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STUDY OF DIVERSITY AND GENETIC STRUCTURE OF CAGAITA, CAJUZINHO-DO-CERRADO AND PEQUI POPULATIONS FOR CONSERVATION AND SUSTAINABLE MANAGEMENT PURPOSES

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ABSTRACT

Objective: The aim of this study is to investigate the genetic diversity and population structure of cagaita, cajuzinho-do-cerrado, and pequi populations, with the purpose of providing scientific foundations for the conservation and sustainable management of these plant species.

Theoretical Framework: Investigations into the genetic diversity of species such as cagaita, cajuzinho-do-cerrado, and pequi are fundamental for elucidating their population structures and genetic variability. These studies employ population genetics models and gene flow analyses, forming a robust foundation for determining the conservation and sustainable management needs of these species, which are vital aspects for the preservation of the Cerrado's biodiversity.

Method: Leaf samples were collected from adult individuals of the three species, maintaining a minimum distance of 200 meters between trees, in three locations with different levels of anthropization in the Cerrado. DNA extraction followed the procedures outlined by Doyle and Doyle (1990), with some adaptations. ISSR oligonucleotides from the UBC Collection were used for the PCR reactions, and the products were analyzed by agarose gel electrophoresis. The generated data were processed using binary matrices and the UPGMA method for genetic similarity analysis.

Results and Discussion: The adapted DNA extraction protocol was effective for cagaita and pequi but inefficient for cajuzinho-do-cerrado due to its leathery leaves, suggesting the use of other tissues like floral buds. Dendrograms showed significant genetic diversity among cagaita accessions and similar diversity in pequi. Clustering by genetic similarity indicated small isolated populations, signaling a risk of genetic erosion if forest fragmentation continues, which could affect genotype distribution and compromise species survival.

Keywords: Conservation, Genetic Diversity, Genetic Erosion, Sustainable Management.

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ESTUDO DE DIVERSIDADE E ESTRUTURA GENÉTICA DAS POPULAÇÕES DE CAGAITA, CAJUZINHO-DO-CERRADO E PEQUI PARA FINS DE CONSERVAÇÃO E MANEJO SUSTENTÁVEL

RESUMO

Objetivo: O objetivo deste estudo é investigar a diversidade genética e a estrutura populacional das populações de cagaita, cajuzinho-do-cerrado e pequi, com o propósito de fornecer fundamentos científicos para a conservação e o manejo sustentável dessas espécies vegetais.

Referencial Teórico: Investigações sobre a diversidade genética de espécies como cagaita, cajuzinho-do-cerrado e pequi são fundamentais para elucidar suas estruturas populacionais e variabilidade genética. Esses estudos utilizam modelos de genética de populações e análises de fluxo gênico, formando uma base robusta para determinar as necessidades de conservação e manejo sustentável dessas espécies, aspectos vitais para a preservação da biodiversidade do Cerrado.

Método: Amostras foliares foram coletadas de indivíduos adultos das três espécies, mantendo uma distância mínima de 200 metros entre as árvores, em três locais com diferentes níveis de antropização no Cerrado. A extração de DNA seguiu os procedimentos descritos por Doyle e Doyle (1990), com algumas adaptações. Oligonucleotídeos ISSR da Coleção UBC foram utilizados nas reações de PCR, e os produtos foram analisados por eletroforese em gel de agarose. Os dados gerados foram processados usando matrizes binárias e o método UPGMA para análise de similaridade genética.

Resultados e Discussão: O protocolo adaptado de extração de DNA foi eficaz para cagaita e pequi, mas ineficiente para cajuzinho-do-cerrado devido às suas folhas coriáceas, sugerindo o uso de outros tecidos, como botões florais. Os dendrogramas mostraram significativa diversidade genética entre os acessos de cagaita e diversidade semelhante nos de pequi. A formação de agrupamentos por similaridade genética indicou pequenas populações isoladas, sinalizando um risco de erosão genética caso a fragmentação florestal continue, o que poderia afetar a distribuição de genótipos e comprometer a sobrevivência das espécies.

Palavras-chave: Conservação, Diversidade Genética, Erosão Genética, Manejo Sustentável.

ESTUDIO DE DIVERSIDAD Y ESTRUCTURA GENÉTICA DE LAS POBLACIONES DE CAGAITA, CAJUZINHO-DO-CERRADO Y PEQUI PARA FINES DE CONSERVACIÓN Y MANEJO SOSTENIBLE

RESUMEN

Objetivo: El objetivo de este estudio es investigar la diversidad genética y la estructura poblacional de las poblaciones de cagaita, cajuzinho-do-cerrado y pequi, con el propósito de proporcionar bases científicas para la conservación y el manejo sostenible de estas especies vegetales.

Marco Teórico: Las investigaciones sobre la diversidad genética de especies como cagaita, cajuzinho-do-cerrado y pequi son fundamentales para elucidar sus estructuras poblacionales y variabilidad genética. Estos estudios emplean modelos de genética de poblaciones y análisis de flujo génico, formando una base robusta para determinar las necesidades de conservación y manejo sostenible de estas especies, aspectos vitales para la preservación de la biodiversidad del Cerrado.

Método: Se recolectaron muestras foliares de individuos adultos de las tres especies, manteniendo una distancia mínima de 200 metros entre los árboles, en tres ubicaciones con diferentes niveles de antropización en el Cerrado. La extracción de ADN siguió los procedimientos descritos por Doyle y Doyle (1990), con algunas adaptaciones. Se utilizaron oligonucleótidos ISSR de la Colección UBC para las reacciones de PCR, y los productos se analizaron mediante electroforesis en gel de agarosa. Los datos generados se procesaron utilizando matrices binarias y el método UPGMA para el análisis de similitud genética.

Resultados y Discusión: El protocolo adaptado de extracción de ADN fue efectivo para cagaita y pequi, pero ineficaz para cajuzinho-do-cerrado debido a sus hojas coriáceas, lo que sugiere el uso de otros tejidos como botones florales. Los dendrogramas mostraron una significativa diversidad genética entre los accesos de cagaita y una diversidad similar en pequi. La agrupación por similitud genética indicó la existencia de pequeñas poblaciones aisladas, señalando un riesgo de erosión genética si la fragmentación forestal continúa, lo cual podría afectar la distribución de genotipos y comprometer la supervivencia de las especies.

