



BETWEEN PHENOLIC COMPOUNDS AND POLYMORPHIC PROFILES: A ROBUST STRATEGY FOR GENETIC STUDIES IN *CARYOCAR BRASILIENSE*

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ABSTRACT

Objective: This study aimed to optimize a leaf DNA extraction protocol based on the CTAB method, with adaptations to mitigate the effects of phenolic compounds, and to evaluate the efficiency of ISSR markers in the genetic discrimination of natural populations of the species.

Theoretical Framework: *Caryocar brasiliense* Cambess., commonly known as *pequizeiro*, is a native species of the Brazilian Cerrado with ecological, economic, and cultural relevance. The genetic characterization of its populations is essential for conservation strategies and genetic improvement programs. However, the presence of secondary compounds in the leaves hinders the extraction of high-quality DNA, compromising molecular analyses.

Method: Samples from five regions in northern Minas Gerais were analyzed, with experimental adjustments to the extraction buffer, deproteinization time, and the number of pellet washing steps. For molecular analysis, 20 ISSR primers were tested, of which 11 produced satisfactory amplification.

Results and Discussion: The samples showed high variability in DNA concentration and purity, with A260/A280 ratios ranging from 0.68 to 2.16, demonstrating the effectiveness of the protocol even in partially degraded leaves. The introduction of BSA (25 mg/mL) in PCR reactions significantly increased amplification efficiency, especially with primer UBC 840, resulting in polymorphic and informative profiles, particularly in the Mirabela-MG population. The results confirm that the optimized protocol is effective in obtaining high-quality DNA, being suitable for genetic studies in *C. brasiliense*. The combination of an adjusted extraction technique and the application of ISSR markers, supported by additives such as BSA, represents a robust and economically viable approach for the genetic characterization of the species, supporting conservation, management, and the valorization of Cerrado genetic resources.

Keywords: *Caryocar Brasiliense*, Genetic Diversity, Cerrado, DNA Extraction, ISSR, BSA.

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ENTRE COMPOSTOS FENÓLICOS E PERFIS POLIMÓRFICOS: UMA ESTRATÉGIA ROBUSTA PARA ESTUDOS GENÉTICOS COM *CARYOCAR BRASILIENSE*

ABSTRACT

Objetivo: Este estudo teve como objetivo otimizar um protocolo de extração de DNA foliar baseado no método CTAB, com adaptações para mitigar os efeitos de compostos fenólicos, e avaliar a eficiência de marcadores *ISSR* na discriminação genética de populações naturais da espécie.

Referencial teórico: *Caryocar brasiliense* Cambess., comumente conhecido como pequizeiro, é uma espécie nativa do Cerrado brasileiro com relevância ecológica, econômica e cultural. A caracterização genética de suas populações é essencial para estratégias de conservação e programas de melhoramento genético. No entanto, a presença de compostos secundários nas folhas dificulta a extração de DNA de alta qualidade, comprometendo as análises moleculares.

Método: Amostras de cinco regiões do norte de Minas Gerais foram analisadas, com ajustes experimentais no tampão de extração, no tempo de desproteinização e no número de etapas de lavagem do pellet. Para a análise molecular, foram testados 20 primers *ISSR*, dos quais 11 apresentaram amplificação satisfatória.

Resultados e discussão: As amostras apresentaram alta variabilidade na concentração e pureza do DNA, com razões A260/A280 variando de 0,68 a 2,16, demonstrando a eficácia do protocolo mesmo em folhas parcialmente degradadas. A introdução de *BSA* (25 mg/mL) nas reações de *PCR* aumentou significativamente a eficiência da amplificação, especialmente com o primer *UBC 840*, resultando em perfis polimórficos e informativos, particularmente na população de Mirabela-MG. Os resultados confirmam que o protocolo otimizado é eficaz na obtenção de DNA de alta qualidade, sendo adequado para estudos genéticos em *C. brasiliense*. A combinação de uma técnica de extração ajustada e da aplicação de marcadores *ISSR*, com o suporte de aditivos como a *BSA*, representa uma abordagem robusta e economicamente viável para a caracterização genética da espécie, subsidiando a conservação, o manejo e a valorização dos recursos genéticos do Cerrado.

Palavras-chave: *Caryocar Brasiliense*, Diversidade Genética, Cerrado, Extração de DNA, *ISSR*, *BSA*.

