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Multivariate analysis in the selection of baru genotypes

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Abstract
In terms of extension and flora, the *cerrado* is the second largest Brazilian biome, which harbors native fruit species that produce fruits with different characteristics, attractive colors, and exclusive flavors, such as baru. However, the uncontrolled and unsustainable exploitation of resources in this biome has been a cause of great concern worldwide. In view of the importance and productive potential of baru, breeding programs should characterize its fruits and seeds in seedling formation by univariate and multivariate analyses and frequency histograms. This study describes an experiment laid out in a completely randomized design with seven populations of baru from the south and southeast regions of the state of Goiás, Brazil. Five replicates were used, totaling 35 experimental plots, with 15 fruits per replicate. For estimated 100-fruit weight, estimated 100-seed weight, and pulp thickness, the populations of Caldas Novas and Bom Jesus stood out with the highest means for some of the evaluated traits. As for frequency intervals, the population of Caldas Novas showed the largest amplitude for seed length, whereas the Edéia population exhibited the greatest amplitude for seed width. In the analysis of the fruit, the population of Caldas Novas showed the largest amplitudes for both length and width. After genetic dissimilarity analysis, the UPGMA clustering method grouped the Caldas Novas and Bom Jesus populations into one group and the others into another. The highest correlation indices observed were between 100-fruit weight and 100-seed weight (87%) and between seed length and fruit length (86%). Clustering based on the k-means method resulted in two groups formed: one comprising the populations of Caldas Novas, Aloândia, and Bom Jesus the other the remaining populations.
Keywords: biometrics; clustering; *Dipteryx alata* Vogel; plant breeding.

Análises multivariadas na seleção de genótipos de barueiro

Resumo:
Em termos de extensão e flora, o cerrado constitui o segundo maior bioma brasileiro e apresenta espécies frutíferas nativas, que produzem frutos com características diferenciadas, cores atraentes e sabores exclusivos, como o barueiro. Contudo, a exploração descontrolada e insustentável dos recursos deste bioma vem sendo motivo de grande preocupação em todo mundo. Diante da importância e potencial produtivo do barueiro, torna-se necessário a caracterização de frutos e sementes na formação de mudas através de análises univariadas, multivariadas e histogramas de intervalo de frequência em programas de melhoramento. O delineamento utilizado foi inteiramente ao acaso, com sete populações de barueiros provenientes das regiões sul e sudeste do estado de Goiás, com cinco repetições totalizando trinta e cinco parcelas experimentais, com quinze frutos por repetição. Verificou-se que para massa estimada de 100 frutos, massa estimada de 100 sementes e espessura de polpa, as populações de Caldas Novas e Bom Jesus se destacaram por apresentarem maiores médias em algumas características avaliadas. Quanto aos intervalos de frequência, a maior amplitude para sementes foi observada na população de Caldas Novas, enquanto para a largura, maior amplitude foi observada na população de Edéia. Para frutos, as maiores amplitudes foram observadas em relação ao comprimento e largura para a população de Caldas Novas. Após a análise de dissimilaridade genética, o método de agrupamento por UPGMA agrupou as populações de forma que um grupo foi formado pelas populações Caldas Novas e Bom Jesus e o outro pelas demais. Os

maiores índices de correlação observados foram massa de cem frutos e massa de cem sementes (87%) e comprimento de sementes e comprimento de frutos (86%), respectivamente. No sistema de agrupamento pelo método k-means houve a formação de dois grupos com Caldas Novas, Aloândia e Bom Jesus em um dos grupos e o outro grupo formado pelas demais populações.
Palavras-chave: agrupamento; biometria; Dipteryx alata Vogel; melhoramento vegetal.

Introduction

Habitat destruction is a threat that causes the extinction of endemic species and characterizes the emergence of areas in urgent need of attention (OLIVEIRA *et al*., 2008), and anthropogenic activities greatly contribute to this process. The *cerrado* is considered a biome where biodiversity is continually compromised by the exploitation of its areas (OLIVEIRA *et al*., 2008).

In this respect, from the 1970s onwards, incentives were given to the expansion of the agricultural frontier, which led to the occupation of *cerrado* areas. These areas possess soil characteristics that are favorable to the practice of agriculture. However, little is known about the potential of use of the natural *cerrado* resources, particularly plant species that occur naturally in this biome (ZUFFO *et al.*, 2014). Some species serve reforestation purposes, e.g., baru (*Dipteryx alata* Vogel), a fruit tree that occurs in the forest, savanna, and *cerradão* biomes. Baru is at risk of extinction due to the great demand for wood and deforestation as its trees are located in fertile soils, which are of great interest to farmers (CARRAZA; ÁVILA, 2010).

The process of adaptation to the cultivation of promising species, such as those native to the *cerrado*, is a fundamental tool to reduce the risk of extinction of these species. For Junqueira *et al*. (2010), the detection of genetic diversity between accessions provides good prospects for further research on the potential of species and accessions as commercial fruit species. Vieira *et al*. (2010) cited at least 16 fruit species native to the *cerrado* region of Brazil as promising considering agronomic, nutritional, economic, technological, and social aspects, and the list includes baru.

However, fruits of native species—mainly those found in the *cerrado*—have non-uniform vegetative and reproductive traits, which warrants further studies to establish parameters that allow selection based on attributes such as color, size, thickness, among others (BORGES *et al*., 2010). According to Pereira *et al*. (2019), knowledge of the genetic variability between genotypes, as in "cajuzinho do cerrado" (*Anacardium humile* A. St.-Hil), is important to establish pre-breeding and use strategies in a breeding program.