Palabras clave: Conservación, Diversidad Genética, Erosión Genética, Manejo Sostenible.

1 INTRODUCTION

The preservation of biodiversity and the sustainable management of natural resources have become priority issues in a world increasingly aware of environmental impacts (Cimatti; Kramer; Marco, 2023). The Cerrado, Brazil's second-largest biome, hosts an extraordinary diversity of plant species, many of which play key roles in ecosystems and the livelihoods of local communities. Among these species, the cagaita (*Eugenia dysenterica*), the cajuzinho-do-cerrado (*Anacardium othonianum*), and the pequi (*Caryocar brasiliense* Camb.) stand out, being native fruits of significant socioeconomic and cultural importance (Noleto et al., 2022).

However, these species have been facing increasing pressures due to habitat loss, environmental degradation, and disorganized exploitation (Chaves et al., 2023). To ensure the conservation and sustainable management of these species, it is essential to understand the genetic diversity and population structure of their respective populations. The genetic diversity of tree species is crucial for studying conservation methods and managing natural populations, important for biodiversity conservation and for developing appropriate conservation strategies, as well as for promoting more diverse and locally adapted agriculture (Gomes et al., 2022).

Studies guiding the analysis of genetic diversity in plant species often employ Inter Simple Sequence Repeats (ISSR) oligonucleotides. These are favored for their independence from prior genomic information and their reproducibility across various laboratories (Ng; Tan, 2015). This technique enables the evaluation of intrapopulational genetic diversity, aiding in the identification of patterns such as the geographical distribution of this variability. Furthermore, ISSR markers are particularly valuable for generating reliable and comprehensive data on genetic diversity, crucial for devising conservation strategies and understanding evolutionary processes in plant populations.

These aspects are crucial for the adaptability and viability of species in the face of current and future threats. Therefore, this study aims to investigate the genetic diversity and population structure of cagaita, cajuzinho-do-cerrado, and pequi in the North of Minas Gerais.

Understanding these genetic and population dynamics is essential not only for the preservation of these species but also for their potential contributions to local and global ecosystems.

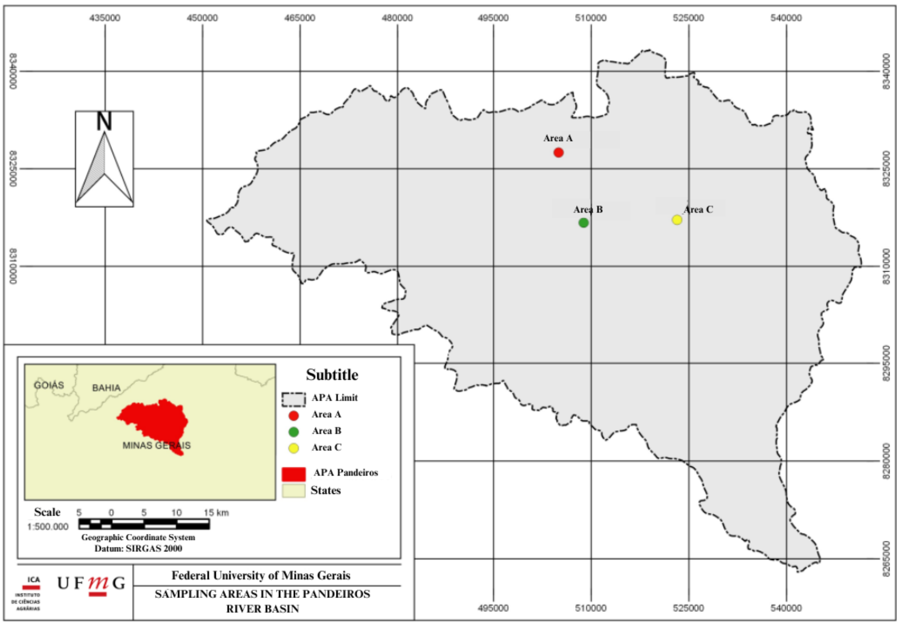
2 METHODOLOGY

2.1 FIELD MATERIAL COLLECTION

Leaf samples were collected from adult individuals of the three species, maintaining a minimum distance of 200 m between trees (Figure 1) in three distinct locations with varying degrees of anthropization in the Cerrado. In each of the three defined sampling areas, 8 to 15 individuals of each species were sampled per area. Leaves were collected from the youngest branches of the trees, without apparent damage from pathogens and/or insects, showing good vegetative vigor, and no apparent signs of nutritional deficiency. Young leaves (10 leaves/individual) of each species were collected, identified, and placed in thermal boxes with ice, then transported to the laboratory.

Figure 1

Map illustrating the three leaf sample collection sites for cagaita, cajuzinho-do-cerrado, and pequi in the Pandeiros River basin, in the municipality of Bonito de Minas – MG, at the Community of São Domingos (A), the Community of Água Doce (B), and Fazenda Santa Maria da Vitória (C).



The plant material collections were carried out in the following areas, classified as follows: (1) preserved Cerrado, with the presence of arboreal individuals (Figures 2 and 5A), (2) Cerrado in the process of natural regeneration (Figures 3 and 5B), and (3) degraded Cerrado (Figures 4 and 5C). Thus, three locations were defined for the collection of plant material: São Domingos Community, sampling species in an area classified as degraded Cerrado; Sêo Santino's property in Água Doce Community, collecting plant material in Cerrado in the regeneration stage; and Fazenda Santa Maria da Vitória, belonging to the Non-Governmental Organization (NGO) Save Cerrado, which had a remnant of Cerrado in a more preserved state. In this context, the area to be sampled was expanded. It is worth noting that the sampling required a significant amount of time due to the difficult access to the properties, caused by road conditions (Figure 5D).

Figure 2

Map illustrating the collection points of leaf samples from cagaita, cajuzinho-do-cerrado, and pequi in the Pandeiros River basin, in the municipality of Bonito de Minas – MG, at the São Domingos Community.

