PHENOTYPIC VARIABILITY OF THE FRUIT IN A NATURAL POPULATION OF *Caryocar brasiliense* CAMB AS A CONSERVATION STRATEGY

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ABSTRACT – This study aimed to estimate the phenotypic variation between genotypes of *C. brasiliense* through morphological descriptors of the fruit. In a natural population were sampled 47 genotypes, based on 17 physical parameters related to fruit and seed. The data (average of 20 fruits) were analyzed using multivariate methods (cluster analysis according to the method based on average linkage between groups - UPGMA, begin as measure of dissimilarity the Average Standardized Euclidean distance and Principal Component Analysis). The study reveals significant phenotypic variability, particularly concerning fruit weight, peel weight, seed weight, and peel and pulp thickness. Correlation analyses highlight important relationships, such as the positive correlation between fruit weight and peel weight, and between peel weight and fruit diameter. The formation of six similarity groups based on genetic dissimilarity may assist in selecting genotypes for germplasm banks to maximize genetic diversity. Considerable similarity was observed among genotypes sampled within a distance of less than 20 meters, suggesting kinship or vegetative reproduction of *C. brasiliense* in the area.

Keywords: Multivariate Analysis, Savana, Pequi, Genetic Resources.

VARIABILIDADE FENOTÍPICA DO FRUTO EM UMA POPULAÇÃO NATURAL DE *Caryocar brasiliense* CAMB COMO ESTRATÉGIA DE CONSERVAÇÃO

RESUMO –Este estudo teve como objetivo estimar a variabilidade fenotípica entre genótipos de *C. brasiliense*, por meio de descritores morfológicos do fruto. Em uma população natural foram amostrados 47 genótipos, com base em 17 parâmetros físicos, referentes ao fruto e a semente. Os dados (média de 20 frutos) foram analisados utilizando-se métodos de análise multivariada (análise de agrupamento de acordo com base no Método da Ligação Média entre Grupos - UPGMA, tendo como medida de dissimilaridade a distância Euclidiana Média Padronizada e Análise de Componentes Principais). O estudo revela uma variabilidade fenotípica significativa principalmente em relação ao peso dos frutos, peso da casca, peso dos caroços, e espessura da casa de da polpa. Análises de correlação destacam relações importantes, como a correlação positiva entre peso do fruto e peso da casca, e entre peso da casca e diâmetro do fruto. A formação de seis grupos de similaridade com base na dissimilaridade genética pode auxiliar na seleção de genótipos para bancos de germoplasma visando maximizar a diversidade genética. Observou-se considerável similaridade entre genótipos amostrados a uma distância inferior a 20 metros, sugerindo parentesco ou reprodução vegetativa de *C. brasiliense* na área.

Palavras-chave: Analise Multivariada, Cerrado, Pequi, Recursos Genéticos.

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INTRODUCTION

According to AGEITEC – Embrapa Information Technology Agency, pequi (Caryocar brasiliense Camb.) is part of one of the most important families of plant species in the Cerrado, belonging to the Caryocaraceae family and the genus Caryocar (MIRANDA and VERAS, 2021). The species is found throughout the Brazilian Cerrado, with distribution in the states of Bahia, Ceará, Distrito Federal, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Pará, Piauí, Rondônia, Rio de Janeiro, São Paulo, and Tocantins (ALMEIDA et al., 1998).

Its importance is expressed by Federal Ordinance 54, dated March 5, 1987, from the former Brazilian Institute of Forestry Development (IBDF), now the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA), which prohibits the cutting and commercialization of its wood throughout the national territory (RIBEIRO, 2003).

The *C. brasiliense* is a plant that can reach up to 10 meters in height. Its fruits have a greenish pericarp (peel) with orange-yellow pulp, and its almond is covered with thin spines measuring 2 to 5 mm in length. Each fruit contains 2 to 4 seeds weighing 100 to 150 grams each (LIMA et al., 2007; SANTOS et al., 2013).

