H_E), Observed Heterozygosity (H_O) under Hardy-Weinberg Equilibrium (HWE), Number of Effective Alleles (Ae), Number of Private Alleles (Ap), and Mean Number of Alleles (A) per Population with *f*requency >=5%. Allelic richness (Ar) was analyzed using Fstat 2.9.4[24]. Estimation of F_{ST} and F_{IT} [25,26] as well as deviation from HWE, were tested using Fstat 2.9.4 [24] with Bonferroni correction [27]. A significance level of 5% (P value = 0.05) was employed for statistical tests. AMOVA (Analysis of Molecular Variance) was performed in GenAlEx 6.5 [21] to understand how genetic diversity was structured among populations, with an allelic distance matrix for pairwise F_{ST} analysis. The two genetically closest population clusters were assessed and calculated using Fstat 2.9.4 [24].

RESULTS

Genetic Diversity and Hardy-Weinberg Equilibrium

No allele drop-out, null alleles, or stuttering were detected among the codominant genotypes using Micro-checker 2.2.3 [20]. From the seven tested SSR, four were polymorphic

and amplified a total of 46 different alleles, with an average number of nine per polymorphic loci, ranging from 99 to 276 base pairs (bp). The markers with the highest number of sampled alleles were Aacu10 (11) and Aacu12 (10). In total, 1352 alleles were sampled and the distribution of them across the natural populations of *A. aculeata* is showed in Table 1.

Table 1. Characterization of microsatellite loci used in the study for *A. aculeata*.

Locus	Allele size range (bp)	Registered Alleles	Mean H_E	Mean H_O	F_{ST}
Aacu07	136 - 152	9	0.505	0.555	0.322
Aacu10	146 - 164	11	0.567	0.327	0.238
Aacu12	99 - 192	10	0.484	0.184	0.314
Aacu26	242 - 276	9	0.428	0.385	0.416

Expected heterozygosity (H_E);

Observed heterozygosity (H_O);

Wright's Fixation index (F_{ST}) to indicate genetic differentiation between SSR.

Aiming to estimate the genetic diversity based on microsatellite data in each population over a short time scale [15,28], genetic descriptive parameters were analyzed among the sampled populations and are presented in Table 2.

Table 2. Genetic descriptive parameters assessed with microsatellite (SSR) in natural populations of *A. aculeata*.

Population	Parameters	Locus				Mean
		Aacu07	Aacu10	Aacu12	Aacu26	
ESP	H_O	0.08	0.3	0.07	0.33	0.2 ± 0.07
	H_E	0.08	0.3	0.52	0.35	0.4 ± 0.11
	HWE	0.043	24.07***	38.72***	0.02	62.85***
	f	-0.03	0.49	0.86	0.04	0.34 ± 0.21
	A	-	-	-		3.25 ± 0.48
	Ae	1.08	2.42	2.10	1.53	1.78 ± 0.30

	Ar	1.32	2.8	2.13	1.9	2.04
	Ap	1	1	-	1	0.75
MIR	H_O	0.48	0.64	-	0.52	0.41 ± 0.14
	H_E	0.48	0.82	0.24	0.61	0.54 ± 0.12
	HWE	0.12	35.70	60***	9.45	105.27***
	f	-0.01	0.22	1.0	0.14	0.34 ± 0.23
	A	-	-	-	-	4.75 ± 1.18
	Ae	1.91	5.68	1.31	2.54	2.86 ± 0.97
	Ar	2.33	4.75	2.0	2.96	3.01
	Ap	1	2	-	2	1.25
CLP	H_O	0.64	0.20	0.28	0.12	0.31 ± 0.12
	H_E	0.55	0.58	0.58	0.22	0.48 ± 0.09
	HWE	10.77	26.11***	99.58***	75.05***	211.51***
	f	-0.17	0.65	0.52	0.46	0.37 ± 0.18
	A	-	-	-	-	5.25 ± 0.95
	Ae	2.22	2.35	2.37	1.28	2.06 ± 0.26
	Ar	2.9	2.68	4.42	1.91	2.98
	Ap	-	1	2	-	0.75
GRM	H_O	0.78	0.06	0.24	0.54	0.40 ± 0.16
	H_E	0.66	0.42	0.43	0.50	0.50 ± 0.06
	HWE	7.53	13.60***	58.56***	1.61	81.29***
	f	-0.17	0.87	0.43	-0.07	0.27 ± 0.24
	A	-	-	-	-	4.25 ± 0.85
	Ae	2.96	1.74	1.74	2.01	2.11 ± 0.29
	Ar	3.28	1.96	2.35	2.48	2.52
	Ap	-	-	-	1	0.25
ITA	H_O	0.79	0.44	0.33	0.41	0.49 ± 0.10
	H_E	0.76	0.43	0.65	0.47	0.57 ± 0.08

HWE	7.20	3.75	47.99***	30.07***	89.01***
f	-0.05	-0.03	0.49	0.11	0.13 ± 0.12
A	-	-	-	-	4.75 ± 0.48
Ae	4.11	1.74	2.87	1.87	2.65 ± 0.55
Ar	3.93	2.57	3.15	2.65	3.07
Ap	1	1	1	-	0.75

Observed heterozygosity (H_O); Expected heterozygosity (H_E); X^2 value of the population in relation to Hardy-Weinberg Equilibrium (HWE); Inbreeding Coefficient (*f*); Mean Number of Alleles per population with a frequency >=5% (A); Number of Effective Alleles (Ae); Allelic Richness (Ar); Number of Private Alleles (Ap); Espinosa (ESP); Mirabela (MIR); Claro dos Poções (CLP); Grão Mogol (GRM); Itacambira (ITA). Significant deviation from HWE (* P<0.05, ** P<0.01, *** P<0.001).

