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Genetic Biology

Genetic diversity of native populations of Roncador (*mouriri guianensis* aubl.) from the Amazon and Mato Grosso Pantanal

Diversidade genética de populações nativas de Roncador (*mouriri guianensis* aubl.) da Amazônia e Pantanal Mato-Grossense

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ABSTRACT

Due to the significant ecological, economic, and medicinal potential of roncador (*Mouriri guianensis* Aubl.), it is essential to understand the genetic diversity and distribution of this species, especially as no study has been conducted on its genetic composition. Therefore, the study aimed to evaluate the genetic diversity and structure in natural populations of roncador using ISSR molecular markers. Genetic analyses were performed on roncador populations occurring naturally in the state of Mato Grosso, Brazil, in four locations: Rio Cuiabá (Barão do Melgaço), Rio São Lourenço (Poconé), and Rio Jauru (Cáceres) in the Pantanal Biome, and Rio Sepotuba (Cáceres) in the Amazon. For molecular characterization, eight ISSR primers were selected, resulting in 127 amplified fragments (98% polymorphism). AMOVA showed greater genetic diversity among populations (52%). The highest diversity indices were found for the Rio Jauru population (H=0.28; I=0.41, and P%=71.65). UPGMA clustering analysis formed five distinct genetic groups based on collection locations, while Bayesian analysis formed two groups according to biomes. Principal Coordinates Analysis corresponded with UPGMA and Bayesian clustering, isolating the Rio Sepotuba population (Amazon). ISSR molecular markers revealed genetic diversity among roncador individuals, highlighting population structuring among different collection locations and biomes, suggesting the importance of conserving native populations of the Amazon and Mato Grosso Pantanal.

Keywords: Amazon Biome; Pantanal Biome; Plant conservation; Population genetics; ISSR molecular markers

RESUMO

Em razão do grande potencial ecológico, econômico e medicinal do roncador (*Mouriri guianensis* Aubl.), é fundamental compreender a diversidade genética e distribuição dessa espécie, especialmente, por não apresentar nenhum estudo sobre sua composição gênica. Portanto, o estudo visou avaliar a diversidade e estrutura genética em populações naturais de roncador através de marcadores moleculares ISSR. As análises genéticas foram realizadas em populações de roncador com ocorrência natural no estado de Mato Grosso, Brasil, em quatro localidades: Rio Cuiabá (Barão do Melgaço), Rio São Lourenço (Poconé) e Rio Jauru (Cáceres) do Bioma Pantanal e Rio Sepotuba (Cáceres) na Amazônia. Para a caracterização molecular, oito *primers* ISSR foram selecionados, resultando em 127 fragmentos amplificados (98% de polimorfismo). A AMOVA apresentou maior diversidade genética entre as populações (52%). Os maiores índices de diversidade foram encontrados para a população Rio Jauru (H=0,28; I=0,41 e P%=71,65). A análise de agrupamento UPGMA formou cinco grupos genéticos distintos com base nas localidades de coleta, enquanto a análise bayesiana formou dois grupos de acordo com os biomas. A PCOA, correspondeu com o agrupamento UPGMA e Bayesiano, isolando a população Rio Sepotuba (Amazônia). Os marcadores moleculares ISSR revelaram diversidade genética entre os indivíduos de roncador, evidenciando estruturação populacional entre diferentes locais de coleta e bioma, sugerindo a importância da conservação das populações nativas da Amazônia e Pantanal Matogrossense.

Palavras-chave: Bioma Amazônico; Bioma Pantanal; Conservação vegetal; Genética de populações; Marcadores moleculares ISSR

1 INTRODUCTION

Conservation of genetic resources of plant species is of great importance, as research has increasingly focused on quantifying genetic diversity and understanding its magnitude, nature, and distribution among and within populations (Perez, 2008). It is important to understand how genetic diversity is distributed in populations of species of interest (Garcia et al., 2020; Wade et al., 2020). Additionally, such analyses enable the determination of population structure and genetic variability (Ma, Khayatnezhad & Minaeifar, 2021).

Based on this information, strategies for conservation, management for sustainable use of native biodiversity, and genetic improvement can be developed (Motahari et al., 2020; Leal et al., 2021; Pádua et al., 2021). Conservation becomes a fundamental tool even for populations with high rates of genetic variability that are not listed as endangered species (Freitas et al., 2006). Thus, preserving high levels of genetic diversity maintains the evolutionary potential of a species or population (Mendonça, Mendonça & Pagotto, 2023).

One way to assess the genetic diversity and structure of native populations, as well as the relationship between genotypes, is using molecular markers (Silva Júnior et al., 2021; Orasmo et al., 2022). Molecular markers offer advantages such as obtaining an unlimited number of polymorphisms and information in a single amplification, and the possibility of analysis from small amounts of material at any stage of plant development (Gelotar et al., 2019).

Among molecular markers are Inter Simple Sequence Repeats (ISSRs), which amplify a DNA segment between two microsatellites (SSR sequences) identical in opposite directions (Gebrehiwet et al., 2019). These DNA regions are amplified by PCR (Polymerase Chain Reaction) (Reddy, Sarla & Siddiq, 2012). ISSRs are characterized by simple handling, efficiency in terms of time and cost, and are not influenced by the environment (Giachino, 2020; Abirami et al., 2021). Furthermore, they do not require prior genetic information (Morillo, Mora & Morillo, 2022).