C. brasiliense is one of the Cerrado species identified as economically viable due to its great fruiting potential. The pequi plant can be used in its entirety, where the peels can be used in the manufacture of utensils (boats, pestles, among others), the leaves for dyeing and the fruit for human and industrial consumption (ALVES et al., 2014). The forms of use most commonly found in the food industry are in the production of preserves, jellies and liqueurs, prepared by extractive communities or cooperatives (SANTOS et al., 2013). However, several studies are still necessary for its exploration sustainability, inclusion in the industry and preservation of the Cerrado (CARRAZZA; ÁVILA, 2010a).

It has been observed that the Brazilian Cerrado provided, during the last half of the 20th century and the first two decades of the current century, a significant part of the land destined for the expansion of the agricultural frontier in the country. Of the 110 million hectares of arable land in the Cerrado biome, approximately 50 million they are already busy with human activities, especially those related to agriculture. Part of this expansion took place without due care for the preservation of existing natural resources, notably genetic resources. This accelerated occupation favors the erosion process genetics of each of the species of the biome, causing irreparable losses for their agronomic development, thus, studies that characterize plant genetic resources are conservation strategies, aimed at the rational exploitation of potential species in the region (ROCHA et al., 2019).

Genetic erosion makes it impossible to fully understand the genetic resources of the species and, therefore, all existing genetic variability. The amount of genetic variation within natural populations and the forces that limit and shape it is of primary interest to population geneticists, as this variability underlies all evolution, and the range of this variation within a population contributes to the potential adaptation to environmental changes, which allows it to evolve through the accumulation of mutations in its gene pool, through natural selection (MOTTA, 2004).

Knowledge of genetic variability in natural populations enables the development and monitoring of germplasm banks (CRUZ and CARNEIRO, 2003), generating useful information for the preservation and use of accessions (TOQUICA *et al*., 2003).

This study aimed to evaluate the phenotypic variability in a natural population of *C. brasiliense*, located in the municipality of Porto Nacional - TO, state of Brazil, based on physical characteristics of fruits, in order to contribute to the management and conservation of the species.

MATERIALS AND METHODS

The work was conducted on a natural population of Caryocar brasiliense Camb. surveyed in a legal reserve area of approximately 30 hectares, located in a rural property 10 km from the city of Porto Nacional – Tocantins, BR., on the banks of the TO 255 highway, which connects Porto Nacional - TO to Monte do Carmo - TO. The original vegetation characteristic of the area is predominantly shrubland, which can be classified as Restricted Sense Savana.

From the population, 47 adult plants were sampled randomly. Out of the 47 plants, 41 were sampled at a distance of 20 m from each other, and 6 (46, 8, 26, 28, 29, and 17) were sampled at a distance less than 20 m from plants 4, 7, 25, 27, 30, and 18, respectively, to verify the effect of sampling on the divergence between the plants (Figure 1). The plants were georeferenced with the aid of a Global Positioning System (GPS) receiver (Figure 1).