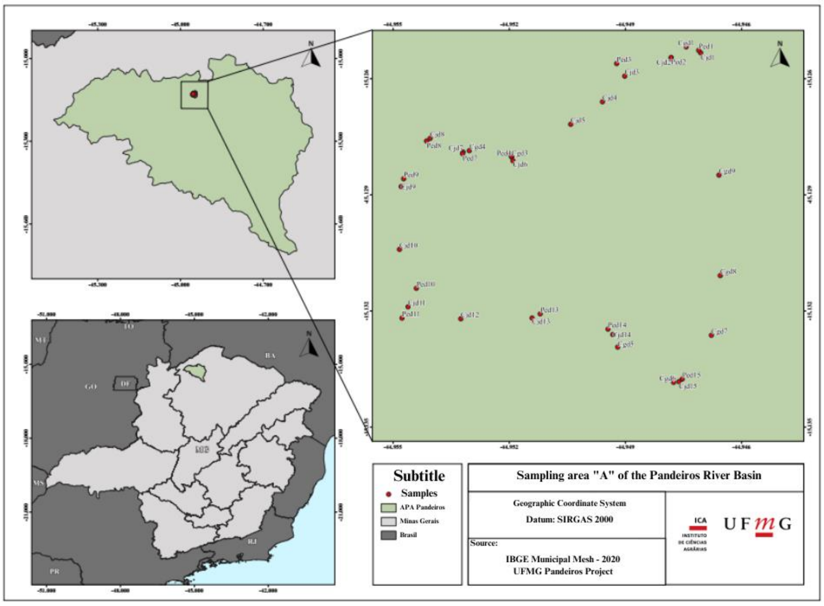


Figure 3

Map illustrating the collection sites of leaf samples from cagaita, cajuzinho-do-cerrado, and pequi in the Pandeiros River basin, in the municipality of Bonito de Minas – MG, at the Água Doce Community.

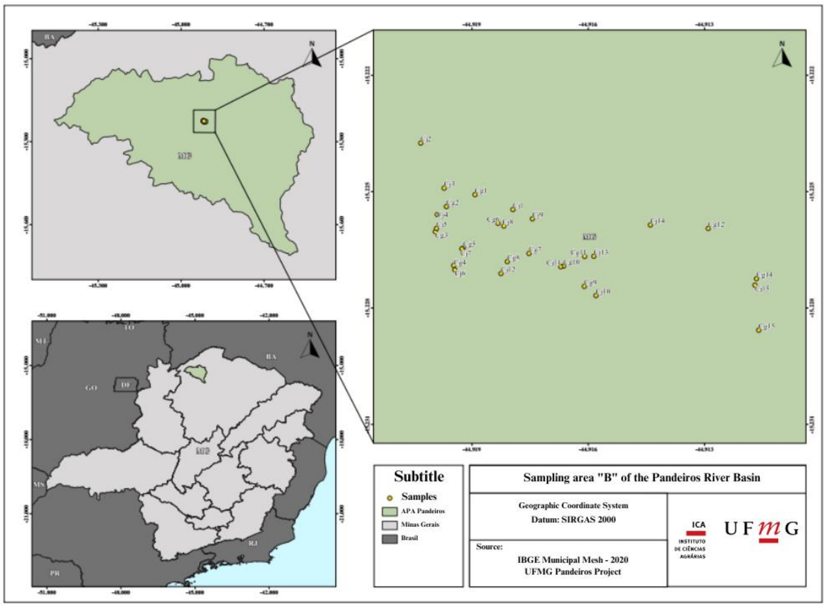


Figure 4

Map illustrating the collection sites of leaf samples from cagaita, cajuzinho-do-cerrado, and pequi in the Pandeiros River basin, in the municipality of Bonito de Minas – MG, at Fazenda Santa Maria da Vitória, NGO Save Cerrado.

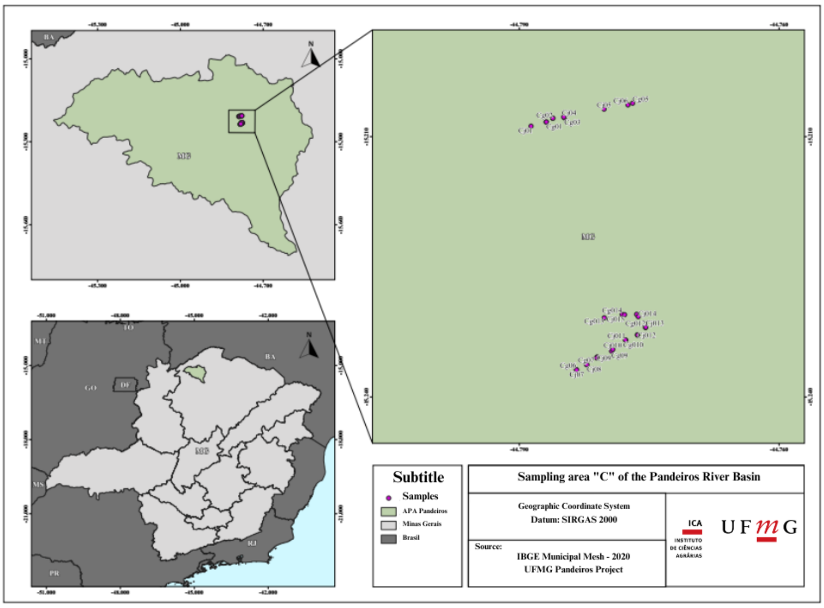
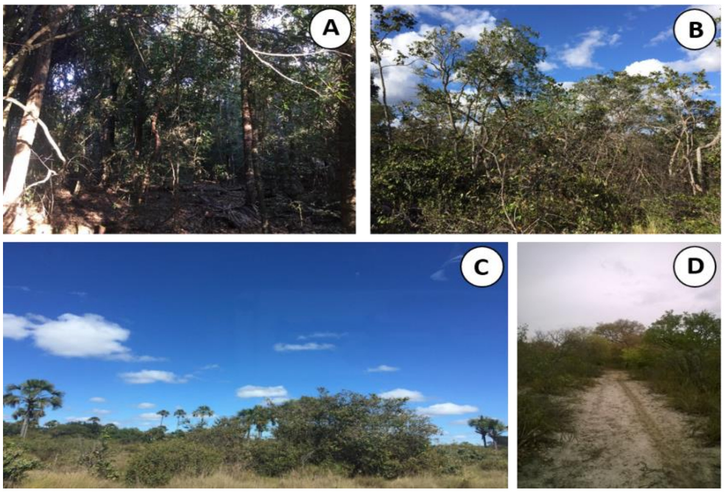


Figure 5

Overview of the areas used for collecting plant material from cagaita, cajuzinho-do-cerrado, and pequi for the assessment of genetic diversity of the species populations in the Pandeiros River basin. A - Preserved Cerrado (Fazenda Santa Maria da Vitória, belonging to the NGO Save Cerrado), B - Cerrado in natural regeneration phase (Sêo Santino's property in the Água Doce Community), C - Degraded Cerrado (São Domingos Community), and D - Roads with difficult access for reaching the sampled areas.