ENTRE COMPUESTOS FENÓLICOS Y PERFILES POLIMÓRFICOS: UNA ESTRATEGIA ROBUSTA PARA ESTUDIOS GENÉTICOS EN *CARYOCAR BRASILIENSE*

RESUMEN

Objetivo: Este estudio tuvo como objetivo optimizar un protocolo de extracción de ADN foliar basado en el método CTAB, con adaptaciones para mitigar los efectos de los compuestos fenólicos, y evaluar la eficiencia de los marcadores *ISSR* en la discriminación genética de poblaciones naturales de la especie.

Marco teórico: *Caryocar brasiliense* Cambess., comúnmente conocido como pequizeiro, es una especie nativa del Cerrado brasileño con relevancia ecológica, económica y cultural. La caracterización genética de sus poblaciones es esencial para las estrategias de conservación y los programas de mejoramiento genético. Sin embargo, la presencia de compuestos secundarios en las hojas dificulta la extracción de ADN de alta calidad, lo que compromete los análisis moleculares.

Método: Se analizaron muestras de cinco regiones del norte de Minas Gerais, con ajustes experimentales en el tampón de extracción, el tiempo de desproteinización y el número de pasos de lavado del pellet. Para el análisis molecular, se probaron 20 cebadores *ISSR*, de los cuales 11 produjeron una amplificación satisfactoria.

Resultados y discusión: Las muestras mostraron una alta variabilidad en la concentración y pureza del ADN, con relaciones A260/A280 que oscilaron entre 0,68 y 2,16, lo que demuestra la eficacia del protocolo incluso en hojas parcialmente degradadas. La introducción de *BSA* (25 mg/mL) en las reacciones de *PCR* aumentó significativamente la eficiencia de la amplificación, especialmente con el cebador *UBC 840*, resultando en perfiles polimórficos e informativos, particularmente en la población de Mirabela-MG. Los resultados confirman que el protocolo optimizado es eficaz para obtener ADN de alta calidad, siendo adecuado para estudios genéticos en *C. brasiliense*. La combinación de una técnica de extracción ajustada y la aplicación de marcadores *ISSR*, con el apoyo de aditivos como la *BSA*, representa un enfoque robusto y económicamente viable para la caracterización genética de la especie, apoyando la conservación, el manejo y la valorización de los recursos genéticos del Cerrado.



Palabras clave: *Caryocar Brasiliense*, Diversidad Genética, Cerrado, Extracción de ADN, ISSR, BSA.

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1 INTRODUCTION

The tree commonly known as *pequizeiro* belongs to the class Magnoliopsida (dicotyledons), order Malpighiales, family Caryocaraceae, and genus *Caryocar* L., which comprises about 16 species, 12 of which occur in Brazil (Lorenzi, 2002). One of these species is *Caryocar brasiliense* Cambess., a tree that can reach 8 to 12 meters in height, with a trunk circumference between two and three meters, rough and wrinkled dark gray bark, and fissured surface. The fruit, known as *pequi*, is a drupe containing one to four seeds surrounded by the mesocarp, which is fleshy and light yellow in color. The mesocarp is divided into two layers: an external whitish layer and an internal yellowish one, both with a strong and characteristic odor and taste (Araújo *et al.*, 2018; Araujo, 1995; Santos *et al.*, 2013). The endocarp is woody, with a muricate surface (covered by short, hard excrescences) and spiny internally, housing subreniform (almost kidney-shaped) seeds with an embryo featuring a straight or slightly curved radicle (Araujo, 1995).

The *pequizeiro* is a native plant of the Cerrado and holds great environmental, social, and economic importance (Araújo *et al.*, 2018; Gribel & Hay, 1993). Its fruits and pulp have high nutritional value and are widely used for oil extraction, both from the mesocarp and the seed (Nascimento-Silva & Naves, 2019). The fruit is traditionally consumed in the Southeast, Center-West, North, and Northeast regions of Brazil, contributing to job creation and income generation for many farming families in these areas (Pinto *et al.*, 2016). Due to its economic value and potential uses, the *pequizeiro* is considered a priority species in domestication, conservation, and genetic improvement programs for native Cerrado species (Collevatti *et al.*, 2001; Gomes *et al.*, 2022; Melo Júnior *et al.*, 2004). Beyond its economic relevance, the *pequizeiro* is also a cultural symbol, regarded as part of the natural heritage of the Cerrado (Pinto *et al.*, 2016).