Some authors indicate univariate and multivariate analyses to group promising species and genotypes, e.g., Coutinho *et al*. (2019), in quince cultivars; Guedes *et al*. (2014), in blackberry cultivars and a red mulberry species (*Rubus rosifolius* Smith); Neitzke *et al*. (2009), in melon landraces; and Faria *et al*. (2012), in accessions of peppers of the genus *Capsicum*. In species native to the *cerrado*, this recommendation was made by Pereira *et al*. (2019), who analyzed the genetic diversity of "cajuzinho do cerrado".

Frequency histograms, in turn, can be used in biometric studies of native species, e.g., Vieira *et al*. (2019) analyzed the biometric characteristics of seeds of *Annona crassiflora* Mart. and *Anonna coriacea* Mart., both native to the *cerrado* and popularly known as "aratincunzeiro"; and Borges *et al*. (2010), examined the width and length of fruits and seeds of savannah cherry (*Eugenia calycina* Camb.).

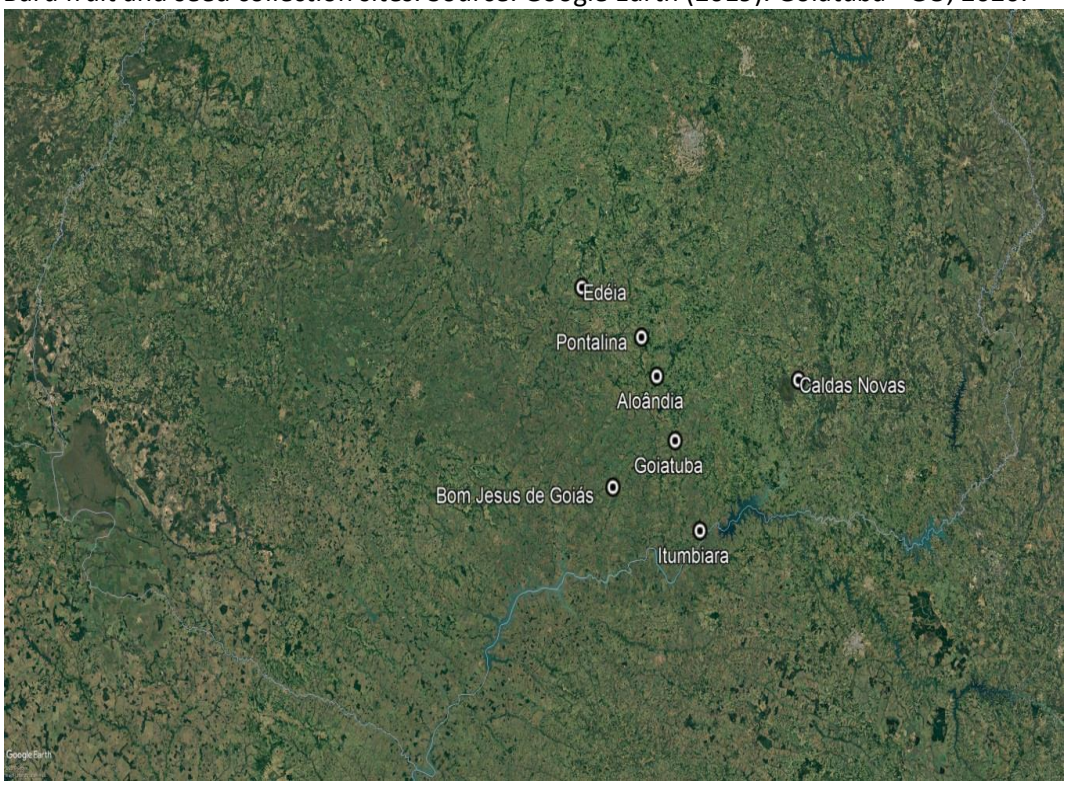
Given the relevance and productive potential of baru, and with a view to sustainable production and the maintenance of natural areas with populations of this species, it is very important to characterize its fruits and seeds for seedling formation. Therefore, the present study proposes to select potential populations for the start of baru breeding programs using multivariate analysis and frequency histograms. The objective was to enable and make its cultivation a profitable alternative for fruit production, thus benefiting small, medium, and large producers.

Material and Methods

Characterization of sampling site and collection period

The study was carried out in native *cerrado* vegetation areas, a common habitat of baru. Baru fruits and seeds were obtained between September and November 2019 in the municipalities of Aloândia, Bom Jesus, Caldas Novas, Edéia, Goiatuba, Itumbiara, and Pontalina (Figure 1).

Figure 1. Baru fruit and seed collection sites. Source: Google Earth (2019). Goiatuba - GO, 2020.



All municipalities, as well as fruit collection sites, have an Aw climate according to the Köppen classification (Table 1), which corresponds to a tropical climate with dry winters (CARDOSO *et al*., 2014).

Table 1. Characterization of fruit collection sites. Source: Google Earth (2019)* and Climate Data (2019)**. Goiatuba - GO, 2020.

Site	South latitude*	West longitude*	Altitude (m)**	Average annual temperature (°C)**	Precipitation (mm)**	Köppen's climatic classification**
Aloândia	17°43'	49° 28'	606	24.1	1337	Aw
Bom Jesus	18° 12'	49° 44'	598	23.9	1408	Aw
Caldas Novas	17° 43'	48° 36'	690	23.8	1347	Aw
Edeia	17° 20'	49° 55'	586	24.1	1423	Aw
Goiatuba	18° 00'	49° 21'	795	23.0	1369	Aw
Itumbiara	18° 23'	49° 13'	443	24.6	1119	Aw
Pontalina	17° 30'	49° 26'	635	23.9	1360	Aw

Material collection

The obtained material was composed of the fruits collected from each population. Two to five individuals were sampled per population. The collection was manual, following the recommendations of Almeida (1998). The fruits were packed in labeled paper bags and stored in a cold chamber at 10 °C. For identification purposes, each municipality was considered a distinct population of plants; thus, the fruits of seven populations were collected. Because the baru fruit has a hard endocarp, a machine specifically designed for this task was used to extract the seeds without damage.