Mouriri guianensis Aubl. (Melastomataceae), commonly known as roncador (MUNIZ et al., 2020), it occurs in Brazil, the Guianas, Trindade and Tobago, Suriname, and Venezuela, occupying a wide range of vegetation types. In Brazil, it is found across the phytogeographic domains of the Amazon, Caatinga, Cerrado, Atlantic Forest, and Pantanal (Voltz; Gondenberg, 2025). According to Pott and Pott (1994), the species grows in seasonally flooded savannas, gallery forests, and secondary forests, with a geographic distribution range extending from Venezuela and the Guianas to Rio de Janeiro, and from the state of Mato Grosso to Bolivia.

Is a fruit-bearing tree whose sweet fruits are sources of natural food (Mors, Rizzini & Pereira, 2000; Cruz & Kaplan, 2004). Besides being consumed by birds and especially by fish, they have an important ecological relationship through ichthyochory, thus favoring the maintenance of economic activities by producing fruits that can be sold by local residents as bait for professional fishing and fishing tourism (Muniz et al., 2020).

Roncador is considered a medicinal plant, as its leaves and bark are used in folk medicine for treating ulcers, vaginal infections, and postpartum baths, as well as for its wood as firewood and charcoal (Mors, Rizzini & Pereira, 2000; Cruz & Kaplan,

2004). It can also be recommended for apicultural purposes (Pott & Pott, 2003), as bees are its main pollinators (Buchmann & Buchmann, 1981).

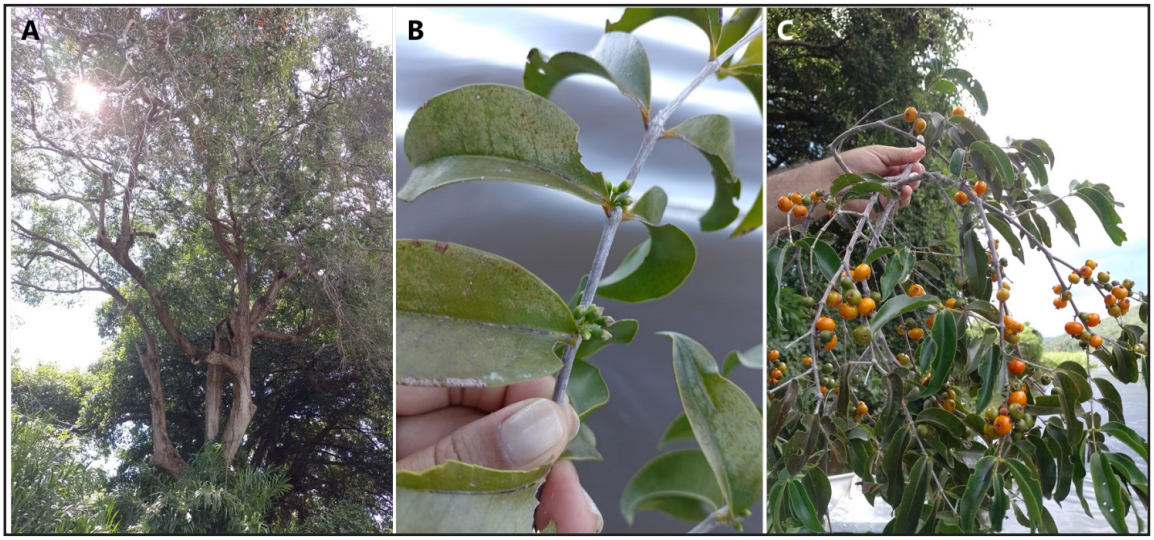
Due to the ecological importance, economic, and medicinal potential of roncador and its relevance to research in plant genetics, this study aims to assess the diversity and genetic structure in natural populations of roncador (*Mouriri guianensis*) from two important Brazilian biomes, the Amazon and the Pantanal, using ISSR molecular markers.