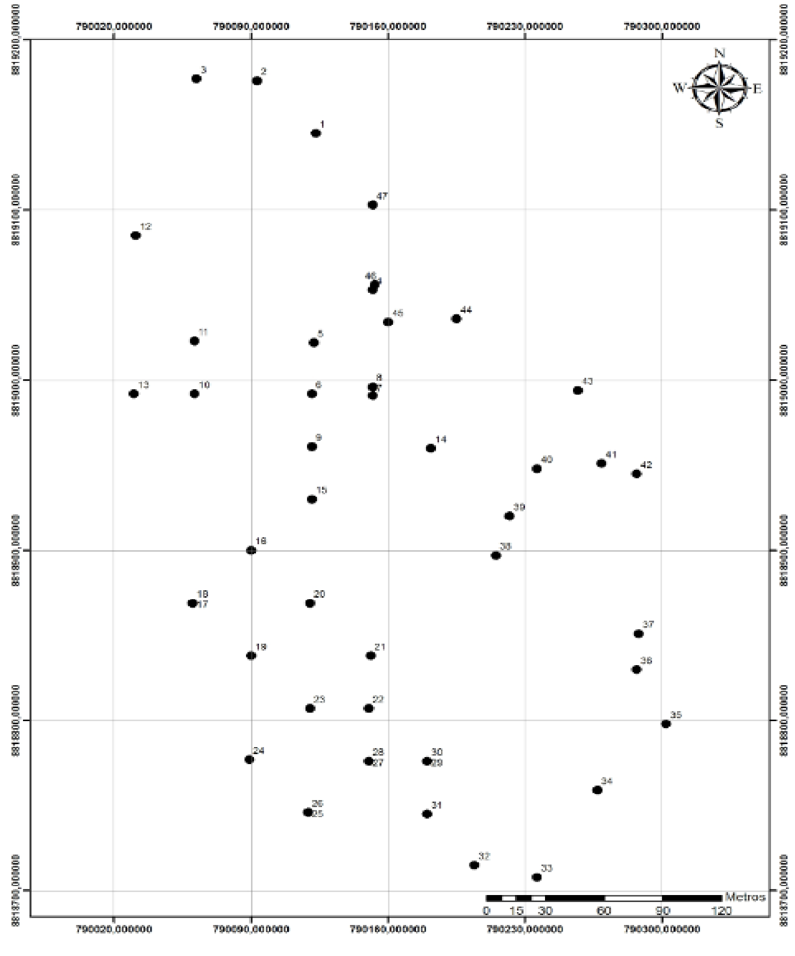

Figure 1. Plotting of the 47 *C. brasiliense* genotypes based on geographic coordinates. Tocantins, BR.

The collections were carried out in October and November 2009. In each plant, 20 fruits at the same physiological maturation stage were randomly sampled and evaluated for the following physical parameters: whole fruit weight (FW, g); fruit length (FL, cm); diameters (DMLF - diameter longitudinal of the fruit – larger, and DMTF - diameter transverse of the fruit – smaller, both obtained at the equatorial position, cm); peel weight (PW, g); greater peel thickness (GPT, cm); lesser peel thickness (LPT, cm); peel yield (PY, %); number of pits (NP, unit); number of “frutilhos" refers to the number of undeveloped pits within a fruit. (NF, unit);

pit weight with pulp (PWP, g); diameters (larger diameter of the pits - DMA, and smaller diameter of the pits - DMI, cm); pulp thickness (PT, cm); spine layer thickness (SLT, cm); and almond diameters (D1A - major diameter and D2A - minor diameter, cm).

The peel yield was obtained using the formula:

$$PY\% = \frac{PW}{FW} x\ 100$$

Weights were obtained using a digital semi-analytical balance, and the other measures were taken using a caliper.

The relationship between the studied traits was estimated by the Pearson correlation coefficient, and the hypothesis that the correlation coefficient is equal to zero was evaluated by the "t" statistic at 1% and 5% probability.

To evaluate the phenotypic diversity among genotypes, a clustering analysis was performed using the Unweighted Pair Group Method with Arithmetic Mean - UPGMA, based on the Standardized Euclidean Mean distance as a dissimilarity measure. To estimate the relative importance of the evaluated traits, a Principal Component Analysis (PCA) was performed.

All statistical analyses were performed using the "Programa Genes" software, Windows version (CRUZ, 2006).

RESULTS AND DISCUSSION

In the 47 evaluated genotypes, a great variation was observed regarding the weight of the peel and the weight of the fruit. The weights of the entire fruits ranged from 66.02 g (genotype 34) to 134.92 g (genotype 43), with an overall mean of 98.73 g (Table 1). Regarding this characteristic, the individuals 43 (134.92 g), 11 (131.89 g), 10 (131.26 g), 3 (128.48 g), 4 (125.14 g), and 46 (120.98 g) showed superiority, presenting fruit weights higher than the mean plus the standard deviation. Vera et al. (2005), working in the municipalities of Mambaí and Araguapaz - GO, found values for this characteristic ranging from 46.38 g to 379.37 g; with an average of 140.7 g, results superior to those presented in this study. According to Barradas (1972), the fruits show a great mass variability (30 to 150 g), with smaller fruits predominating, that is, extremely large fruits are more difficult to occur.