The five natural populations of *A. aculeata* analyzed in this work showed significant deviations from HWE (P<0.001), with a mean H_O of 0.36 ± 0.05 and mean H_E of 0.5 ± 0.04, and an *f* of 0.288 ± 0.08. Regarding the analysis of each population, ITA had the highest number of heterozygous individuals (mean H_O) (0.49 ± 0.10) and the lowest *f* (0.13 ± 0.12), while ESP had the lowest heterozygosity (0.2 ± 0.07). MIR showed the highest number of Ae (2.86 ± 0.97) and ITA had the highest Ar (3.07). ESP had the lowest values for both Ae (1.78 ± 0.30) and Ar (2.04). The presented data lead to the conclusion that ITA is the most genetically diverse population, and ESP is the least diverse.

Genetic Structure

Significant genetic structure and differentiation were observed among geographical populations and within individuals, characterized by a mean F_{ST} (Coefficient of inbreeding by population subdivision between populations) of 0.329. Additionally, these populations do not function as subpopulations within a metapopulation, as indicated by a F_{IT} (Coefficient of inbreeding by population subdivision and reproductive system) of 0.484, data presented on Table 3. AMOVA analyses were performed to identify populations with greater genetic similarity, and the genetically closest populations were grouped into clusters, namely

GRM+ITA (1) and MIR+CLP (2). Group 1 exhibited a relatedness value of 0.228 and group 2, a relatedness value of 0.222 (Table 3).

Table 3. Inbreeding Coefficient (*f*), Genetic Structure (F_{ST}) and relatedness between analyzed populations and groups.

Identification	*f*	F_{ST}	F_{IT}	Relatedness
All populations	0.231	0.329	0.484	0.444
Groups				
1	0.163	0.147	-	0.228
2	0.228	0.149	-	0.222

The genetic similarity observed in groups 1 and 2 suggests that these populations may have belonged to two distinct metapopulations. Probably as a consequence of habitat fragmentation and environmental changes, subpopulations within groups 1 and 2 have likely reduced gene flow between them, leading to population structure through genetic drift. This scenario is supported by the geographical proximity of these populations. In contrast, the genetic and geographic distance of ESP demonstrates its isolation from other populations, with absent or severely limited gene flow between subpopulations.

DISCUSSION

Considering the relevance of the selected parameters in evaluating the loss of genetic diversity, and that both heterozygosity and allelic richness help maintain high genetic diversity and can increase the effective population size in the long term, thereby minimizing the effects of selective pressures [15,29,30]. Among all the studied populations, Itacambira stands out with the most significant genetic characteristics for seed collection to form a germplasm bank for the species, as it is the most genetically diverse population.

Taking into account that the reduction in heterozygosity negatively influences the fitness of each individual and can, in the long term, reduce the genetic diversity of the species and its adaptive potential in response to environmental changes [30], Espinosa is the population with the greatest need for human intervention. It has demonstrated the lowest values of allelic

richness and effective alleles, along with lower heterozygosity and higher inbreeding compared to the other populations. In this concerning scenario, managing the natural population is essential. If human intervention—such as the introduction of genetically diverse individuals—does not occur, the population could cease to exist in the long term.

The deficiency of heterozygotes and high inbreeding observed in the analyzed populations is likely attributed to self-crossing. Although Macaw has adapted mechanisms to persist in the environment, such as mixed reproductive system and the ability to colonize degraded areas [1,12], the results suggest an inability of the studied natural populations to regenerate through the recruitment of new individuals.

Among the possible causes for the difficulty in the regeneration, we can emphasize seed predation by insects (e.g., *Pachymerus* sp.), which reduces the recruitment of young individuals. Additionally, the absence of dispersers, preventing seeds from finding suitable sites for development[31,32]. And a non-random pollination process caused by restricted dispersion due to asynchronous flowering, leading pollinators to visit nearby flowers because of the reduced number of reproductive individuals[33]. These scenarios, whether isolated or combined, may be responsible for the results found for genetic diversity and also for the significant genetic structure and differentiation in the populations analyzed in this study.

We also emphasize that one of the most likely causes of significant genetic structure and differentiation is the increase of forest fragmentation caused by human activities. This fragmentation leads to a decrease or absence of the environmental services provided by seed-dispersing and pollinating species, resulting in a loss of structural connectivity and habitat for plant species [15]. Consequently, there is a reduction in gene flow, a decrease in individual fitness, and a potential loss of genetic diversity. Given the socio-cultural and economic importance attributed to *A. aculeata*, implementing a management system to reduce the genetic structuring of these populations is more than justified.

CONCLUSIONS

The findings from this study highlight key conclusions. Firstly, natural populations of Macaw in Northern Minas Gerais require conservation efforts, and some are suitable candidates for seed collection to establish a germplasm bank. Secondly, there is a clear need

for restoration efforts in these areas through population management to enhance genetic diversity. These conservation measures are crucial for reducing the populations' susceptibility to environmental changes resulting from human activities.

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