2 MATERIALS AND METHODS

2.1 Study Area

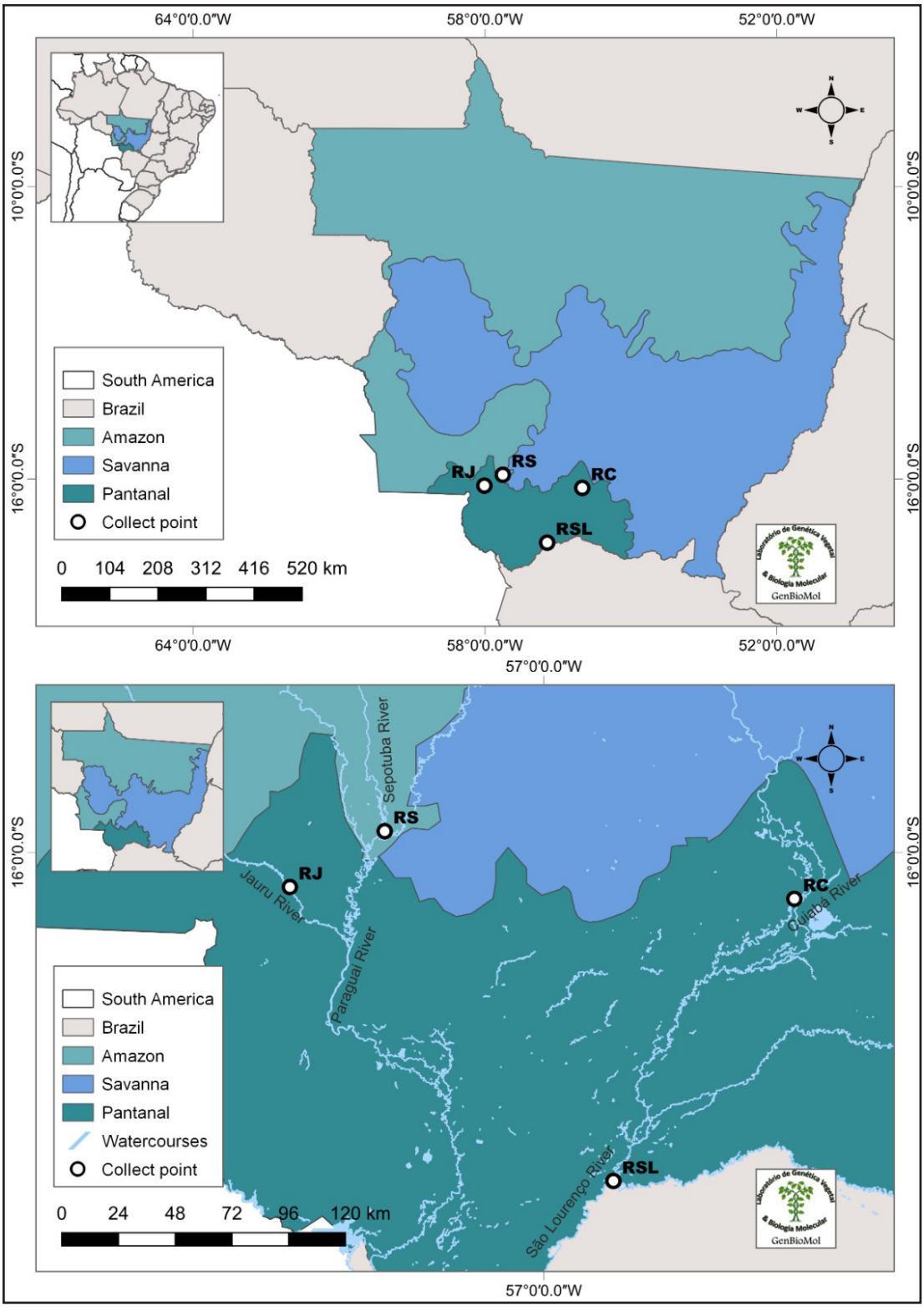
Genetic analyses were conducted on native populations of roncador (Figure 1) occurring in the state of Mato Grosso, Brazil, in four locations: Rio Cuiabá (Barão do Melgaço), Rio São Lourenço (Poconé), and Rio Jauru (Cáceres) within the Pantanal Biome, and Rio Sepotuba (Cáceres) within the Amazon Biome (Figure 2). These populations consisted of nine, four, twelve, and fourteen individuals, respectively. Individuals within each population were randomly selected, with priority given to those with greater geographical spacing between them.

Figure 1 – Roncador (*Mouriri guianensis* Aubl.). (A) Canopy of the roncador tree; (B) Mature leaves of the species; (C) Orange-colored fruits



Source: Authors (2024)

Figure 2 – (A) Geographic distribution of roncador (*Mouriri guianensis* Aubl.) populations, collected in the state of Mato Grosso. (B) Collection points location: Pantanal Biome: RC= Rio Cuiabá (Barão do Melgaço); RJ= Rio Jauru (Cáceres) and RSL= Rio São Lourenço (Poconé). Amazon Biome: RS= Rio Sepotuba (Cáceres)



Source: Authors (2024)

2.2 Plant Material

Leaf material collected from each individual during field expeditions was properly identified, stored in Ziploc® type plastic bags containing silica gel and transported to the Plant Molecular Biology and Genetics Laboratory (GenBioMol) at CEPTAM (Center for Technology and Research of Southern Amazon) at the State University of Mato Grosso "Carlos Alberto Reyes Maldonado," Alta Floresta Campus, MT. Leaf material was dehydrated in silica gel and stored in Ziploc® plastic bags for subsequent total DNA extraction.

Sampling criteria prioritized adult individuals spaced at least 10 meters apart, aiming to minimize the collection of related individuals and to represent local genetic diversity. Site selection was based on previous species records, logistical accessibility, and representation of different biomes. Although limited by field collection constraints, this approach sought to capture the greatest possible genetic variation within the operational conditions of the expedition.

2.3 DNA Extraction

Total DNA extraction was performed using the CTAB (Cetyltrimethylammonium Bromide) method as described by Doyle and Doyle (1990), with modifications: addition of 2% PVP (polyvinylpyrrolidone), increase in β-mercaptoethanol concentration from 2% to 3%, and CTAB concentration from 2% to 5% in the extraction buffer (protocol P4).

The concentration of extracted DNA was estimated by spectrophotometry (ND-3800-OD Nano DOT), and its integrity was analyzed by 1% agarose gel electrophoresis prepared in 1x TBE buffer (Tris-Borate-EDTA) and stained with ethidium bromide (0.6 ng mL-1). After quantification, samples were diluted to reach an ideal concentration (± 30 ng) for PCR amplification and stored at -20 °C for further amplification.

2.3 Primer Selection and PCR Optimization

Based on band intensity, polymorphism, and repetitiveness, eight ISSR primers developed by the University of British Columbia (UBC) (Table 1) were selected for molecular characterization of individuals.