Genetic diversity within a species is essential to ensure its adaptive potential in the face of environmental adversities, reflecting the capacity of populations to remain viable and reproductive over time (Reed *et al.*, 2003; Ribeiro & Rodrigues, 2006). In this sense, studying the diversity and genetic structure of *C. brasiliense* populations is fundamental to understand



how this variability is distributed and which environmental or species-intrinsic factors influence this variation (Gomes *et al.*, 2022). Various molecular techniques are available to assess genetic diversity by detecting polymorphisms in DNA sequences, such as ISSR (Inter Simple Sequence Repeats) and SSR (Simple Sequence Repeats) markers, among others (Ng & Tan, 2015). The ISSR technique is PCR-based and involves the amplification of DNA segments located between two identical microsatellite regions oriented in opposite directions (Reddy *et al.*, 2002).

In general, studies aiming at identifying and characterizing genetic diversity through molecular techniques require the analysis of many individuals, which demands rapid and efficient DNA extraction methods. These methods must provide DNA in adequate quantity and quality, which is challenging in Cerrado plants like *pequizeiro*. Despite the existence of several published protocols for plant DNA isolation, the presence of high levels of secondary compounds in the leaves — a common feature in Cerrado species — (Novaes *et al.*, 2013) poses an obstacle for PCR-based molecular studies. Moreover, in many plant species, adaptations to ISSR protocols and amplification conditions become necessary to optimize data generation.

Given this, the aim of this study was to optimize and establish the leaf DNA extraction protocol for *C. brasiliense* using the CTAB protocol, as well as to obtain useful ISSR markers for the genetic characterization of the species.

2 METHODOLOGY

2.1 SAMPLING PROTOCOL

Prospection trips were carried out for the selection and marking of adult *C. brasiliense* trees in five traditionally pequi-producing regions in northern Minas Gerais, during the fruiting period (December to March) of the years 2022 and 2023 (Table 1). For each selected individual, young leaves were collected, subsequently identified, placed in bags with silica, and stored under refrigeration.



Table 1

*Distribution of *Caryocar brasiliense* sampling areas in northern Minas Gerais, indicating the type of land use and the environmental conservation status at each location.*

Location (cities)	Environmental conservation status	Latitude	Longitude
Japonvar	Preserved	-15.989460°	-44.295875°
	Degraded	-15.973904°	-44.256056°
Conego Marinho	Preserved	-14.927188°	-44.640316°
	Degraded	-15.202066°	-44.435029°
Bonito de Minas	Preserved	-15.284692°	-44.766961°
	Degraded	-15.287448°	-44.813390°
Mirabela	Preserved	-16.427540°	-44.034640°
	Degraded	-16.423076°	-44.036891°
Montezuma	Preserved	-15.165574°	-42.487991°
	Degraded	-15.194133°	-42.631688°