2.2 DNA EXTRACTION

The experiment was conducted at the Biotechnology Laboratory of the Institute of Agricultural Sciences (ICA) at UFMG, Montes Claros Campus. DNA extractions followed the procedures recommended by Doyle and Doyle (1990) with some adaptations. Fragments of leaf samples from the three species were ground in 1.5 mL microtubes using a biological sample disruptor/homogenizer under a fume hood. During the grinding procedure, 750 µL of extraction buffer [2% (w/v) PVP; 100 mM Tris-HCl pH 8.0; 20 mM EDTA pH 8.0; 1.4 M NaCl; 0.2% (v/v) β-mercaptoethanol and 2% (w/v) CTAB] were added to each microtube. The microtubes were then incubated in a water bath at 65°C for 45 minutes, followed by the addition of 750 µL of chloroform:isoamyl alcohol (24:1). To enhance the effectiveness of the chloroform, gentle inversions were performed for five minutes, and the microtubes were centrifuged for 8 minutes (10,000 x g). The supernatants were transferred to new microtubes, with the addition of 600 µL of isopropanol, and left to stand for 12 hours under refrigeration (4°C). After this period, they were centrifuged again for 8 minutes (10,000 x g) to form pellets, and the isopropanol (supernatant) was discarded. The pellets were then washed with 300 µL of PA ethanol (95%) for three repetitions. After discarding the ethanol in the third wash, the pellets were resuspended with 300 μL of TE (10 mM Tris-HCl, 1 mM EDTA pH 8.0) containing Ribonucleases A and K, both at a concentration of 40 μg/mL. The resuspensions were incubated in a water bath at 37°C for 45 minutes. After this, 30 μL of 5M NaCl and 220 μL of isopropanol were added to each microtube and kept refrigerated (4°C) for another 12 hours. After this period, another centrifugation was performed for 8 minutes (10,000 x g) to precipitate the pellets. The pellets were washed again with 300 μL of PA ethanol (95%) for three repetitions. After discarding the ethanol in the third wash, the pellets were finally resuspended with 300 μL of TE (10 mM Tris-HCl, 1 mM EDTA pH 8.0). The DNA concentrations of the samples were quantified using a spectrophotometer by reading the absorbance at 260 nm, with each unit of absorbance corresponding to a concentration of 50 ng/mL of double-stranded DNA (Sambrook et al., 1989).

2.3 AMPLIFICATION OF GENETIC MATERIAL THROUGH PCR

In the PCR (Polymerase Chain Reaction) reactions, 37 (thirty-seven) oligonucleotides (primers) ISSR (Inter Simple Sequences Repeats) from the UBC Collection (University of British Columbia, Canada) were used (Table 1).

Table 1

Oligonucleotides (primers) ISSR (Inter Simple Sequence Repeats) developed by the University of British Columbia (UBC primer set #9, Vancouver, Canada). N = (A, G, C, T); R = (A, G); Y = (C, T); B = (C, G, T) (I. e. not A); D = (A, G, T) (I. e. not C); H = (A, C, T) (I. e. not G); V = (A, C, G) (I. e. not T).

Nº	Sequence 5' → 3'	Nº	Sequence 5' → 3'
802	ATA TAT ATA TAT ATA TG	**846**	CAC ACA CAC ACA CAC ART
805	TAT ATA TAT ATA TAT AC	**847**	CAC ACA CAC ACA CAC ARC
807	AGA GAG AGA GAG AGA GT	**848**	CAC ACA CAC ACA CAC ARG
808	AGA GAG AGA GAG AGA GC	**849**	GTG TGT GTG TGT GTG TYA
813	CTC TCT CTC TCT CTC TT	**850**	GTG TGT GTG TGT GTG TYC
815	CTC TCT CTC TCT CTC TG	**851**	GTG TGT GTG TGT GTG TYG
816	CAC ACA CAC ACA CAC AT	**855**	ACA CAC ACA CAC ACA CYT
822	TCT CTC TCT CTC TCT CA	**856**	ACA CAC ACA CAC ACA CYA
825	ACA CAC ACA CAC ACA CT	**866**	CTC CTC CTC CTC CTC CTC
827	ACA CAC ACA CAC ACA CG	**870**	TGC TGC TGC TGC TGC TGC
828	TGT GTG TGT GTG TGT GA	**871**	TAT TAT TAT TAT TAT TAT
831	ATA TAT ATA TAT ATA TYA	**873**	GAC AGA CAG ACA GAC A
832	ATA TAT ATA TAT ATA TYC	**875**	CTA GCT AGC TAG CTA G
833	ATA TAT ATA TAT ATA TYG	**877**	TGC ATG CAT GCA TGC A
836	AGA GAG AGA GAG AGA GYA	**882**	VBV ATA TAT ATA TAT AT
838	TAT ATA TAT ATA TAT ARC	**888**	BDB CAC ACA CAC ACA CA
840	GAG AGA GAG AGA GAG AYT	**894**	TGG TAG CTC TTG ATC ANN NNN
843	CTC TCT CTC TCT CTC TRA	**900**	ACT TCC CCA CAG GTT AAC ACA
845	CTC TCT CTC TCT CTC TRG		

Source: Authors, 2024.

The selection of ISSR markers was based on the independence from prior genomic information. ISSR-based polymorphism analysis generates robust, highly polymorphic data for intra- and interspecific analyses. Additionally, ISSRs are considered neutral genome markers, an important characteristic for studying patterns of genetic dispersal, diversity, and natural population genetic structure.

The population amplification procedures, whose conditions are described in Table 2.

Tabela 2

PCR (Polymerase Chain Reaction) amplification conditions for ISSR oligonucleotides (primers), involving populations of cagaita, cajuzinho-do-cerrado, and pequi.