Table 1 – ISSR primers used in the molecular characterization of roncador (*Mouriri guianensis* Aubl.) individuals, their respective sequences, and anneling temperatures

Primer ID	Sequence (5'----3')	TA (°C)
UBC 810	GAGAGAGAGAGAGAGAT	48,0
UBC 825	ACACACACACACACACT	48,0
UBC 890	VHVGTGTGTGTGTGTGT	52,2
UBC 891	HVHTGTGTGTGTGTGTG	52,2
TriAGG - TriAGG3'RC	AGGAGGAGGAGGAGGRC	55,0
TriCGA - TriCGA3'RC	CGACGACGACGACGARC	62,0
DiCA5'CY	CYCACACACACACACACA	58,8
TriATG3'RC	ATGATGATGATGATGRC	52,2

Source: Authors (2024)
Legend: TA = Annealing Temperature. *Y = (C or T); R = (A or G)

PCR amplification reactions were carried out in a final volume of 15 µL in an AerisTM thermocycler, containing 2 µL of DNA (± 30 ng), 1.5 µL of 10x buffer (1 mM KCl; 1 mM Tris-HCl pH 8.3; 10% Tween 20), 3.0 µL of $MgCl_2$ (25 mM), 2.3 µL of primer (2 µM), 3.0 µL of dNTPs (1 mM of each dNTP), 0.75 µL of DMSO, 0.12 µL of Taq polymerase (5 U µL-1), and Milli-Q water. The amplification program proposed by Maltezo et al. (2021) was used. Amplification cycles consisted of initial denaturation at 94 °C for 1.5 minutes, followed by 35 cycles of 94 °C for 45 seconds, annealing at 48 – 62 °C (depending on the primer used) for 45 seconds, extension at 72 °C for 1.5 minutes, and a final extension of five minutes at 72 °C.

The amplification products were separated by electrophoresis on 1.5% agarose gel in 1X TBE buffer (Tris-Boric Acid-EDTA) at a constant voltage of 80V using a horizontal electrophoresis system LCH 20x25 (Loccus Biotecnologia®). After electrophoresis, the gel was stained in ethidium bromide solution (0.6 ng mL-1) for 20 minutes and then visualized, photographed, and edited on a transilluminator with UVB light LTB-20x20 STi,

photodocumentor, and L-Pix STi software, respectively (Loccus Biotecnologia®). Fragment sizes were estimated by comparison with the 100 bp DNA Ladder molecular marker.

2.4 Data Analysis

Data were obtained by visual assessment of the bands of sampled individuals. The amplified products constituted the presence (1) and absence (0) matrix of bands. The total number of amplified bands (NTB), number of polymorphic bands (NBP), percentage of polymorphism (%P), and polymorphic information content (PIC) were also described.

2.5 Statistical Analysis

The genetic dissimilarity matrix between each pair of individuals was obtained using the arithmetic complement of the Jaccard Index. This analysis compares the number of common band presences and the total number of bands, excluding the number of joint absences (Meyer et al., 2004). Based on the matrix obtained by the Jaccard index, cluster analysis of individuals was performed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), and the Cophenetic Correlation Coefficient (CCC) was obtained. These analyses were performed using the GENES program (Cruz, 2013).

The "POPGENE" 1.31 program (Yeh, 2000) was used to estimate population diversity parameters through the Shannon (I) and Nei (H) indices. For the analysis of population genetic structure, the "STRUCTURE" 2.3.4 program (Pritchard, Stephens & Donnelly, 2000), based on Bayesian statistics, was employed to infer the number of groups (K). This analysis was conducted with 20 iterations, 200,000 burn-ins, and 500,000 Monte Carlo simulations of Markov Chains (MCMC). The most likely K value, relative to the proposed ones, was determined using the criteria described by Pritchard and Wen (2004) and Evanno, Regnaut and Goudet (2005).

The analysis of molecular variance (AMOVA) was used to reveal the distribution of genetic diversity within and among populations, performed according to Excoffier and Heckel (2006). The genetic relationships among all roncador individuals (*M. guianensis*)

were visualized through Principal Coordinates Analysis (PCoA) conducted according to Peakall and Smouse (2012), using GenAlEx 6.5 software.

3 RESULTS AND DISCUSSION

The genetic characterization of the 39 roncador individuals using eight ISSR primers yielded a total of 127 amplified fragments, of which 98% were polymorphic. The highest number of amplified bands (24) was achieved with the DiGA5'CY primer, while the lowest (10) was with the TriATG3'RC primer. With this variation, an average of 16 fragments per primer was obtained. The UBC 890, UBC 891, TriCGA3'RC, and TriAGG3'RC primers showed the highest percentage of polymorphism (100%) for the individuals analyzed (Table 2).

Table 2 – Primers, total number of bands (NTB), number of polymorphic bands (NFP), percentage of polymorphism (%P), and polymorphic information content (PIC)

Primer	**NTB**	**NFP**	**%P**	**PIC**
TriATG3'RC	10	9	90	0,56
UBC 890	16	16	100	0,62
UBC 891	14	14	100	0,70
UBC 825	12	11	92	0,54
TriCGA3'RC	16	16	100	0,61
TriAGG3'RC	17	17	100	0,53
DiGA5'CY	24	23	96	0,74
UBC 810	18	17	94	0,54
Total	127	123	-	-
Average	16	16	98	0,58

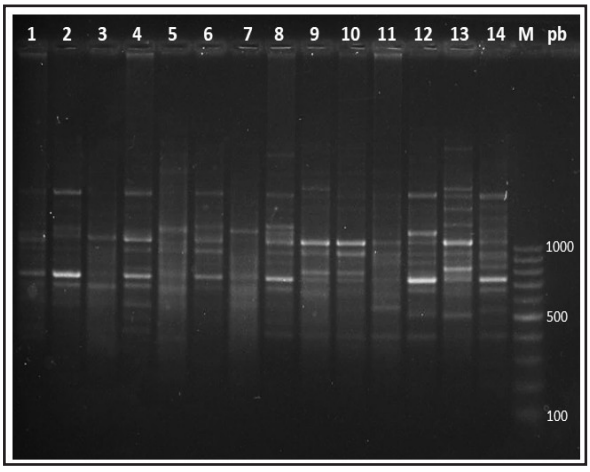
Source: Authors (2024)

To evaluate the efficiency of ISSR primers in indicating polymorphism between two individuals, based on the absence or presence of fragments, the PIC (Polymorphic Information Content) was calculated, which ranged from 0.53 to 0.74, depending on the primer used, with an average of 0.58. The PIC value ranges from "0" for monomorphic profiles to "1" for highly polymorphic profiles. Thus, this value is related to the number of alleles, which in turn is directly associated with genetic diversity and the number of genotypes under study (Malone et al., 2007).