2.2 DNA EXTRACTION

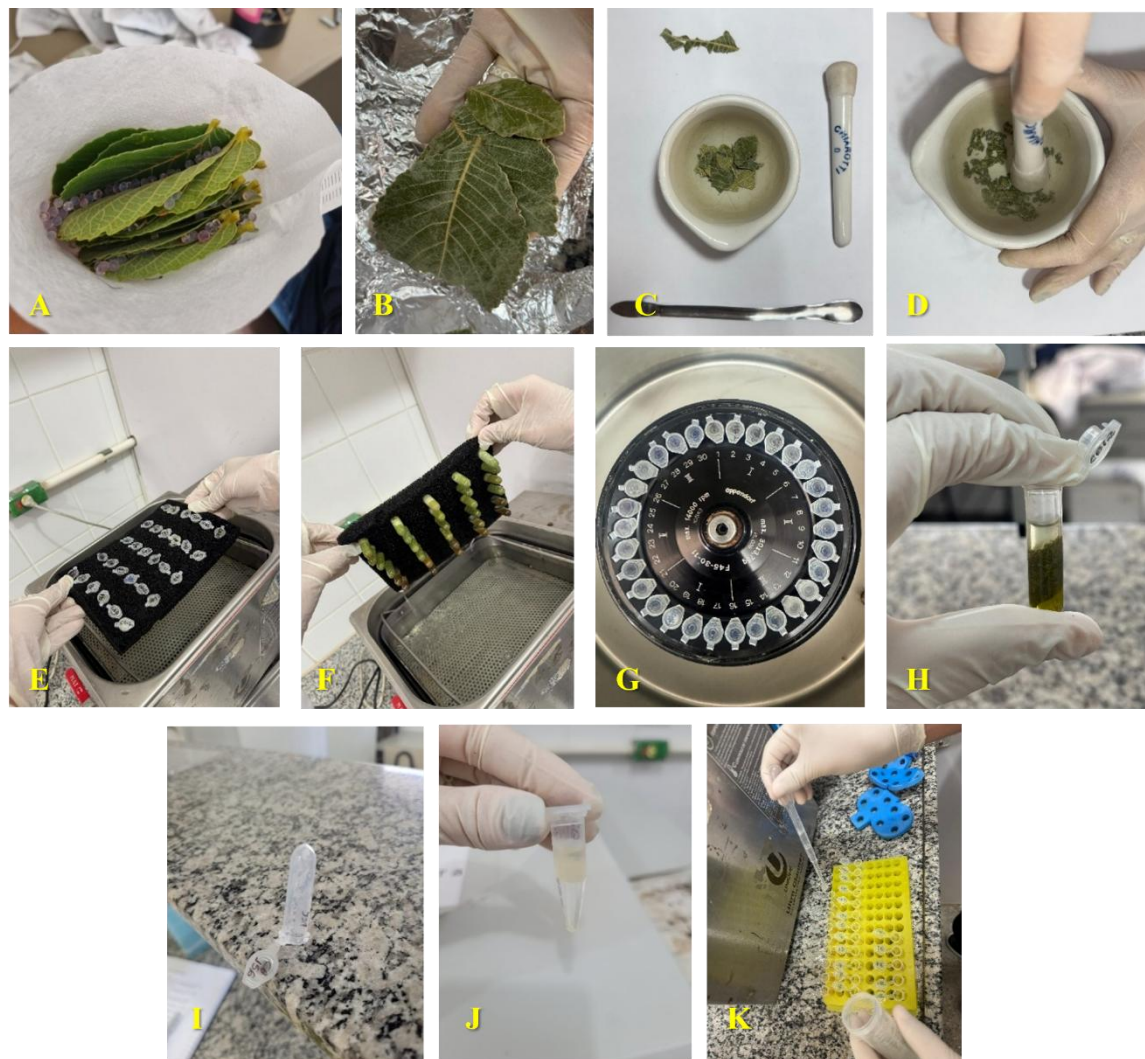
The experiment was conducted at the Laboratory of Bioprospecting and Genetic Resources of the Universidade Estadual de Montes Claros (UNIMONTES). DNA extraction followed the protocol of Doyle and Doyle (1987), with some modifications (Fig. 1). The tissues were macerated in a container using liquid nitrogen, followed by the addition of 800 µL extraction buffer (2% CTAB, NaCl, 1% PVP [w/v], 0.2% β-mercaptoethanol [v/v], 20 mM EDTA, 100 mM Tris-HCl). These samples were then incubated in a water bath at 65°C for at least 30 minutes and up to one hour, being agitated every 10 minutes. After incubation, proteins were removed by adding 600 µL of chloroform:isoamyl alcohol (CIA) in a 24:1 ratio. The tubes were gently agitated for five minutes by inverting them at least 20 times until a homogeneous emulsion was formed. Subsequently, the samples were centrifuged at 12,000 rpm for 10 minutes. The supernatant was then transferred to new tubes and incubated in a water bath at 37°C for 30 minutes with the addition of 10 µL of RNase (10 mg/mL). To the aqueous phase, 1/10 of the volume (60 µL) of 10% CTAB solution (1.4 M NaCl) was added and mixed for five minutes until completely homogenized. The extraction was repeated with 600 µL of CIA. After this step, the samples were centrifuged at 12,000 rpm for 10 minutes at 4°C. The supernatant was then transferred to new tubes, and the nucleic acids were precipitated by adding 450 µL of chilled isopropanol. The obtained samples were kept at -20°C for at least two hours and then centrifuged at 14,000 rpm for 10 minutes at 4°C. After centrifugation, 200 µL of 70% chilled ethanol was added to the DNA pellet, keeping the samples at room temperature for 5 to 10 minutes, followed by centrifugation at 14,000 rpm for 10 minutes at 4°C. Finally, the pellets were dried under a laminar flow hood for approximately 30 minutes and resuspended in 50 µL



of TE/RNase solution (5 μ L of RNase + 49.95 mL of TE) for DNA solubilization and subsequent quantification.