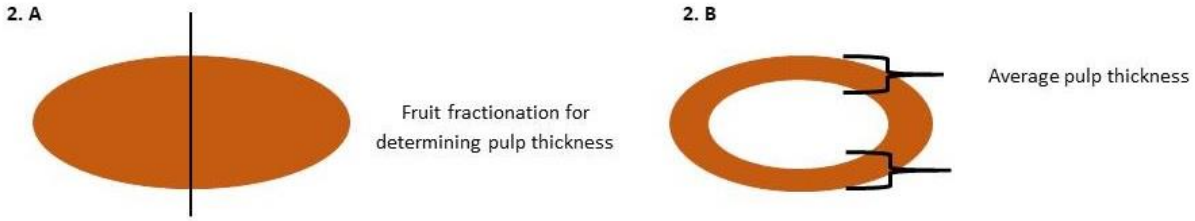
Different populations of individuals are herein considered so according to the location and existing geographic distance between them, with a minimum distance of 20 km adopted as the reference between one population and another.

Evaluated traits

The evaluated fruit traits were length (mm), width (mm), pulp thickness (mm), and estimated average 100-fruit weight (g). Seeds were evaluated as to width (mm), length (mm), and estimated average 100-seed weight (g).

Length and width measurements were performed with a digital caliper. Then, the fruits were sectioned in half to determine pulp thickness, where two points were sampled and averaged (Figure 2).

Figure 2. Determination of average pulp thickness of baru fruits (**2. A.** fruit fractionation and **2. B.** measurements for determining pulp thickness). Goiatuba, GO - 2020.



Experimental design

The experiment was laid out in a completely randomized design with seven populations of baru trees from municipalities located in the south and southeast regions of the state of Goiás, Brazil. Five replicates were used, totaling 35 experimental plots with 15 fruits per replicate, except for the population of Bom Jesus, where 12 fruits were obtained per plot, totaling 510 fruits.

Statistical analysis

Data were subjected to frequency analysis on Excel software (MICROSOFT CORPORATION, 2010), using histograms to determine the highest frequency shown regarding biometric measurements of fruits and seeds (longitudinal diameter and transverse diameter).

Subsequently, the traits of 100-fruit weight (g); 100-seed weight (g); pulp thickness (mm); longitudinal (mm) and transverse (mm) seed lengths; and longitudinal (mm) and transverse (mm) fruit lengths were subjected to genetic dissimilarity analysis and hierarchical clustering of average linkage or Unweighted Pair-Group Method with Arithmetic Mean (UPGMA). To validate the formed groups, as well as to check the hierarchy efficiency of the clustering method, the cophenetic correlation coefficient was calculated using PAST software version 4.02 (HAMMER *et al*. 2001). Cluster analysis was also performed using the k-means method and Pearson's correlation analysis was carried out at the 0.05 probability level using R® software (R CORE TEAM, 2021).

Results and Discussion

Some baru populations showed to be promising with respect to fruits and seeds, standing out for these traits. It can thus be inferred that these individuals can be selected for the breeding of the species, given their superior results (Figures 3 and 4).

Figure 3. Fruits of baru populations from different municipalities in the south and southeast regions of the state of Goiás (**3. A.** Aloândia; **3. B.** Bom Jesus; **3. C.** Caldas Novas; **3. D.** Edéia; **3. E.** Goiatuba; **3. F.** Itumbiara; and **3. G.** Pontalina). Goiatuba - GO, 2020.