According to Botstein et al. (1980), PIC values above 0.50 are classified as highly informative. This parameter assists in classifying primers according to their efficiency in detecting polymorphism, serving as a basis for selecting primers for future genetic diversity studies (Costa et al., 2015). In this study, all markers are highly informative, indicating that they are efficient in detecting polymorphism and thus recommended for roncador genetic diversity studies.

In addition to polymorphic information content, the quality of amplification was also a criterion for primer selection. The electrophoretic profile of 14 roncador individuals using the TriCGA3'RC primer represents the standard obtained in this study and can be observed in Figure 3.

Figure 3 – Electrophoresis of 14 roncador individuals (*Mouriri guianensis* Aubl.) from the Rio Sepotuba population (Cáceres) (Amazon Biome) using the TriCGA3'RC primer. M: 100 bp DNA Ladder marker



Source: Authors (2024)

The highest estimates of Nei (H) and Shannon (I) diversity indices were found for the Rio Jauru population (H=0.28; I=0.41 and P%=71.65), while the Rio São Lourenço population showed the lowest genetic diversity index and consequently the lowest percentage of polymorphism (H=0.17; I=0.25 and P%=32.28). At the species level, genetic diversity was H=0.39, I=0.57, and P%=96.85 (Table 3).

Table 3 – Genetic diversity within the four roncador populations (*Mouriri guianensis* Aubl.)

Population	H	I	P%
Rio Sepotuba (RS)	0,20	0,30	51,97
Rio Cuiabá (RC)	0,23	0,34	51,18
Rio São Lourenço (RSL)	0,17	0,25	32,28
Rio Jauru (RJ)	0,28	0,41	71,65
Species level	0,39	0,57	96,85

Source: Authors (2024)
Legend: H= Nei's index; I= Shannon's index; P%= polymorphism

Nei and Shannon index values can vary from 0 to 1, with 1 representing the maximum genetic diversity that can occur within a population (Lewontin, 1972). The Rio Sepotuba population, the only one in the Amazon Biome, obtained values of H=0.20; I=0.30; and P%=51.97, indicating genetic diversity within the population. This result highlights the importance of the Amazon Rainforest as a reservoir of genetic information and the need for the conservation of this biome (Tiago et al., 2018). However, it is not the only biome of great importance, as the population with the highest diversity was found in the Rio Jauru, located in the Pantanal.

The Pantanal is a fire-dependent region from an ecological perspective (Pivello et al., 2021), and fire is part of the landscape, existing in this location before the earliest records of human presence in the Pantanal area (Damasceno-Junior et al., 2021). However, high-intensity fires can have catastrophic effects, as seen in the fire events recorded in 2020 (Silva et al., 2023). Thus, it is important to prioritize the conservation and management of roncador in the Amazon and Pantanal biomes. Further investigation into the areas where this species occurs can contribute significantly, especially considering the species' genetic variability and its specific adaptation capacity to each environment or biome.

The analysis of molecular variance (AMOVA) revealed that 52% of the observed genetic variation stemmed from differences between the studied populations, while the remaining 48% was attributed to variations within these populations, as shown in Table 4. These results support the information presented in the diversity indices (Table 3), reinforcing the existence of genetic diversity in the roncador populations.

Table 4 – Analysis of Molecular Variance (AMOVA) among the four roncador populations (*Mouriri guianensis* Aubl.) using ISSR molecular markers

Source of variation	DF	SS	MS	CV	TV (%)	F_{st}	P
Among populations	3	613,80	204,60	20,11	52%	0,523	<0,001
Within populations	35	640,77	18,31	18,31	48%		
Total	38	1254,56		38,41	100%		