Reagents	**Working Concentration**	**Final Concentration**	**Volumes per Reaction**
H_2O ultrapure	-	-	12,4 uL
$MgCl_2$	20,0 mM	2,8 mM	3,5 uL
Tris / KCl pH 8,3	100 mM / 500 mM	10 mM / 50 mM	2,5 uL
d'NTP (A, T, C, G)	2,5 mM (each)	0,1 mM (each)	1,0 uL
Primer ISSR	4,0 uM	0,4 uM	2,5 uL
Taq polymerase	1,0 un/uL	1,0 un	0,1 uL
DNA	10 ng/uL	30 ng	3,0 uL
Total			**25,0 uL**

The reactions were programmed with an initial denaturation phase at 94°C for 5 min; followed by 35 cycles of [denaturation (94°C / 1 min), annealing (46°C to 59°C / 1 min), and extension (72°C / 2 minutes)]; and a final extension phase at 72°C for 7 min. Following these cycling steps, the apparatus maintained the reactions at 4°C until sample retrieval.

2.4 ELECTROPHORESIS AND PHOTODOCUMENTATION

The resulting amplification products were separated by horizontal electrophoresis using 1.2% (w/v) agarose gels immersed in TBE buffer (Tris-Borate 90 mM, EDTA 1 mM). At the time of application, 3 µL of type IV dye [0.125% (w/v) bromophenol blue and 10% (w/v) sucrose] and 5 µL of GelRed™ will be added to each sample. The gels will be subjected to a voltage of 120 V for 3 hours. Following electrophoresis, the gels will be analyzed using a photodocumentation system.

2.5 ANALYSIS OF GENETIC DIVERGENCE

The data were analyzed using matrices of binary data, considering the presence (1) and absence (0) of bands observed in the aforementioned gels. The coefficient used for calculating genetic similarity was the arithmetic complement of the Jaccard index (Sokal; Rohlf, 1962; Cruz et al., 2011). Based on the matrices, the hierarchical method of UPGMA (Unweighted Pair-Group Method Using Arithmetic Average) for genetic distance was applied, using the complement of the Jaccard coefficient as the dissimilarity coefficient. The average coefficient of similarity was determined by the arithmetic mean of all similarities between samples. The analyses were performed using the computational program GENES (Cruz, 2013).

The UPGMA method is commonly used in genetic diversity studies as it considers the arithmetic averages of dissimilarity measures, not characterizing dissimilarity by extreme values among individuals. Thus, this method allows for the grouping with maximum similarity within a group and dissimilarity between groups.

3 RESULTS AND DISCUSSIONS

3.1 GENETIC DIVERSITY IN THE POPULATION OF CAJUZINHO-DO-CERRADO

The adapted DNA extraction protocol provided concentrated and pure DNA samples for the cagaita and pequi samples, with all samples resulting in DNA concentrations above 150 ng/µL of TE. The A260/A280 ratio was 1.8 for most samples, indicating no significant protein contamination. However, for cajuzinho-do-cerrado, the use of the technique did not provide a sufficient amount of high-quality DNA. This was due to the leathery constitution of its leaves. In this regard, the recommendation would be to use other types of plant tissues, such as floral buds.

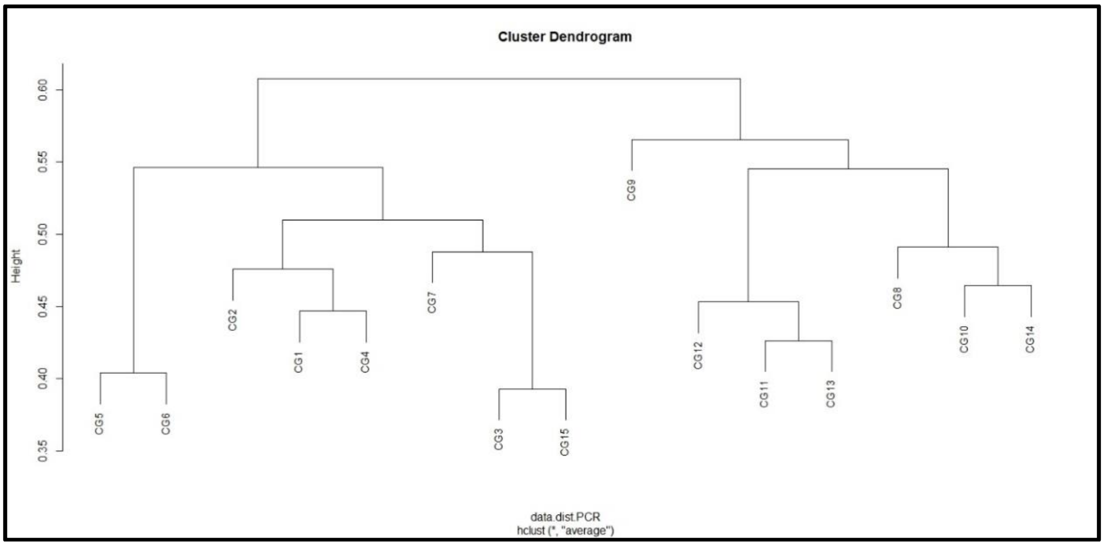
In order to address this issue, adaptations were made to the currently used protocol. The main modifications focused on the maceration steps (replacing liquid nitrogen) and sample cooling (using an ultrafreezer), reducing the process by 48 hours. As previously mentioned, validation of this modified protocol yielded positive results only for cagaita and pequi, enabling the identification of molecular polymorphisms in accesses with low morphological differentiation. The band patterns were clear and reproducible after integrating gel images using a photodocumentation system (Loccus). This indicates that the obtained DNAs have purity and concentration suitable for amplification routines and diversity studies.