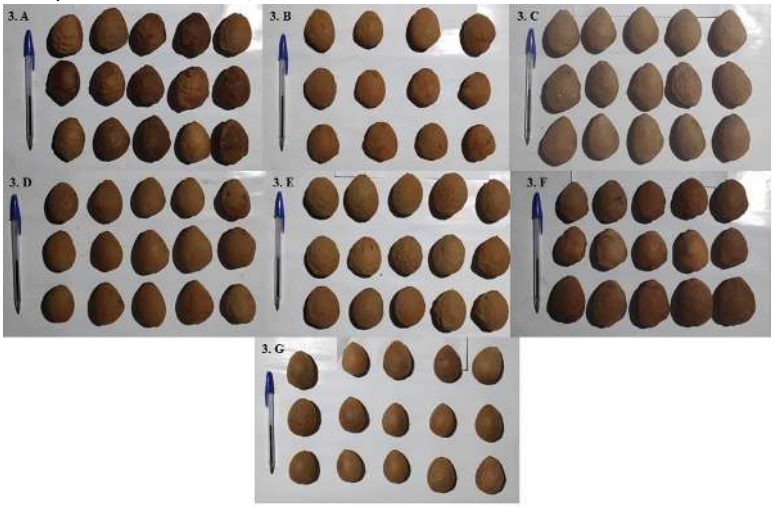
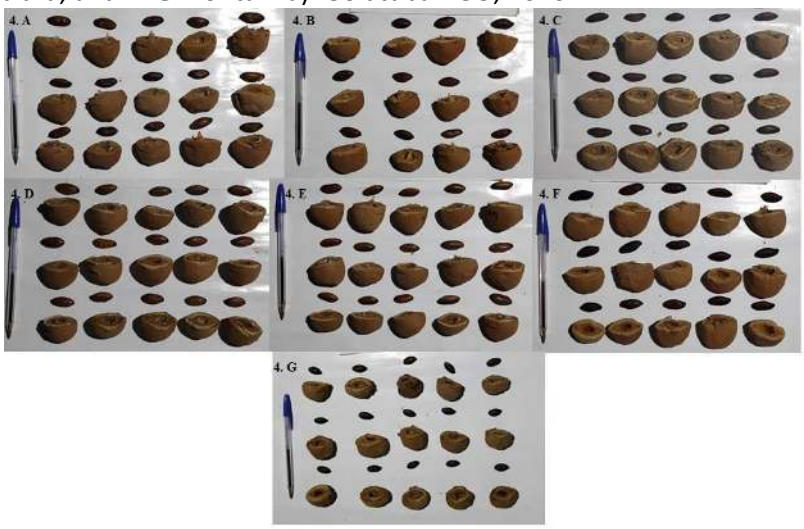
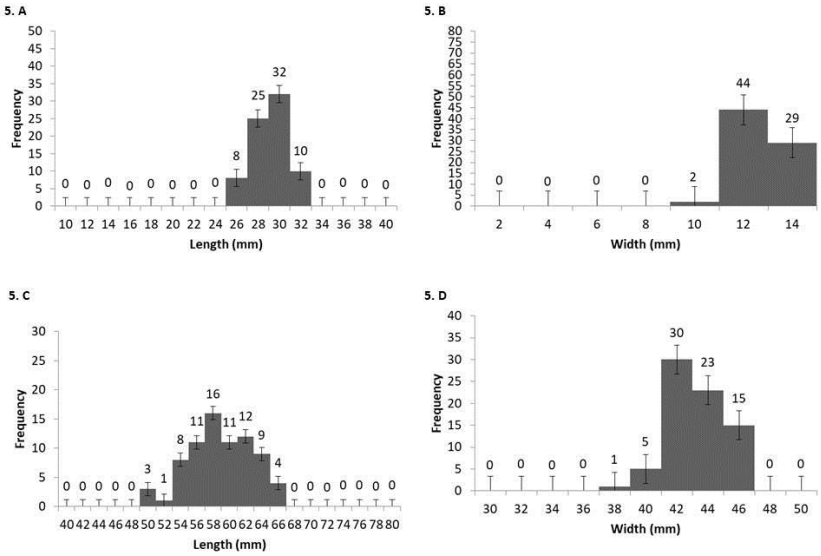


Figure 4. Fractionated fruits and seeds of baru populations from different municipalities in the south and southeast regions of the state of Goiás (**4. A.** Aloândia; **4. B.** Bom Jesus; **4. C.** Caldas Novas; **4. D**. Edéia; **4. E.** Goiatuba; **4. F.** Itumbiara; and **4. G.** Pontalina). Goiatuba - GO, 2020.



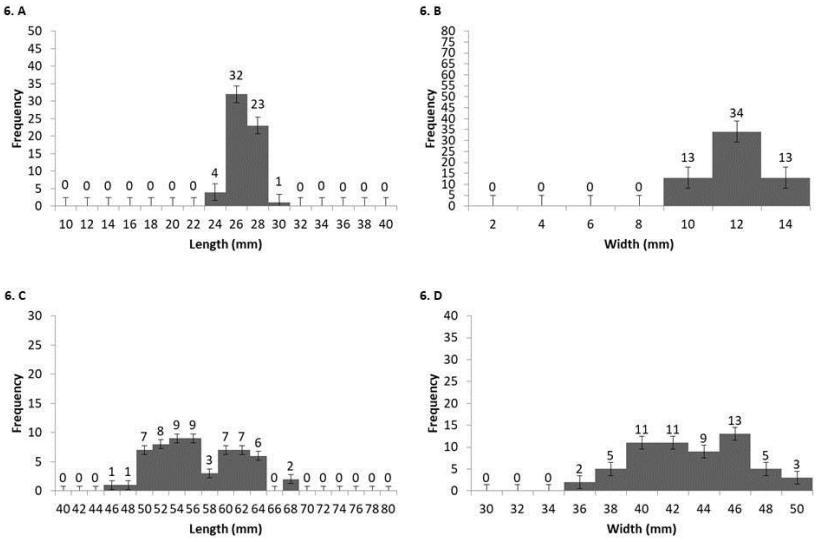
Biometric analysis revealed that among the seeds of the Aloândia population, the greatest amplitude for seed length (Figure 5. A.) was between 28 and 30 mm, in 32 seeds. As for seed width (Figure 5. B.), the largest amplitude was between 10 and 12 mm, in 44 seeds. In the fruits, the greatest amplitudes for length (Figure 5. C.) were between 56 and 58 mm (16 fruits) and for width (Figure 5. D.) between 40 and 42 mm (30 fruits).

Figure 5. Length (**5. A.**) and width (**5. B.**) of seeds and length (**5. C.**) and width (**5. D.**) of fruits of baru in the municipality of Aloândia, Goiás. Goiatuba - GO, 2020.