Figure 1

Steps of the DNA extraction from *Caryocar brasiliense* leaves.



A – collected leaves stored in paper filters with silica to retain moisture, B – leaves frozen in a freezer (-20°C), C – leaves in a crucible ready for maceration, D – mechanical maceration with liquid nitrogen, E – leaf extract with extraction buffer (2% CTAB) incubated in a water bath, F – agitation of the samples during the water bath process, G – centrifugation of the samples, H – samples after centrifugation, I – sample after supernatant removal, addition of CIA, and new centrifugation, J – addition of 10% CTAB, K – DNA pellet drying after the extraction process.

The DNA concentrations of the samples were quantified using a spectrophotometer by measuring absorbance at 260 nm, with each absorbance unit corresponding to a concentration of 50 ng/mL of double-stranded DNA



2.2 DNA AMPLIFICATION THROUGH POLYMERASE CHAIN REACTION (PCR)

Twenty primers were tested, of which 14 belong to the University of British Columbia (UBC) collection, along with the primers MANNY, GOOLY, BECKY, OMAR, and CHRIS (Table 2), with 11 showing amplification for *Caryocar brasiliense*. To assess the viability of the extracted DNA and select the ISSR primers, two PCR protocols were used (Tables 3 and 4), with a specific amplification program for each protocol.

Table 2

ISSR (Inter Simple Sequence Repeats) oligonucleotide primers tested for Caryocar brasiliense, with their respective sequences and annealing temperatures (AT). Primers that did not show amplification in the species are indicated.

Primer	Sequence 5'-3'	AT (°C)
BECK	CAC ACA CAC CAC CAC A YC	52°
CHRIS	CAC ACA CAC ACA CAY G GAG	52°
GOOLY	GTG TGT GTG TGT GTYG	Does not amplify in the specie
MANNY	CAC CAC CAC CCA RC	54°
OMAR	GAG GAG GAG GAG RC CAC	59°
TERRY	GTG GTG GTG GTG GGT GRC	Does not amplify in the specie
UBC 809	AGA GAG AGA GAG AGA GG	56°
UBC 810	GAG AGA GAG AGA GAG AT	Does not amplify in the specie
UBC 821	GTG TGT GTG TGT GTG TT	Does not amplify in the specie
UBC 827	ACA CAC ACA CAC ACA CG	50°
UBC 840	GAG AGA GAG AGA GAG AYT	54°
UBC 851	GTG TGT GTG TGT GTG TYG	Does not amplify in the specie
UBC 855	ACACACACACACACACYT	47°
UBC 856	ACA CAC ACA CAC ACA CYA	54°
UBC 880	GGA GAG GAG AGG AGA	52°
UBC 882	VBV ATA TAT ATA TAT AT	Does not amplify in the specie
UBC 883	BVB TAT ATA TAT ATA TA	Does not amplify in the specie
UBC 891	HVH TGT GTG TGT GTG TG	52°
UBC 899	CAT GGT GTT GGT CAT TGT TCC A	Does not amplify in the specie

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)



Table 3

PCR I (Polymerase Chain Reaction) conditions for amplification with ISSR oligonucleotides in Caryocar brasiliense populations.

Reagent	Stock concentration	Final concentration	Volume reaction	per
Ultrapure H ₂ O	-	-	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	
dNTP (A, T, C, G)	2.5 mM (cada)	0.1 mM	1.0 uL	
ISSR primer	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	

Table 4

PCR II (Polymerase Chain Reaction) conditions, with modifications, used for amplification with ISSR oligonucleotides in Caryocar brasiliense populations. This condition includes the addition of BSA for amplification optimization.

Reagent	Stock concentration	Final concentration	Volume reaction	per
Ultrapure H ₂ O	-	-	11.2 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	
dNTP (A, T, C, G)	2.5 mM (cada)	0.1 mM	1.0 uL	
ISSR primer	4.0 uM	0.4 uM	2.5 uL	
BSA	25mg/mL	25mg/mL	1.2 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 PCR reactions were conducted in a thermal cycler under the following amplification program: an initial denaturation step at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing between 47°C and 56°C (according to each primer's temperature) for 1 minute, and extension at 72°C for 2 minutes. At the end of the cycles, a final extension was performed at 72°C for 7 minutes. After amplification, the reactions were maintained at 4°C until sample collection.