Regarding the length of the fruits, the values ranged from 4.71 cm (genotype 45) to 6.22 cm (genotype 5), with an average of 5.44 cm. Silva et al. (1994) reported that the length of C. brasiliense fruits varied from 6.0 cm to 14.0 cm.

Table 1. Descriptive statistics of the physical characteristics of the whole fruit and the peel of *C. brasilienses*, Tocantins, BR.

Plants	FW (g)	FL (cm)	DMLF (cm)	DMTF (cm)	PW (g)	GPT (cm)	LPT (cm)	PY (%)
Minimum	66.02	4.71	5.02	4.36	37.22	0.72	0.45	52.28
Maximum	134.92	6.22	7.16	5.86	102.22	1.41	0.98	82.09
Average	98.73	5.44	6.12	5.21	67.66	1.13	0.72	68.26
DP	19.5	0.39	0.47	0.38	15.89	0.16	0.12	6.25
CV%	19.75	7.16	7.67	7.29	23.48	14.15	16.66	9.15

FW - fruit weight, FL - fruit length, DMLF - diameter longitudinal of the fruit (larger), DMTF - diameter transverse of the fruit – (smaller), PW - peel weight, GPT - greater peel thickness, LPT - lesser peel thickness, PY- peel yield.

The larger diameter of the fruits varied from 5.02 cm (genotype 34) to 7.16 cm (genotype 11), with an average of 6.12 cm. For the smaller diameter of the fruit, the variation was from 4.36 cm (genotype 38) to 5.86 cm (genotype 4), with an average of 5.21 cm, which gives a somewhat spherical conformation to the fruit. However, it is known that the size of the fruit is highly variable among natural populations of *C. brasiliensis*, as observed in the study conducted by Santana and Naves (2003), where they worked with twenty populations in the state of Goiás, and found diameters ranging from 3.2 cm to 30.0 cm.

Regarding the weight of the peel, the values ranged from 37.22 g (genotype 38) to 102.22 g (genotype 4), with an average of 67.6 g. Vera et al. (2005) found an average weight of 95.40 g for the weight of peel. Since the economic interest in the plant lies in the pulp of the fruit, it becomes interesting to analyze different uses for the peel, given that large quantities of this material are generated. Moreira et al. (2020), when using a substrate for vegetable seedling production enriched with pequi peel, stated that this substrate presented positive characteristics regarding the composition of macro and micronutrients, C/N ratio, and organic matter. Additionally, it positively influenced the growth of the studied seedlings.

The greatest thickness of the peel varied from 0.72 cm (genotype 38) to 1.41 cm (genotype 4), with an average of 1.13 cm, a value close to that found by Vera et al. (2005), which was 1.14 cm. Regarding peel yield, the lowest proportion found was 52.28% (genotype 38) and the highest proportion was 82.09% (genotype 4), with an average of 68.26%.

The average number of frutilhos found in this study was 2.04 per fruit (Table 2), which is close to the value reported by Vera et al. (2005), with an average of 2.25 small fruits per *C. brasiliense* fruit. However, it should be noted that this characteristic has little relevance, since the small fruits have little commercial use (VERA et al., 2005).

Table 2. Descriptive statistics of the physical characteristics of the stone and almond of C. brasiliense, Tocantins, BR., 2009.

Plants	NF	NP	PWP (g)	DMA (cm)	DMI (cm)	PT (cm)	SLT (cm)	D1A (cm)	D2A (cm)
Minimum	1.78	1	14.79	3.41	2.57	0.21	0.32	1.75	0.85
Maximum	2.73	2	43.83	4.61	3.52	0.48	0.6	3.31	1.45
Average	2.04	1.3	30.65	4.16	3.06	0.35	0.49	2.36	1.11
DP	0.18	0.23	7.27	0.31	0.24	0.08	0.06	0.31	0.13
CV%	8.82	17.69	23.71	7.45	7.84	22.85	12.24	13.13	11.71

NF: number of frutilhos; NP: number of pits; PWP: pit weight with pulp; DMA: larger diameter of the pit; DMI: smaller diameter of the pit; PT: pulp thickness; SLT: spine layer thickness; D1A: almond major diameter; D2A: almond minor diameter.