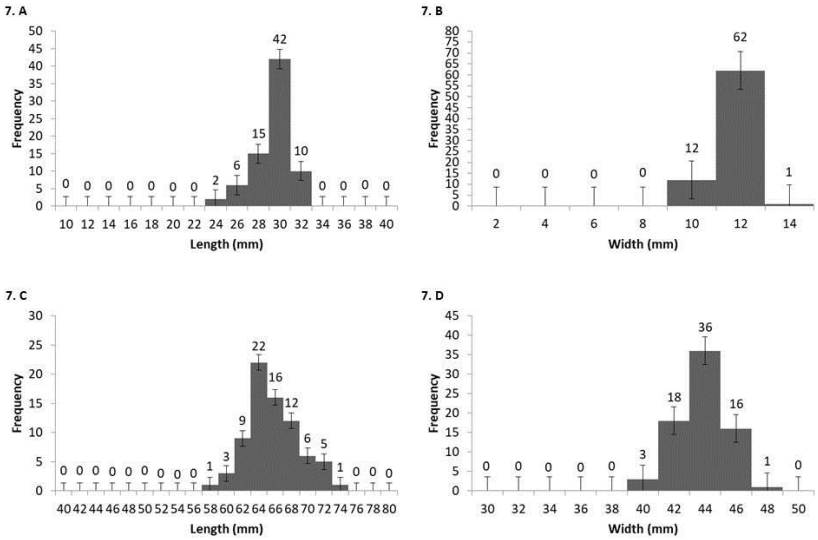
In the population of Bom Jesus, the greatest amplitude for seed length (Figure 6. A.) was between 24 and 26 mm, in 32 seeds; for seed width (Figure 6. B.), between 10 and 12 mm, in 34 seeds. The largest amplitudes for fruit length (Figure 6. C.) were between 52 and 56 mm (18 fruits); for fruit width (Figure 6. D.), between 44 and 46 mm (13 fruits).

Figure 6. Length (**6. A**.) and width (**6. B.**) of seeds and length (**6. C**.) and width (**6. D.**) of fruits of baru in the municipality of Bom Jesus, Goiás. Goiatuba - GO, 2020.



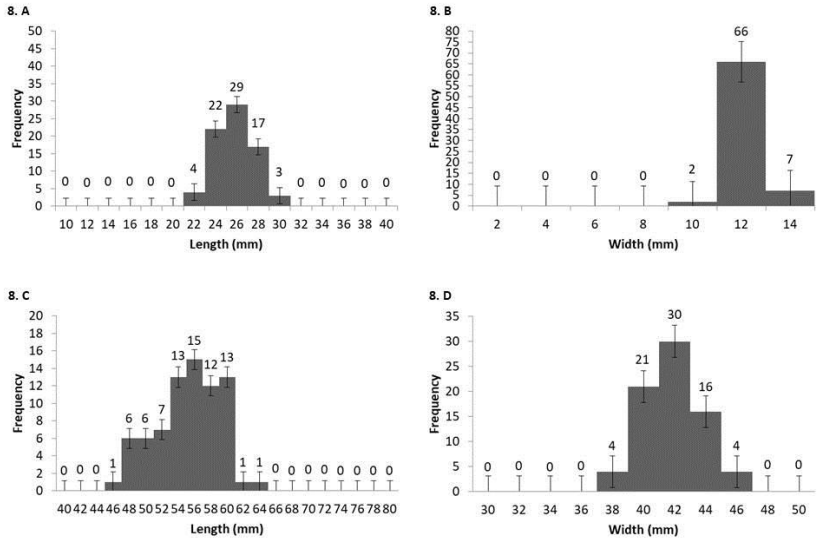
In the population of Caldas Novas, the biometric analyses showed that the greatest amplitude of seed length (Figure 7. A.) was in the interval between 28 and 30 mm, in 42 seeds. For seed width (Figure 7. B.), the largest amplitude was in the interval between 10 and 12 mm, in 62 seeds. In the case of fruits, the greatest amplitudes for length (Figure 7. C.) were between 62 and 64 mm (22 fruits); and for width (Figure 7. D.), between 42 and 44 mm (36 fruits).

Figure 7. Length (**7. A.**) and width (**7. B.**) of seeds and length (**7. C.**) and width (**7. D**.) of fruits of baru in the municipality of Caldas Novas, Goiás. Goiatuba - GO, 2020.



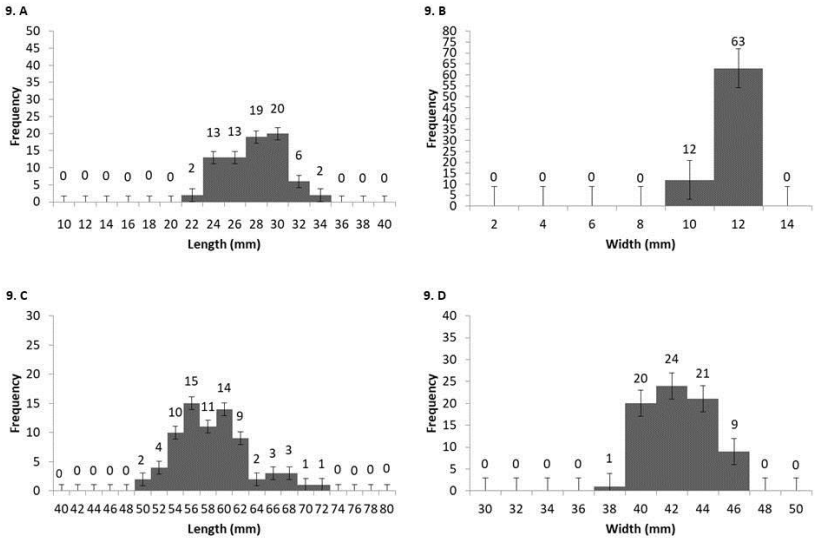
Among the seeds of the Edéia population, the greatest amplitude for length (Figure 8. A.) was in the interval between 24 and 26 mm, in 29 seeds. For seed width (Figure 8. B.), the largest amplitude was between 10 and 12 mm, in 66 seeds. In the fruits, the greatest amplitudes for length (Figure 8. C.) were between 54 and 56 mm (15 fruits) and for width (Figure 8. D.), between 40 and 42 mm (30 fruits).