2.3 ELECTROPHORESIS AND DOCUMENTATION

The resulting amplification products were separated by horizontal electrophoresis using 1% (w/v) agarose gels immersed in 1X SB (Sodium Borate) buffer. At the time of loading, 3 µL of loading dye type IV (0.125% [w/v] bromophenol blue and 10% [w/v] sucrose) and 5 µL of GelRed™ were added to each sample. The gels were subjected to a voltage of 120 V for 1



hour and 30 minutes. After electrophoresis, the gels were analyzed using a photodocumentation system — Locus Biotechnologia L-PIX.

2.4 STATISTICAL ANALYSIS

To estimate the polymorphic information content (PIC) of the ISSR markers used in this study, the formula proposed by ROLDÁN-RUIZ *et al.*, 2000, recommended for dominant markers, was applied. Since these markers do not allow distinguishing between heterozygous and dominant homozygous individuals, the PIC was calculated based on band presence frequencies as follows: $PIC_i = 2f_i(1 - f_i)$, where f_i represents the frequency of band presence (dominant allele) at a given locus. This approach allows evaluating the discriminatory power of each marker, with PIC values closer to 0.5 indicating greater genetic variability among the analyzed individuals.

3 RESULTS AND DISCUSSION

During the process of isolating and identifying DNA from *pequizeiro* leaves, it is crucial to adequately select the leaf tissue, giving preference to younger leaves due to the high concentration of secondary compounds that accumulate throughout leaf development in this species (Cruz *et al.*, 2022). These secondary metabolites play an essential role in the plant's chemical defense mechanisms and tend to concentrate in structures most relevant to its growth and propagation, such as leaves and seeds (Fonseca *et al.*, 2018; Novaes *et al.*, 2013). Leaves, in particular, accumulate high levels of phenolic compounds, which act as defenses against pathogens and protect against intense solar radiation (Erb & Kliebenstein, 2020). Such contaminant compounds adhere firmly to nucleic acids during DNA isolation, directly or indirectly interfering with the enzymatic reactions involved in the extraction and amplification stages of the genetic material.

The logistics of collecting and transporting plant material, especially in studies of geographically distributed natural populations, present another important challenge to be considered. In many cases, it is not always possible to strictly follow all the ideal conservation parameters, resulting in samples with varying degrees of leaf integrity. Therefore, DNA extraction from *Caryocar brasiliense* leaves requires methodological adjustments that ensure the viability of analysis, even under less-than-ideal material preservation conditions.