The number of putamen (pits) did not vary much among the plants, resulting in an overall average of 1.30 putamen per fruit. The studies by Barradas (1972), Almeida (1998), and Silva et al. (2001) also observed a higher incidence of fruits with only one developed stone.

The pits had an average mass of 30.65 g. Vera et al. (2007) found mean values of 2.49 cm and 2.52 cm for the larger and smaller diameter of the pits in the Araguapaz region of Goiás, respectively, and mean values of 3.10 cm and 3.34 cm for the same measurements in the Mambaí region of Goiás.

The thickness of the pulp of *C. brasiliense* had an average of 0.35 cm, ranging from 0.21 cm (genotype 43) to 0.48 cm (genotype 19). Genotypes 19 (0.4 cm), 27 (0.48 cm), 5 (0.47 cm), 2 (0.46 cm), 22 (0.45 cm), and 9 (0.45 cm) stood out. In this study, the thickness of the spiny endocarp layer of the pits was also measured, which had an average of 0.49 cm, ranging from 0.32 cm (genotype 25) to 0.60 cm (genotypes 32 and 43).

The average diameters of the almond of *C. brasiliense* ranged from 2.36 cm (major diameter) to 1.11 cm (minor diameter). According to Oliveira (1998), the larger size of the almond is important for the formation and survival of seedlings in nurseries.

The approximate average distribution of the components of the *C. brasiliense* fruit in the population of Porto Nacional - TO is composed of 68% shell, 31% pit, and 0.5% undeveloped fruits. It should be noted that the percentage of pits in this study is higher than that found by Vera et al. (2005), who reported the following values for the fruit: 76.7% shell, 21.6% pit, and 1.7% undeveloped fruits.

Through the analysis of correlation among the physical characteristics of the fruit (Table 3), it was observed a high positive and significant correlation between fruit weight and peel weight (0.93); peel weight, larger (0.72) and smaller (0.90) fruit diameter; peel weight and fruit length (0.80); greater peel thickness and lesser peel thickness (0.84); peel yield and greater (0.81) and lesser (0.82) peel thickness; pit weight and larger fruit diameter (0.80); diameter minor and pit weight with pulp (0.72); smaller diameter of the pits and larger diameter of the pits (0.84).

Indeed, these relationships between variables are relevant because they allow us to select commercially important fruit characteristics, such as pulp yield based on the evaluation of the number of pits, fruit weight, height, and the smaller diameter of the fruit.

In the correlation analysis, it was found that seven characteristics: fruit weight - FW; peel yield - PY; number of frutilhos - NF; number of pits - NP; pit weight - PWP; pulp thickness - PT; and spine layer thickness of the pit - SLT; did not correlate significantly with the other characteristics.

Observing the relative importance of the characteristics in the last eigenvectors (data not shown), it can be seen that the peel yield, longitudinal fruit diameter, and seed weight have lower importance, therefore being the ones that contribute the least to the discrimination of the genotypes. However, when these variables are eliminated, alterations in the grouping of genotypes are observed, and it is not recommended to discard these variables for the study of phenotypic variability in this population of *C. brasiliense.*

Table 3. Estimates of Pearson's correlation coefficients (r) between pairs of fruit physical characteristics of 47 genotypes of *C. brasiliense* native to the region of Tocantins, BR.