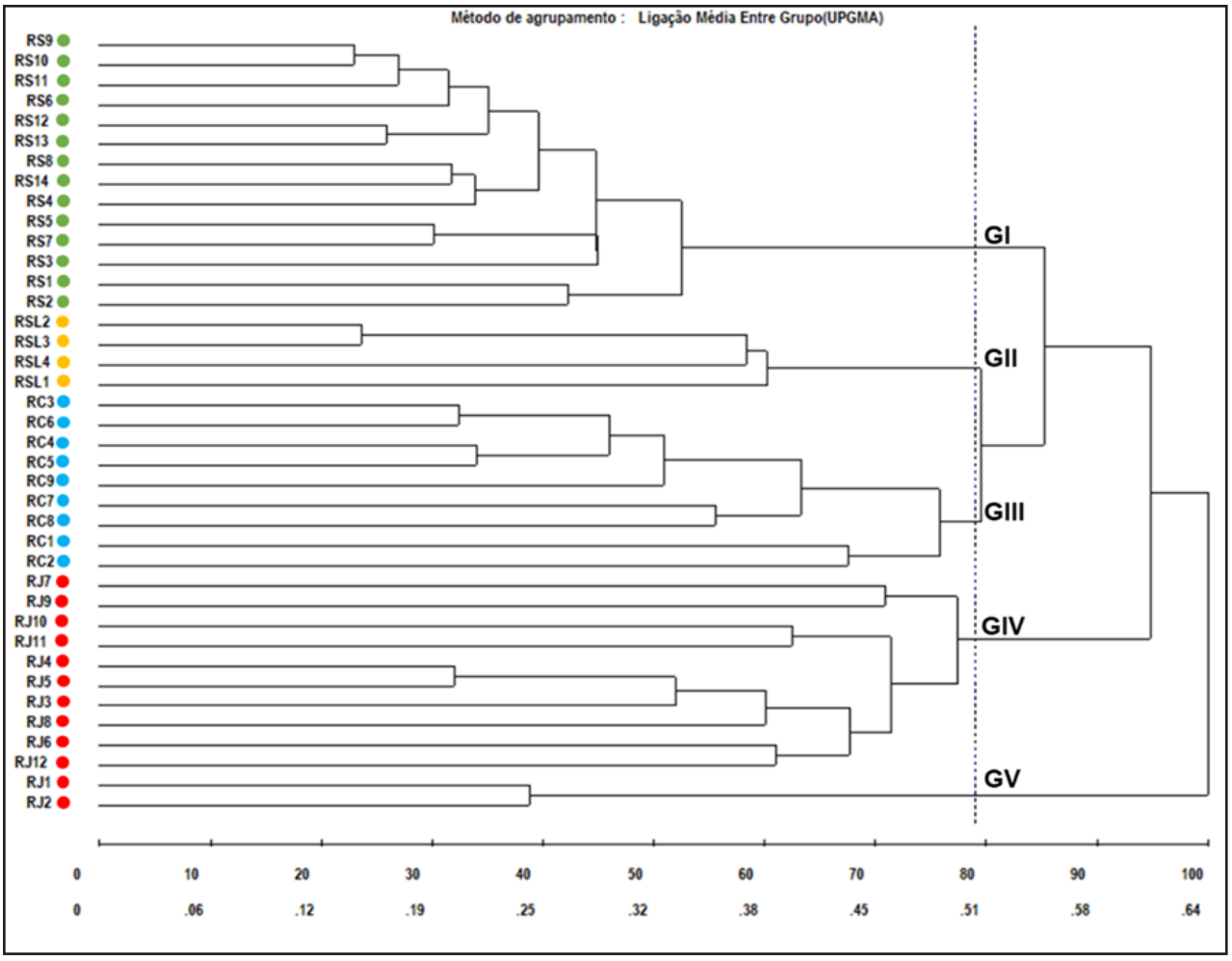
Source: Authors (2024)
Legend: DF= Degrees of freedom; SS = Sum of squares; MS = Mean square; CV = Variance component; TV = Total variance; P = Probability of having a variance component P = Probability of having a variance component greater than the observed values by chance

The levels and distribution of genetic variability among populations, as well as within them, can be influenced by a variety of factors, such as seed dispersal method, successional stages, reproduction system, and geographic distribution (Nybom, 2004). Variation between populations can be explained by low gene flow, as well as the action of selective forces in different environments, given that we have populations belonging to different biomes.

For the dissimilarity matrix, the smallest genetic distance occurred between individuals nine and ten (0.15), both from the same population (Rio Sepotuba), possibly indicating related individuals. Meanwhile, individuals five (Rio Sepotuba) and 32 (Rio Jauru) showed the greatest distance (0.76). Genetic distance indicates the genetic difference between individuals/populations, which can be classified as low (0.05 to 0.15), moderate (0.15 to 0.25), or high (>0.25) (Wright, 1978). The analyzed parameters did not present genetically identical individuals, thus reinforcing the genetic diversity existing among the individuals within the analyzed populations.

Using the UPGMA method, the distribution of individuals among five genetic groups was demonstrated (Figure 4). This consistency in the grouping pattern was observed through the values obtained for the CCC (0.86), distortion (1.67), which according to Regazzi and Cruz (2020), the higher the CCC value, the lower the distortion caused by the grouping of individuals, indicating greater consistency in grouping patterns.

Figure 4 – Dendrogram obtained by the UPGMA method with 39 roncador individuals (*Mouriri guianensis* Aubl.), based on ISSR molecular markers. RS= Rio Sepotuba (green); RSL= Rio São Lourenço (yellow); RC= Rio Cuiabá (blue); and RJ= Rio Jauru (red). Cophenetic correlation coefficient (CCC) = 0.86; Distortion (%) = 1.67; Stress (%) = 12.94; Cutting point 79.02% (Mojena, 1977)