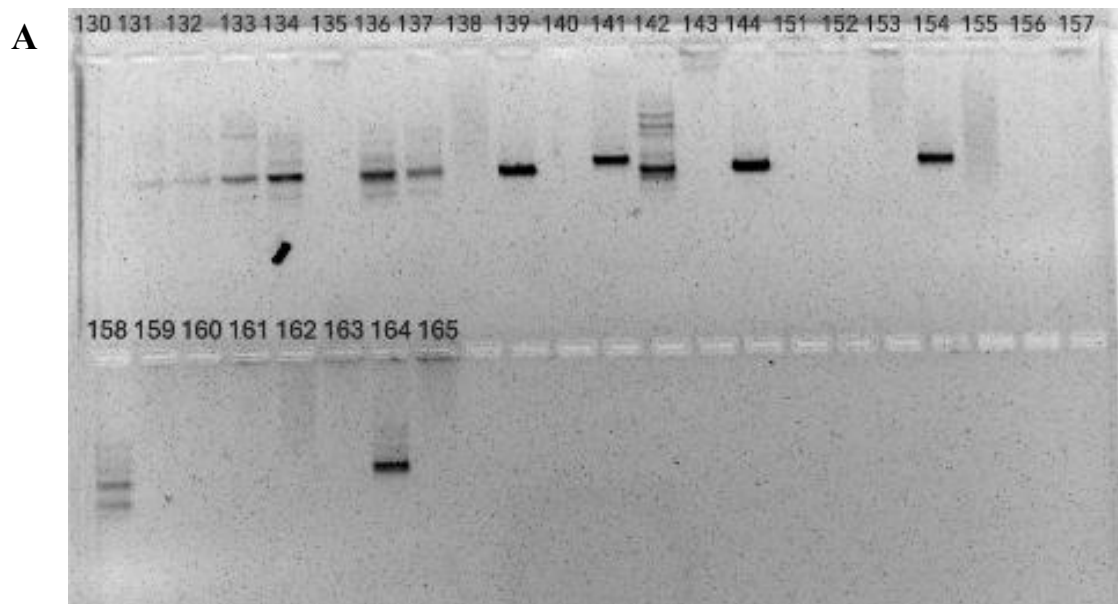


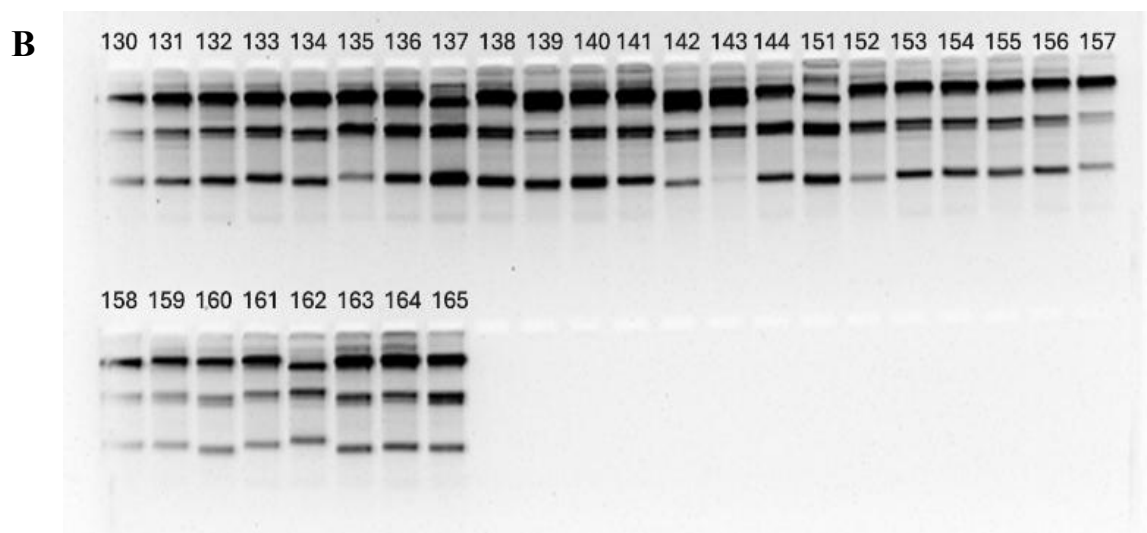
To overcome these limitations, modifications were made to the DNA extraction protocol, including increasing the volume of extraction buffer, extending the deproteinization time, and reducing the number of washes with 70% ethanol. These changes allowed for the extraction of intact DNA with satisfactory quality, as verified by spectrophotometry. Most samples showed an A260nm/A280nm ratio between 1.2 and 2.0 — a range recommended by Ferreira & Grattapaglia (1998) as indicative of adequate purity for molecular analyses (see Supplementary Material).

Regarding DNA fragment amplification, the introduction of BSA in the PCR reactions significantly contributed to increased yield and clearer band definition (Figure 2B). Similar results were reported by Farell & Alexandre (2012), who highlighted the role of BSA as an effective coadjuvant, especially in systems with high phenolic content and GC-rich genomic regions, promoting efficient amplification and clear visualization of products in the gel.

Figure 2

*Band profiles obtained by 1.2% agarose gel electrophoresis from the PCR reaction using primer UBC 840 without (A) and with (B) addition of BSA (25 mg/mL), performed on individuals from the *Caryocar brasiliense* population in the municipality of Mirabela – MG. The annealing temperature was 56 °C. The numbers above the lanes correspond to the individuals identifiers.*





The amplification of genomic DNA from *Caryocar brasiliense* individuals from the Mirabela – MG population using the UBC 840 primer revealed marked differences between reactions conducted with and without the addition of BSA, as shown in Figure 2 (A and B). In Figure 2A, which corresponds to PCR without BSA, a poorly informative band profile is observed, with sparse amplifications and a low number of bands per sample. Several individuals showed no visible bands (such as those identified between numbers 145 and 165), indicating failures or inefficiency in amplification. Even among individuals that displayed bands (such as 133, 135, 137, 140, 141, and 143), the intensity was variable and generally weak, suggesting possible Taq polymerase inhibition, DNA degradation, or the presence of inhibitory compounds co-extracted with the DNA — common in plant species.