	FW	FL	DMLF	DMTF	PW	GPT	LPT	PY	NP	NF	PWP	DMA	DMI	PT	SLT	D1A
FH	0.78**															
DMLF	0.88**	0.58**														
DMTF	0.88**	0.82**	0.68**													
PW	0.93**	0.80**	0.72**	0.90**												
GPT	0.56**	0.57**	0.28	0.63**	0.76**											
LPT	0.56**	0.61**	0.28	0.69**	0.78**	0.84**										
PY	0.23	0.36*	-0.06	0.39**	0.55**	0.81**	0.82**									
NP	-0.13	0.012	-0.22	-0.05	-0.02	0.01	0.21	0.21								
NF	0.31*	-0.12	0.54**	0.02	0.10	-0.05	-0.17	-0.38*	-0.39**							
PWP	0.64**	0.37*	0.80**	0.42**	0.33*	-0.16	-0.16	-0.56**	-0.30*	0.57**						
DMA	0.42**	0.45**	0.50**	0.42**	0.24	-0.17	-0.11	-0.33**	-0.06	-0.19	0.60**					
DMI	0.50**	0.52**	0.50**	0.48**	0.29*	-0.16	-0.07	-0.42**	-0.02	-0.05	0.72**	0.84**				
PT	0.05	0.17	0.15	0.12	-0.07	-0.10	-0.15	-0.25	-0.35*	0.15	0.30*	0.25	0.27			
SLT	0.15	0.19	0.20	0.08	0.05	-0.20	-0.10	-0.27	0.24	-0.08	0.30*	0.46**	0.49**	-		
D1Am	0.24	0.11	0.23	0.17	0.17	-0.09	-0.05	-0.14	0.06	-0.19	0.24	0.55**	0.40**	-	0.47*	
D2Am	0.29*	0.20	0.20	0.19	0.21	-0.06	-0.006	-0.16	0.02	-0.13	0.29	0.45**	0.50**	-0.27	0.43*	0.74**

****: Significant at 1% probability by t test *: Significant at 5% probability by t test. FW - fruit weight, FL- fruit length, DMLF - diameter longitudinal of the fruit (larger), DMTF - diameter transverse of the fruit – (smaller), PW - peel weight, GPT - greater peel thickness, LPT - lesser peel thickness, PY- peel yield. NF: number of frutilhos; NP: number of pits; PWP: pit weight with pulp; DMA: larger diameter of the pits; DMI: smaller diameter of the pits; PT: pulp thickness; SLT: spine layer thickness; D1A: almond major diameter; D2A: almond minor diameter.**

Carrying out the grouping based on the Method of Average Connection between Groups - UPGMA, using the Standardized Mean Euclidean distance as a measure of dissimilarity, and considering the cutoff point the average of the distances of the grouping matrix (1.33), the formation of six dissimilarity groups is observed (Figure 2).