3.2 GENETIC DIVERSITY IN THE CAGAITA POPULATION

The dendrograms generated for cagaita allowed us to observe genetic diversity among the accessions/matrices analyzed in the two locations, Comunidade Água Doce and Comunidade São Domingos. The greatest dissimilarity was observed among the accessions sampled in Comunidade Água Doce (Figure 6). In this population, it was possible to divide it into two large groups, each containing some subgroups. Matrix CG9 corresponds to the genetically most different accession from the others. On the other hand, matrices CG3 and CG15 are the most similar to each other. These two matrices are geographically among those with the greatest distances between them. The similarity between them is most likely due to the fruit dispersion method of the species, which is zoochory. Additionally, being in an area with regenerating Cerrado, the population of animals frequenting the area may contribute to the dispersion of genetic variability in the surrounding areas. In the same vein, we can infer that the greater genetic dissimilarity for the tree matrix CG9 is associated with the same fact. This is evidenced by the fact that the matrix is located in one of the farthest points from the property's dwelling, which facilitates the circulation of animals and thus favors fruit dispersion.

Figure 6

Dendrogram obtained by the UPGMA method from dissimilarity matrices based on molecular data among 14 cagaita matrices (CG1, CG2, CG3, CG4, CG5, CG6, CG7, CG8, CG9, CG10, CG11, CG12, CG14, CG15) sampled in Comunidade Água Doce, Bonito de Minas - MG.

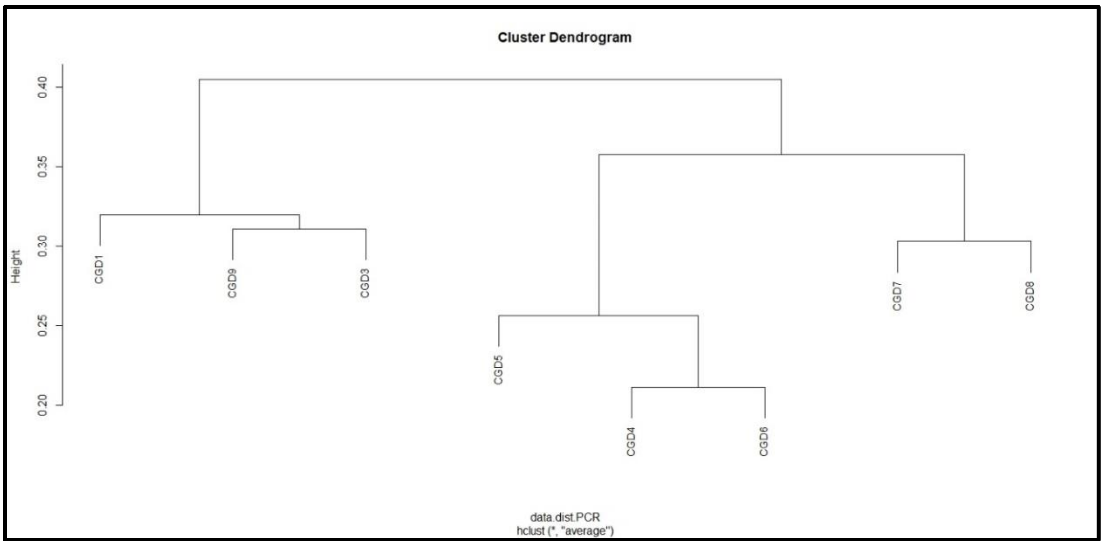


Regarding the analysis of genetic diversity for the matrices sampled in Comunidade São Domingos, the accessions were divided into three groups, with the members of the second group being more genetically divergent (Figure 7). For this location, accessions CGD4 and CGD6 show the highest genetic similarity within the evaluated population. These matrices are geographically distant in the sampled area. However, since it is a location where the Cerrado was deforested for wood charcoal production and pasture opening, it is presumed that even though there is genetic diversity among the individuals, events causing mortality of these matrices inevitably pose an imminent risk of losing this diversity. This scenario is concerning due to the extensive livestock farming activities in the area, which hinder the natural regeneration of forest species. It worsens due to the agricultural exploitation model, where rural producers culturally associate tree development in pastures as detrimental to grasses.

Additionally, as it is an area with a community, the harvesting of Cerrado fruits can be predatory to the point of hindering natural fruit dispersion and the natural regeneration of the species.

Figure 7

Dendrogram obtained by the UPGMA method from dissimilarity matrices based on molecular data among 8 cagaita matrices (CGD1, CGD3, CGD4, CGD5, CGD6, CGD7, CGD8, CGD9) sampled in Comunidade São Domingos, Bonito de Minas - MG.



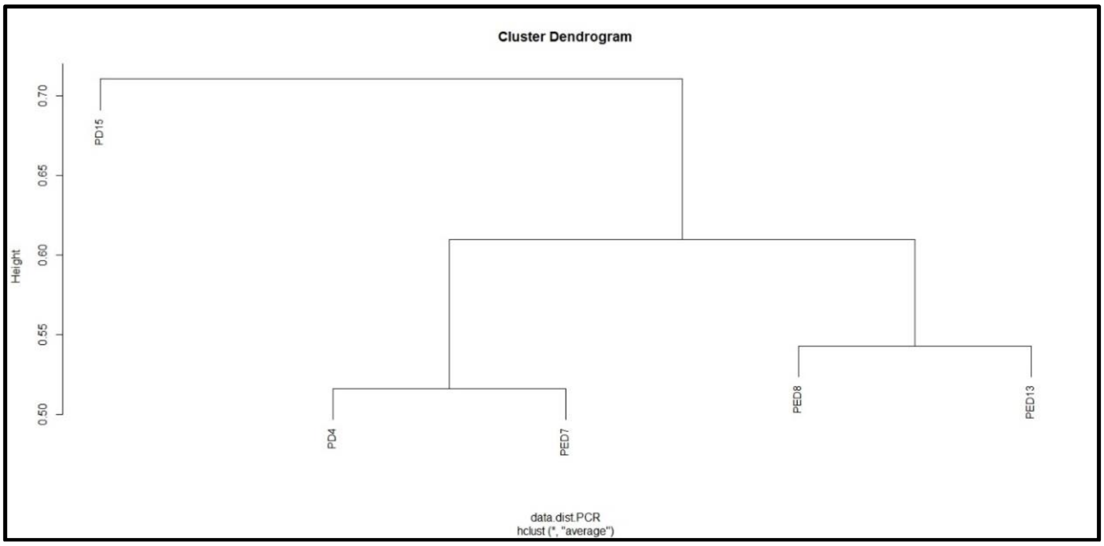
The molecular analyses for all samples collected at Fazenda Santa Maria da Vitória did not allow for DNA extraction and therefore could not proceed with amplification and microsatellite diversity studies. The collections for this location were made during a period of intense heat, and the location was situated in an area with difficult access. Consequently, two consecutive days of collection were required. It is important to note that the collection site (Bonito de Minas - MG) is approximately 250 km away from the laboratory where the molecular analyses were performed (Montes Claros – MG). This distance has an impact, as the samples were stored in a styrofoam box for an extended period, exceeding 48 hours. Therefore, these factors (time and dehydration of plant material) likely caused damage to the samples, leading to issues in laboratory analyses.