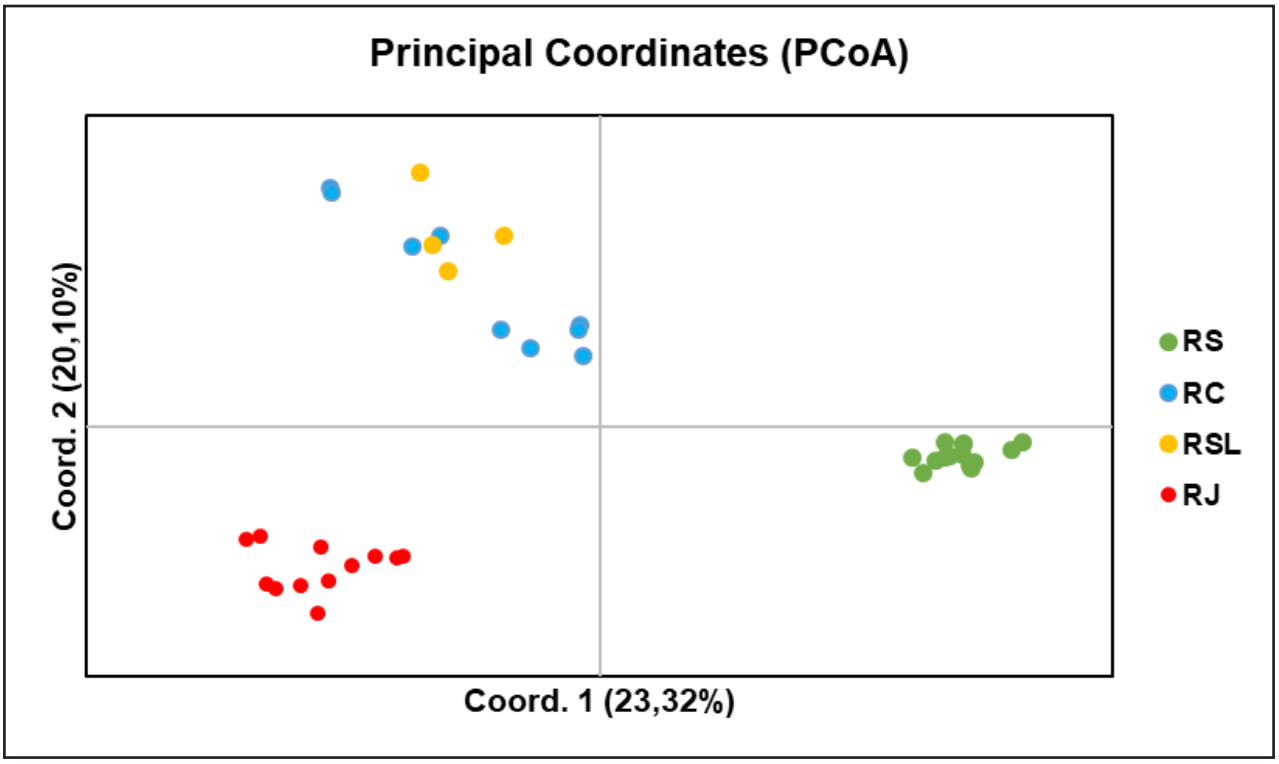
Source: Authors (2024)

Group I (GI) consisted of 14 roncador individuals from the Rio Sepotuba population, correlating with the collection location and origin. The same was observed for the other groups, each allocating individuals collected in the same region, except for individuals one and two from the Rio Jauru population, which were designated as a separate group (GV), genetically distinct from the rest of the population. Silva (2022) observed the same phenomenon for his data when assessing the genetic diversity

of cassava, finding that landraces were allocated according to the collection location. Overall, the results demonstrate that ISSR markers can differentiate roncador genotypes according to their origin, thus geographical distance affected group formation.

For Principal Coordinates Analysis (PCoA), the first coordinate (Coord. 1) explained 23.32% of the variation among individuals. The second coordinate (Coord. 2) contributed 20.10% to the total variation. Both coordinates explained 43.42% of the genetic variation (Figure 5).

Figure 5 – Scatter plot from principal coordinate analysis among the 39 roncador individuals (*Mouriri guianensis* Aubl.) collected from four populations: RS= Rio Sepotuba (green), RC= Rio Cuiabá (blue), RSL= Rio São Lourenço (yellow), and RJ= Rio Jauru (red) in the state of Mato Grosso



Source: Authors (2024)

In genetic terms, the farthest points in Figure 5 are considered the most divergent individuals, while the closest points are the most similar to each other. The clustering of individuals in PCoA corresponded to the UPGMA clustering analysis, highlighting the

isolation of the Rio Sepotuba and Rio Jauru populations and the proximity of the Rio São Lourenço and Rio Cuiabá populations, corresponding to GII and GIII in UPGMA, thus demonstrating consistency in both clustering approaches with molecular data.

The populations collected along the margins of the Rio Sepotuba and Rio Jauru showed genetic distance (Figure 5), although they are geographically close (Figure 2). This likely occurs because Jauru is to the left of the Paraguay River, and Sepotuba is above it, and they are not tributaries of each other, i.e., they are independent, although both are tributaries of the Paraguay River (Calheiros & Fonseca, 1996). It is worth noting that these populations belong to different biomes, with Rio Jauru in the Pantanal and Sepotuba in the Amazon, which will be further discussed later.

On the other hand, a closer genetic proximity was observed between the populations of Rio Cuiabá and Rio São Lourenço (Figure 5), despite being geographically further apart (Figure 2) compared to the Jauru and Sepotuba rivers. The Rio São Lourenço is a tributary of the Rio Cuiabá, which in turn is a tributary of the Paraguay River (Calheiros & Fonseca, 1996).

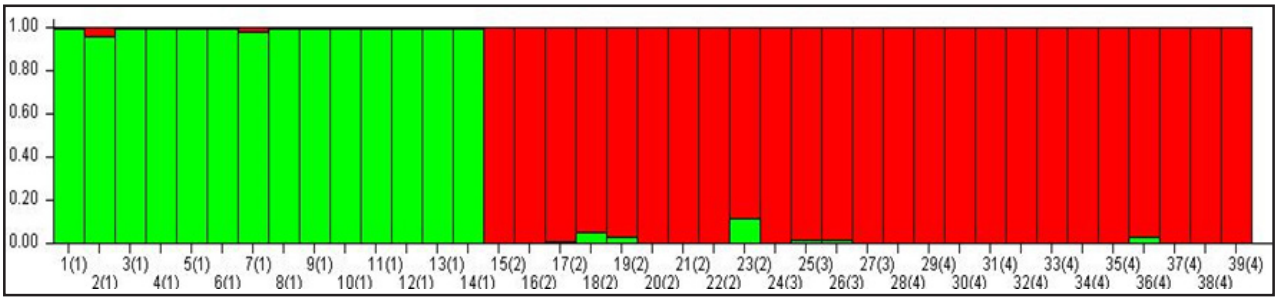
It is suggested that gene flow occurred between the populations of the São Lourenço and Cuiabá rivers, likely facilitated by the dispersal of roncador seeds by fish that feed on the fruit, as they can carry fruits and seeds both upstream and laterally (Muniz et al., 2020). Additionally, it is worth mentioning that the populations of the São Lourenço and Cuiabá rivers belong to the same biome (Pantanal).