Figure 8. Length (**8. A**) and width (**8. B**) of seeds and length (**8. C**) and width (**8. D**) of fruits of baru trees in the municipality of Edéia, Goiás. Goiatuba - GO 2020.



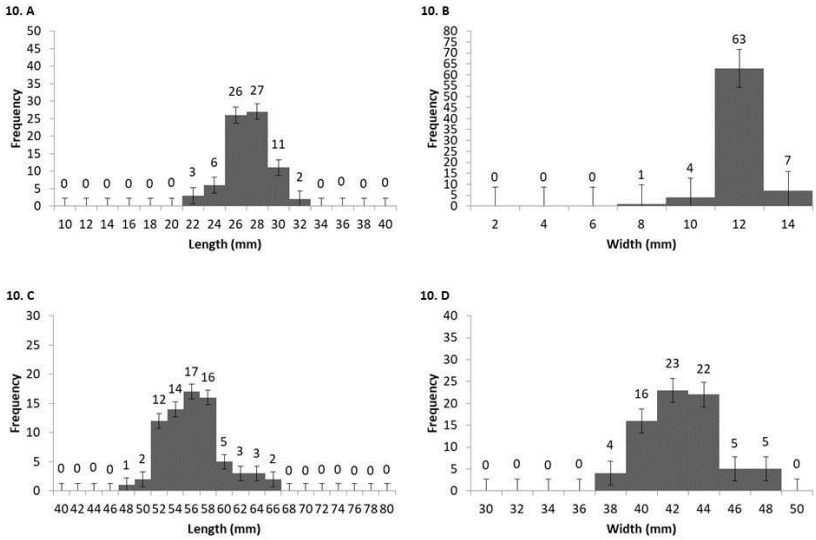
In the population of Goiatuba, the largest amplitude for the seed length (Figure 9. A) was between 28 and 30 mm, in 20 seeds; for seed width (Figure 9. B), between 10 mm and 12, in 63 seeds. In the case of fruits, the greatest amplitudes for length (Figure 9. C) were between 54 and 56 mm (15 fruits), and for width (Figure 9. D), between 40 and 42 mm (24 fruits).

Figure 9. Length (**9. A**) and width (**9. B**) of seeds and length (**9. C**) and width (**9. D**) of fruits of baru trees in the municipality of Goiatuba, Goiás. Goiatuba - GO, 2020.



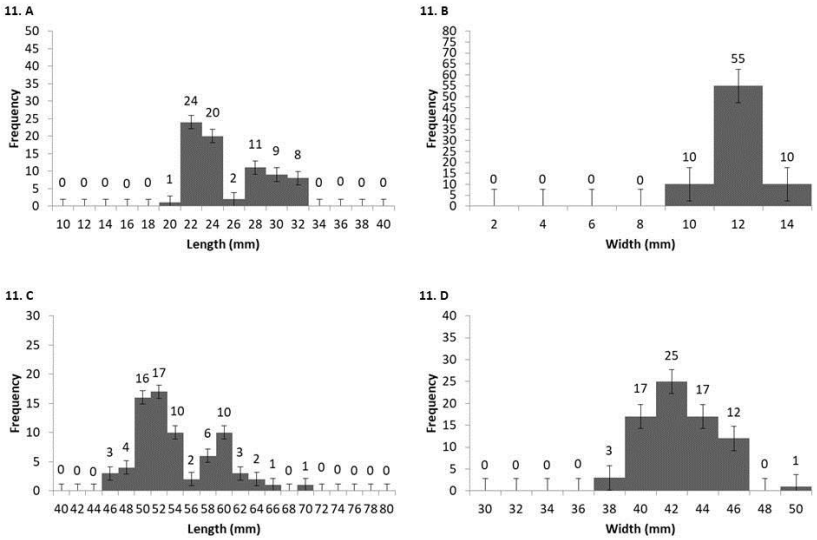
In the population of Itumbiara, the greatest amplitude for seed length (Figure 10. A) was between 26 and 28 mm, in 27 seeds, and for seed width (Figure 10. B), between 10 and 12 mm, in 63 seeds. In the fruits, the largest amplitudes for length (Figure 10. C) were between 54 and 56 mm (17 fruits), and for width (Figure 10. D), between 40 and 42 mm (23 fruits).

Figure 10. Length (**10. A**) and width (**10. B**) of seeds and length (**10. C**) and width (**10. D**) of fruits of baru trees in the municipality of Itumbiara, Goiás. Goiatuba - GO 2020.



Finally, in the population of Pontalina, the largest amplitudes found in the seed were between 20 and 22 mm for length (24 seeds) (Figure 11. A) and between 10 and 12 mm for width (55 seeds) (Figure 11. B). In the fruits of this population, the observed largest amplitudes were between 50 and 52 mm for length (17 fruits) (Figure 11. C) and between 40 and 42 mm for width (25 fruits) (Figure 11. D).

Figure 11. Length (**11. A**) and width (**11. B**) of seeds and length (**11. C**) and width (**11. D**) of fruits of baru trees in the municipality of Pontalina, Goiás. Goiatuba - GO 2020.



Thus, the greatest amplitude in the frequency interval for seed length was observed in the population of the municipality of Caldas Novas, which showed 42 seeds between 28 and 30 mm, whereas for width, the greatest amplitude was observed in the population of Edéia, with 66 seeds in the range between 10 and 12 mm. As for the fruits, the largest amplitudes were found in the population of Caldas Novas, both for length (between 62 and 64 mm, 22 seeds) and width (between 42 and 44 mm, 36 seeds). Vieira *et al*. (2019) used frequency range measurements in two species of *Annonna* from the *cerrado* (*Annona crassiflora* Mart. and *Anonna coriacea* Mart.) and observed that *A. coriacea* seeds had a greater size/width ratio than *A. crassiflora* seeds.