3.3 GENETIC DIVERSITY IN THE POPULATION OF PEQUI

Similarly to what was observed for cagaita, the accessions of pequi also exhibit high diversity. The population from Comunidade de São Domingos shows the highest genetic dissimilarity, with matrix PD15 being the most genetically distant (Figure 8). The genetic difference between matrices may be associated with the geographic distance between them. Matrix PD15 corresponds to a large isolated individual, distant from the other matrices, which had not been affected by deforestation activities.

Figure 8

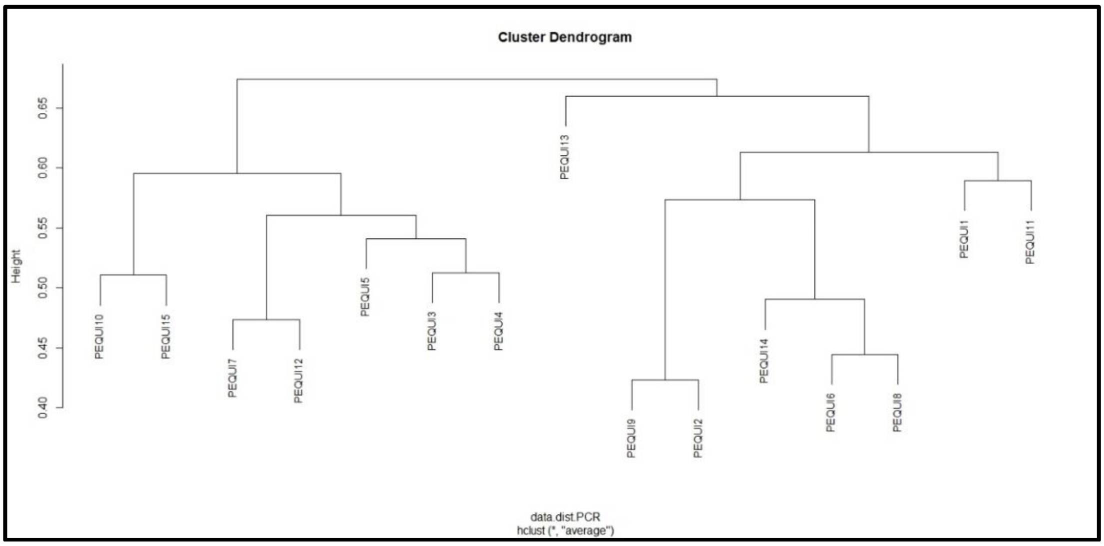
Dendrogram obtained by the UPGMA method from dissimilarity matrices based on molecular data among 5 pequi matrices (PED4, PED7, PED8, PED13, PED15) sampled in Comunidade São Domingos, Bonito de Minas - MG.



In Comunidade Água Doce, the separation of the maternal plants into two large groups was observed, where the accession PEQUI13 stands out with the highest genetic dissimilarity and is not grouped in any cluster (Figure 9). On the other hand, accessions PEQUI2 and PEQUI9 are the most genetically similar. The genetic similarity between these two matrices, which are geographically distant in the sample collection area and considering it is a location where the Cerrado is in a regenerating stage, indicates fruit dispersion and consequently, greater distribution of genetic diversity. Therefore, this result highlights that preservation initiatives preventing forest fragmentation are sufficient to contribute to an increase in genetic diversity in a given locality.

Figure 9

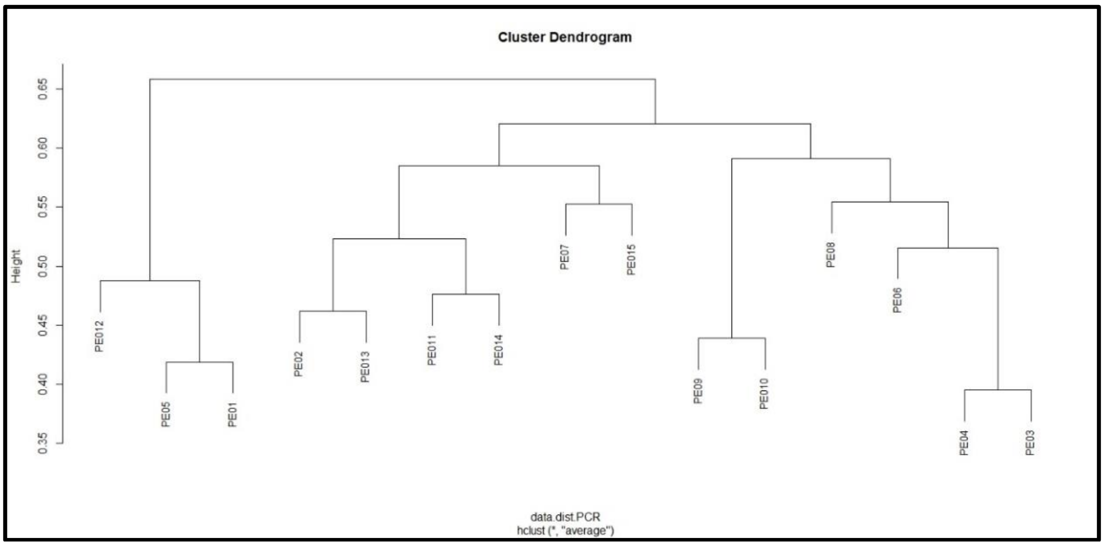
Dendrogram obtained by the UPGMA method from dissimilarity matrices based on molecular data among 15 pequi matrices (PEQUI1, PEQUI2, PEQUI3, PEQUI4, PEQUI5, PEQUI6, PEQUI7, PEQUI8, PEQUI9, PEQUI10, PEQUI11, PEQUI12, PEQUI13, PEQUI14, PEQUI15) sampled in Comunidade Água Doce, Bonito de Minas - MG.