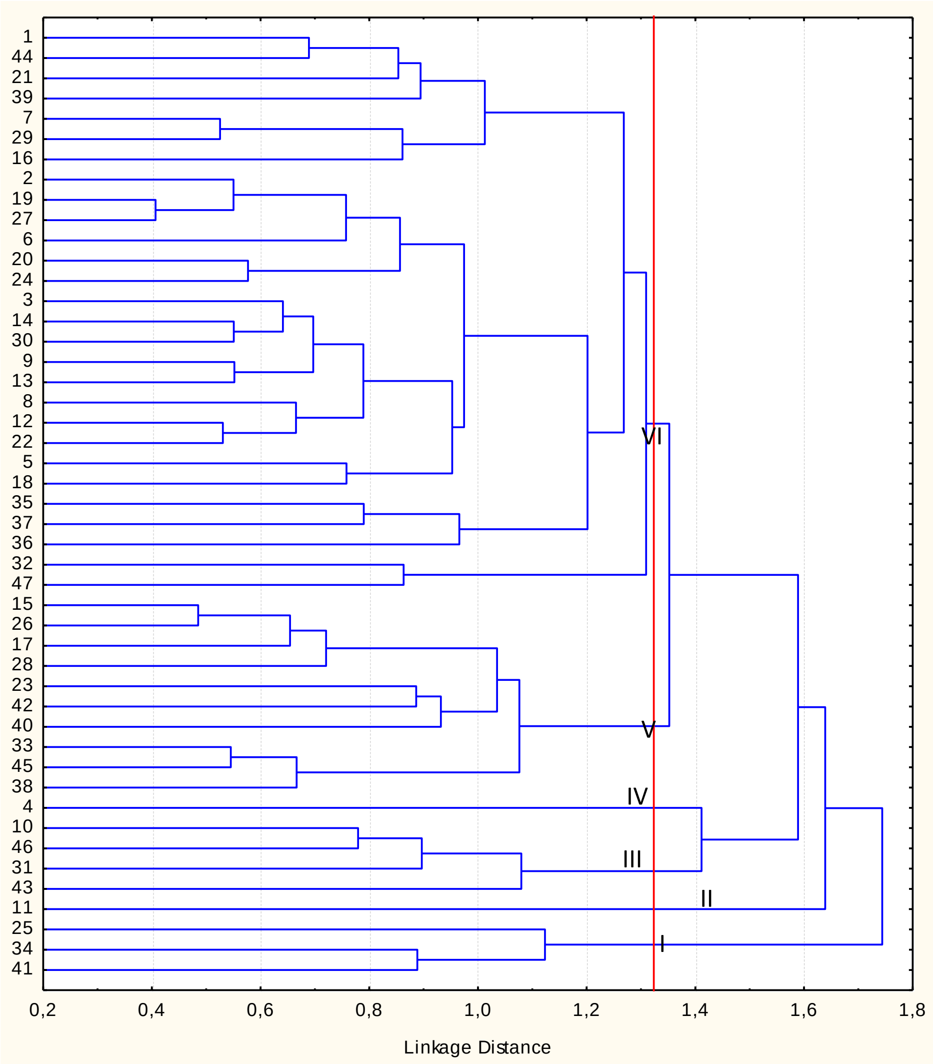

Figure 2. Clustering of 47 genotypes from a population of *C. brasiliense* based on the Mean Linkage Method between Groups – UPGMA.

This type of analysis helps us in the sampling of genotypes for the establishment of germplasm banks, with the minimum possibility of maintaining replicas, sampling phenotypically distant individuals.

Regarding sampling at a distance of less than 20 m, it was found that the plants showed considerable similarity. As can be observed in Figure 2, where genotypes 8 and 7 are allocated in the same similarity group, as well as genotypes 29 and 30. A certain genetic proximity is also observed between genotypes 4 and 46 and between 17 and 18. This similarity is probably caused by kinship or possible vegetative reproduction of *C. brasiliense.*

It can be observed that regarding the larger size of the pit, genotypes 3 (4.72 cm), allocated in group VI, and genotype 43 (4.62 cm), allocated in group I, stand out. These genotypes are therefore quite dissimilar and can be used in crosses aimed at developing populations with larger pits.

As previously mentioned, regarding the pulp thickness, genotypes 19 (0.48 cm) and 5 (0.47 cm), 2 (0.46 cm), 9 (0.45 cm) and 22 (0.45 cm) located in group VI stand out. Therefore, these genotypes show high similarity. In this case, interpopulation crossings should be carried out to increase pulp thickness, that is, these genotypes should be crossed with promising genotypes from other populations.

CONCLUSION

The study reveals significant phenotypic variability, particularly concerning fruit weight, peel weight, seed weight, and peel and pulp thickness.

Correlation analyses among the physical characteristics of the fruits enable the visualization of important relationships, such as the high positive correlation between fruit weight and peel weight, and between peel weight and fruit diameter.

The clustering of genotypes based on dissimilarity led to the formation of six similarity groups, which could be useful in selecting genotypes for germplasm banks to maximize genetic diversity.

Considerable similarity was observed among genotypes sampled within a distance of less than 20 meters, suggesting kinship or vegetative reproduction of *C. brasiliense* in this area.

The results allow us to conclude that the population has sufficient variability to be used in breeding programs or for providing accessions for the composition of germplasm banks.

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