According to Borges *et al*. (2010), *Eugenia calycina* seeds showed large amplitudes, which ranged from 8 to 14 mm for width and from 7 to 14 mm for length. This was also observed in the present study, as all populations exhibited greater amplitudes in the seed biometrics frequency distribution.

The highest estimated mean Euclidean distances of dissimilarity were found to be between the municipality of Bom Jesus and the others, as shown in Table 2.

Table 2. Estimation of mean Euclidean distances of dissimilarity between the baru populations. Goiatuba, GO, 2020.

	Distance between genotypes						
POP.	**GOI.**	**PON.**	**CAL.**	**ALO.**	**ITU.**	**EDE.**	**BOM.**
GOI.	0.00	244.64	358.75	161.38	330.88	186.98	704.88
PON.		0.00	603.04	85.34	86.89	57.76	949.39
CAL.			0.00	520.06	689.51	545.55	346.90
ALO.				0.00	170.02	29.14	866.01
ITU.					0.00	144.03	1035.75
EDE.						0.00	891.81
BOM.							0.00

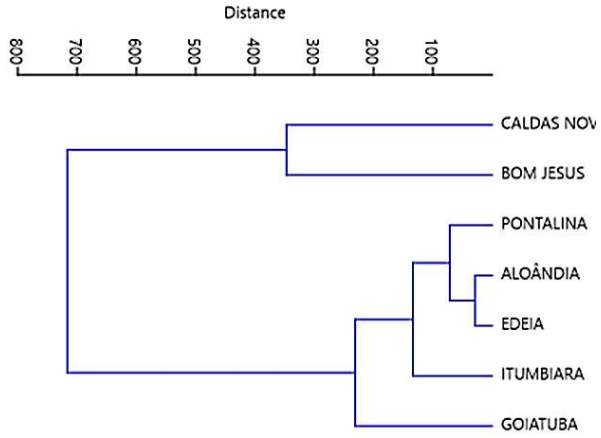
POP. = population; GOI. = Goiatuba; PON. = Pontalina; CAL. = Caldas Novas; ALO. = Aloândia; ITU. = Itumbiara; EDE. = Edéia; BOM. = Bom Jesus.

The higher the estimated Euclidean distance, the less similar (more dissimilar) the municipalities are. At first, high divergence makes it possible to recommend crossing, in aiming to maximize heterosis and increase the possibility of occurrence of segregants in advanced

generations due to the different numbers of loci in which dominance effects are evident (CRUZ *et al*. 2004).

Hierarchical clustering given by the UPGMA average linkage method based on the Euclidean distance allowed grouping the populations through the dissimilarity measures, with a resulting cophenetic correlation coefficient of 0.8895. Therefore, the groups are valid (Figure 12).

Figure 12. Genetic dissimilarity dendrogram based on the Euclidean distance between baru populations of different municipalities in the south and southeast regions of the state of Goiás, constructed from a dissimilarity matrix. Goiatuba - GO, 2020.

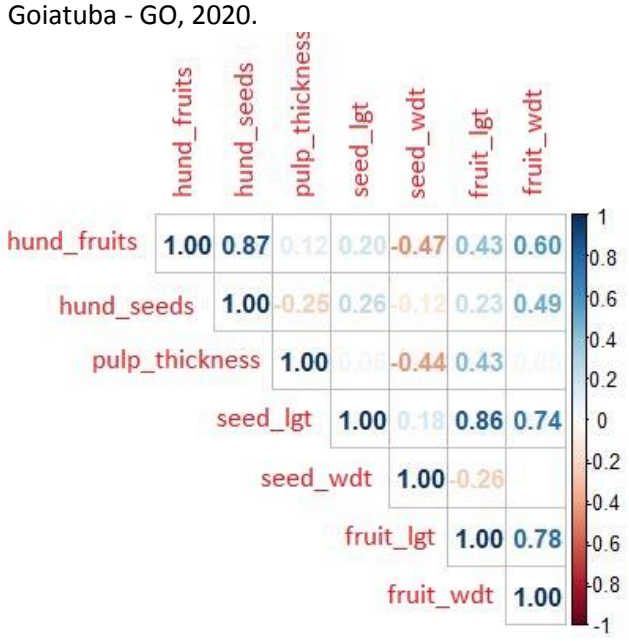


Because the cut-off line was not crossed, the groups were not separated in the figure. However, considering the optimization of the goodness of fit of the clustering pattern and based on the greater amplitude in the junction distances of the formed groups, two main groups were formed: one consisted of the populations of Caldas Novas and Bom Jesus and the other comprised the populations of Pontalina, Aloândia, Edéia, Itumbiara, and Goiatuba. Unweighted Pair-Group with Arithmetic Mean (UPGMA) is a simple agglomerative hierarchical clustering method. It is generally attributed to Sokal and Michener (1958) and its purpose is to group the treatments based on the dissimilarity shown between the traits of each one.

In quince cultivars, Coutinho *et al*. (2019) reported that UPGMA cluster analysis based on the Gower distance resulted in three groups formed, with a cophenetic correlation coefficient of 0.80. It is worth mentioning that the dendrogram allowed visualizing the similarity between the populations of Caldas Novas and Bom Jesus.

As illustrated below, Pearson's linear correlation analysis showed high correlation indices between some of the analyzed traits, which can be of great use in the selection of the most promising genotypes for breeding programs for the species (Figure 13).