Overall, although the four rivers where the collections were made are tributaries of the Paraguay River and belong to the Paraguay River basin, the individuals from populations collected along these rivers had differentiated genetic distances, likely due to gene flow influenced by the direction of the rivers, as well as by the biomes, rather than geographical distance. This demonstrates the ecological role that fish play in the dispersal of roncador along the riverbanks where they occur.

Bayesian analysis revealed the formation of two main genetic groups, according to the ΔK value obtained by the Structure program. Subsequently, a histogram

showing the genetic composition of each individual and their respective grouping by similarity was obtained (Figure 6).

Figure 6 – Clustering of 39 roncador individuals (*Mouriri guianensis* Aubl.) according to molecular bases with eight ISSR primers using Bayesian analysis, assuming K=2 (groups) according to the Structure program. The numbers in parentheses represent the populations: (1) Rio Sepotuba (green); (2) Rio Cuiabá; (3) Rio São Lourenço; and (4) Rio Jauru (red). The same color for a different population indicates that they belong to the same group



Source: Authors (2024)

The first group (green) consisted of 14 roncador individuals from the Rio Sepotuba population, and the second group (red) comprised the remaining 25 individuals, thus the Bayesian grouping formed groups according to the different biomes. Thus, the Rio Sepotuba population represented the first group because they are from the Amazon Biome, while individuals from the populations of Rio Cuiabá, Rio São Lourenço, and Rio Jauru, despite the geographical distance between them, were grouped in the second group because they belong to the Pantanal Biome.

The results of the Bayesian analysis partially corroborated with the results of UPGMA and PCoA, which also allocated all individuals from the Rio Sepotuba population to one group. UPGMA also separated groups by biomes; however, within the Pantanal Biome, there was subdivision into collection areas. Considering the diverse nature of the biomes and the different environmental aspects they present in the regions, it was expected that these differences would favor the genetic variation of individuals, and consequently, populations would be genetically distinct between the Amazon and Pantanal Biomes.

Turchetto-Zolet et al. (2012), evaluating the genetic structure of *Schizolobium parahyba*, reported biogeographical differences between biomes, demonstrating high structuring and separation between populations of the Amazon and Atlantic Forest. The ecology of different biomes is an important factor in the evolution process within a context of biogeographic patterns (Pennington & Levin, 2015). And historical and environmental conditions (edaphoclimatic conditions, temperature, soil fertility) between biomes can also influence differentiation over time (Melo, 2016).

Despite the limitations imposed by reduced number of individuals per locality, a direct consequence of the logistical challenges faced during fieldwork, the data obtained reveal genetic diversity and some level of structure among *M. guianensis* populations. The representativeness of the samples may be partially compromised, which could limit the full detection of intra and interpopulation genetic variability (Hale et al., 2012). Nevertheless, the patterns observed appear to reflect not only the geographic distance between populations but also the ecological heterogeneity between the Amazon and Pantanl biomes.

The species' discontinuous distribution in seasonally flooded environments, along with naturally low population density, aligns with previous findings in the literature, such as those by Ikeda-Castrillon et al. (2011), who reported its presence on riverine islands along the Paraguay River, and Morais et al. (2013) who identified high abundance in semideciduous forests of the Poconé region in the Pantanal.

Additionally, the geographic distance between sampled populations varied significantly, ranging from approximately 47 km (between RS and RJ) to over 210 km (between RC and RJ). This spatial variation, along with natural ecological barriers, may have influenced the genetic clustering patterns observed. According to the isolation-by-distance model (Wright, 1943), more distant populations tend to exhibit greater genetic differentiation due to reduced gene flow, a pattern also observed in other tropical species, which reinforce the importance of geographic and environmental factors in shaping genetic variability (Diniz-Filho et al., 2009; Ramos et al., 2016). Our results underscore the importance of considering both

spatial configuration and ecological variability in interpreting genetic structure and informing conservation strategies for the species.

Population genetic parameters estimated from molecular markers, as emphasized by Avise and Hamrick (1997) and Newton et al. (1999), are valuable tools for conservation planning, particularly in threatened biomes, as they reveal genetic differentiation among populations and therefore require conservation strategies in their habitat (in situ) or outside of it (ex situ). Rodrigues et al. (2021) reports that the loss of a plant species leads to the loss of years of evolutionary history, which over time have become specialized, especially in the Amazon, where evolutionary pressure is very high.

4 CONCLUSION

The roncador individuals exhibited genetic diversity among them, representing the first evidence of population structuring among different collection sites and biomes. Most of the diversity was found at the interpopulation level, with the Pantanal population of Rio Jauru showing the highest diversity indices, and the most genetically isolated from the others being the population from the Amazon Biome (Rio Sepotuba).

For this reason, they become relevant for species conservation measures as they show potential to be utilized to ensure the maintenance of genetic variability and the effective conservation of the native species of the Amazon and Mato Grosso Pantanal.

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