In the population sampled at Fazenda Santa Maria da Vitória, the formation of three groups was observed, with the third being the most diverse among the grouped accessions (Figure 10). In this population, accessions PE04 and PE03 are the most similar due to their geographic proximity. In this location, where the Cerrado exhibits a higher degree of preservation, the formation of groups of genetic similarity among geographically distant matrices is noticeable. This corroborates the previous comments regarding fruit dispersion and consequently, greater distribution of genetic variability in the sampled area. In this context, the preservation of native vegetation formations is indispensable for the maintenance of genetic diversity and species conservation. In the case of the Pandeiros River basin, where there are rural communities, awareness-raising actions regarding predatory harvesting and the implementation of production models that promote the preservation of forest remnants are crucial.

Figure 10

Dendrogram obtained by the UPGMA method from dissimilarity matrices based on molecular data among 15 pequi matrices (PE01, PE02, PE03, PE04, PE05, PE06, PE07, PE08, PE09, PE010, PE011, PE012, PE013, PE014, PE015) sampled at Fazenda Santa Maria da Vitória, Bonito de Minas - MG.

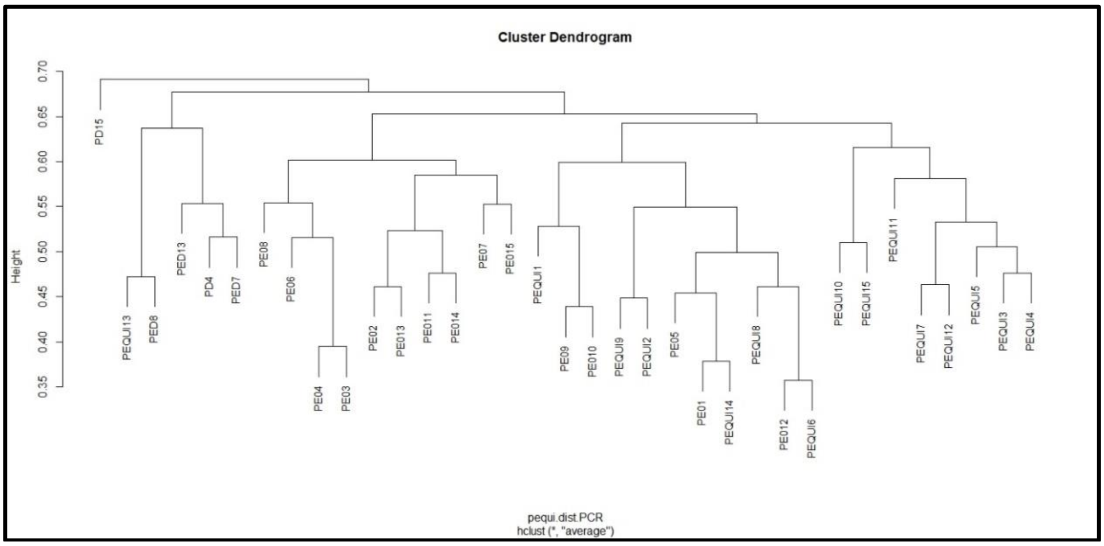


The joint analysis of the three sampled pequi populations revealed the formation of four large groups, with two of them exclusively formed by matrices with the same origin (groups 2 and 4) (Figure 11). This result already indicates a process of reduction in genetic diversity due to the fragmentation of the Cerrado in the Pandeiros River basin. The genetic variability that should be well distributed among the sampled locations does not occur naturally. Thus, there is a trend towards population isolation, which reflects the perpetuation of genotypes with a higher degree of relatedness and, therefore, more prone to environmental factors and Cerrado exploitation impacts.

The process of loss of genetic diversity is evidenced by the results of molecular analyses; however, there are reasons to take initiatives for the recovery of forest remnants and species preservation. The fact that among all the analyzed matrices, PD15 is the most genetically divergent (Figure 11) indicates that there is variability in the Cerrado present in the Pandeiros River basin. In this context, immediate actions to curb predatory exploitation of forest remnants, the adoption of actions to recover degraded areas, and the promotion of a production model in which extractivism is profitable for communities and environmentally friendly are indispensable. This way, it will be possible to ensure the maintenance of genetic diversity present there and, consequently, that species can perpetuate in the environment.

Figure 11

Dendrogram obtained by the UPGMA method from dissimilarity matrices based on molecular data among 40 pequi matrices sampled at Fazenda Santa Maria da Vitória (PE01, PE02, PE03, PE04, PE05, PE06, PE07, PE08, PE09, PE010, PE011, PE012, PE013, PE014, PE015), Comunidade Água Doce (PEQUI1, PEQUI2, PEQUI3, PEQUI4, PEQUI5, PEQUI6, PEQUI7, PEQUI8, PEQUI9, PEQUI10, PEQUI11, PEQUI12, PEQUI13, PEQUI14, PEQUI15), and Comunidade São Domingos (PED4, PED7, PED8, PED13, PED15) in Bonito de Minas - MG.



4 CONCLUSION

The molecular analyses indicated the presence of genetic diversity in the populations of pequi and cagaita analyzed in the three locations. However, it was evidenced that there were groupings by similarity among the matrices, forming small isolated populations. From a conservation standpoint, this corresponds to an indication that if forest fragmentation in the Pandeiros River basin continues, geographical isolation will increasingly influence the distribution of genotypes, and consequently, there will be a greater propensity for lower genetic diversity in forest fragments. Therefore, species will be more prone to genetic erosion risks, with loss of diversity and compromise of species perpetuation in the future.

The genetic diversity analysis of cajuzinho-do-cerrado was not successfully concluded. However, considering that it is a species with fruit dispersion by zoochory, similar to what occurs with cagaita and pequi, its geographical distribution pattern is very close to what is observed for these two species. Thus, it is highly likely that cajuzinho-do-cerrado populations exhibit genetic diversity in the Pandeiros River basin. However, this diversity may be isolated due to forest fragmentation.

In the current scenario, where cagaita and pequi matrices showed genetic variability, actions that contribute to the preservation of these populations are essential, not only through in situ conservation but also through the promotion of future genetic improvement programs of the species, such as the establishment and evaluation of progeny tests, for production or conservation purposes.

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