Figure 13. Pearson's correlation matrix at the 5% probability level. hund_fruits = estimated average weight of 100 fruits (g); hund_seeds = estimated average weight of 100 seeds (g); pulp_thickness = pulp thickness (mm); seed_lgt = seed length (mm); seed_wdt = seed width (mm); and fruit_lgt = fruit length (mm); and fruit_wdt = fruit width (mm). Goiatuba - GO, 2020.

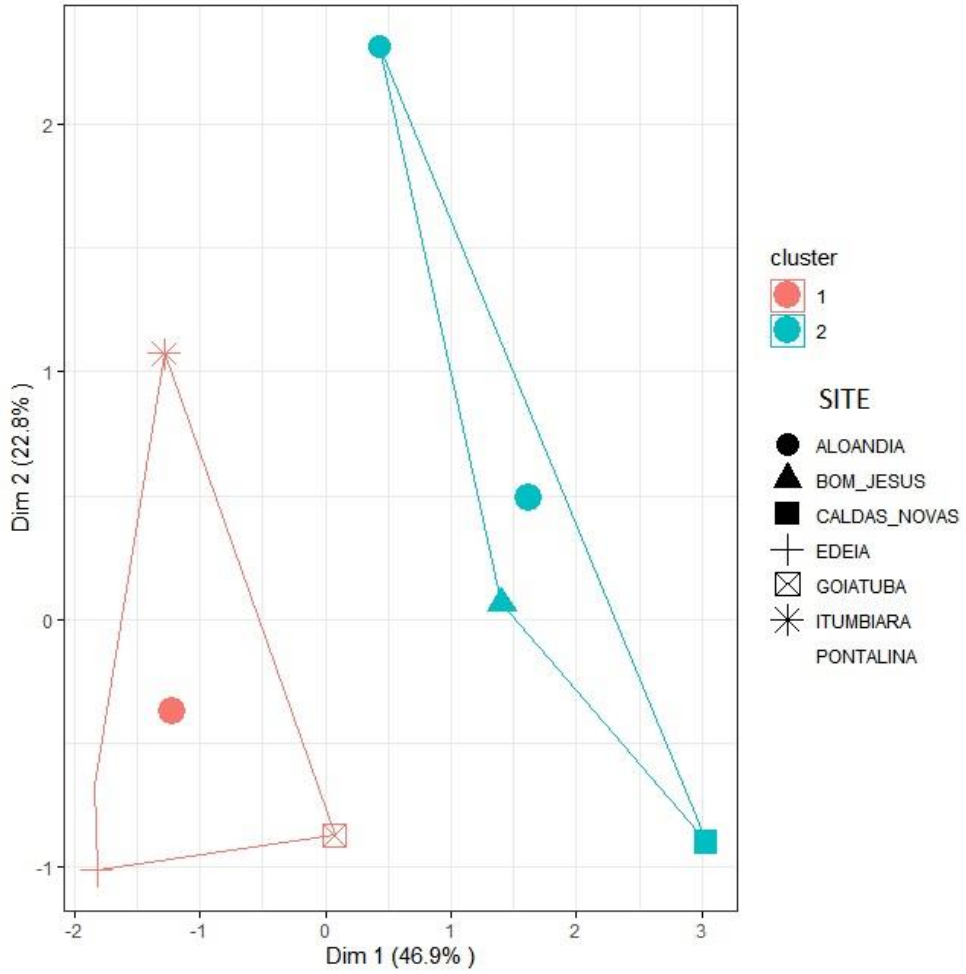


The correlation indices between the traits of 100-fruit weight and 100-seed weight (87%) and seed length and fruit length (86%) are satisfactory. In "gabiroba" (*Campomanesia adamantium* (Cambess.) O. Berg) fruits, Souza *et al*. (2019) noted that among the evaluated physical variables, fruit longitudinal diameter, fruit transverse diameter, and fruit weight showed significant and high correlation values with each other, which demonstrates the dependence of one variable on the other.

Results were subjected to the k-means method, a non-hierarchical data clustering algorithm that uses an iterative technique to partition a dataset. This method was proposed in a pioneering study by S. Lloyd in 1957, but was only published in 1982. The algorithm aims to iteratively minimize the distance of the elements of a dataset with k centers. The k-means method is a way of segregating various data around centers (centroids), creating what we analogously call "clustering" in chemistry, which generates the effect of partitioning n observations between k groups, where each observation belongs to the group closest to the mean. This results in a division of the data space into a Voronoi diagram.

In this situation, it is first necessary to define the number of clusters, and in this case two clusters were considered, i.e., k = 2 (Figure 14).

Figure 14. Application of the k-means method to determine the number of clusters formed from the studied populations. Goiatuba - GO, 2020.



The k-means method divided the groups as follows: the first group was composed of the populations of Goiatuba, Pontalina, Itumbiara, and Edeia. The second group formed comprised the populations of Caldas Novas, Aloândia, and Bom Jesus. In all analyses, the populations of Bom Jesus and Caldas Novas showed a clear dissimilarity, with more prominent genotypes. In this case, their further investigation could suggest promising genotypes for the breeding and development of new baru cultivars.

For Pereira *et al*. (2019), results obtained in the evaluation of genetic diversity of "cajuzinho-do-cerrado" (*Anacardium humile* A. St.-Hil.), by determining physical, chemical, and physicochemical traits of fruits and pseudofruits, can be used to select individuals of the species in future studies for the breeding and conservation of this species.

Conclusion

In terms of frequency intervals, the largest amplitudes were found in the seed and fruit traits evaluated in the population of the municipality of Caldas Novas.

Based on the UPGMA genetic dissimilarity clustering method, artificial crosses between the baru populations of Caldas Novas and Bom Jesus and the other populations are promising for breeding programs.

The clustering system based on the k-means method resulted in the formation of two distinct groups of populations.

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