On the other hand, Figure 2B, which shows the profile obtained with the addition of BSA, reveals significantly more efficient and consistent amplification across samples. All samples exhibit multiple bands with good resolution and intensity, indicating a typical ISSR primer pattern. The addition of BSA proved effective in overcoming potential inhibitors present in the samples, likely phenolic compounds or polysaccharides, which are frequent in Cerrado species (Novaes *et al.*, 2013). Moreover, the use of BSA contributed to better reproducibility and reliability in the amplification profiles, allowing for the identification of greater polymorphism among individuals in the population.

These results corroborate previous studies that highlight BSA as a stability-promoting additive in PCR of tropical plants (Jiang *et al.*, 2023; Samarakoon *et al.*, 2013). Its action in neutralizing inhibitors enhances amplification efficiency and, consequently, the quality of genetic analyses, especially in species originating from environments with a high load of secondary metabolites, such as the Brazilian Cerrado.



The PIC (Polymorphic Information Content) values obtained in this study ranged from 0.2577 to 0.3750, with an overall average of approximately 0.317 (Table 5). According to Botstein *et al.*, 1980, these values indicate that the markers used possess a moderate level of informativeness, making them suitable for genetic diversity studies. When compared to the results of Serrote *et al.*, 2019, who also assessed the genetic variability of *Caryocar brasiliense* populations, it is observed that the values obtained in this work are slightly lower than those reported by the authors, who reached PIC values above 0.4 for several markers. This difference may be related to the type of marker used, the number of loci analyzed, as well as the intrinsic genetic variability of the evaluated populations.

Table 5

Polymorphic information content (PIC) values obtained for each ISSR marker in five natural populations of Caryocar brasiliense located in northern Minas Gerais. PIC values are presented by population, along with the mean PIC value per marker (PIC) ± standard deviation (SD).

Primers	Sampling populations					PIC (± SD)
	Bonito de Minas	Japonvar	Mirabela	Montezuma	Conego Marinho	
809	0.317	0.349	0.383	0.428	0.369	0.369±0.041
827	0.384	0.313	0.353	0.312	0.344	0.341±0.029
840	0.190	0.383	0.416	0.182	0.369	0.308±0.112
855	0.305	0.273	0.246	0.327	0.290	0.288±0.031
856	0.336	0.348	0.287	0.311	0.404	0.337±0.044
880	0.396	0.293	0.403	0.361	0.422	0.375±0.051
891	0.290	0.308	0.310	0.336	0.405	0.329±0.045
Becky	0.239	0.131	0.287	0.365	0.267	0.257±0.08
Chris	0.160	0.390	0.194	0.341	0.223	0.261±0.099
MANNY	0.291	0.231	0.220	0.250	0.407	0.28±0.076
OMAR	0.259	0.322	0.288	0.359	0.273	0.3±0.04

Thus, the obtained data reinforces the existence of genetic diversity among the sampled populations in northern Minas Gerais, highlighting the importance of conservation strategies that consider multiple localities, especially in regions subjected to fragmentation and anthropogenic pressure.

4 CONCLUSION

The results obtained in this study demonstrate that the adaptation of the CTAB protocol for leaf DNA extraction from *Caryocar brasiliense* was successful, especially in light of the challenges posed by the high concentration of secondary compounds typical of Cerrado species.



The applied modifications — such as increasing the volume of extraction buffer, extending the incubation time for deproteinization, and reducing the number of washing steps — contributed significantly to the recovery of DNA with high quality, purity, and concentration, even in partially degraded samples.

The application of ISSR markers, particularly the UBC 840 primer in optimized reactions with the addition of BSA, resulted in clear, reproducible amplification profiles with a high degree of polymorphism among the tested individuals. This performance highlights the potential of the methodology for the genetic characterization of natural *pequizeiro* populations, providing relevant data on intraspecific variability.

The protocol established in this work represents an efficient, robust, and economically viable tool for genetic and molecular studies in *C. brasiliense*, and it can be widely applied in research on genetic diversity, population structure, conservation, and breeding programs for the species. Furthermore, the results reinforce the importance of methodologies adjusted to the physiological particularities of native Cerrado species, contributing to the consolidation of scientific strategies aimed at preserving regional biodiversity.

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