

**Diversidade fenotípica e biometria de frutos e sementes de uma população natural de**

***Cenostigma macrophyllum* Tul.**

**Phenotypic diversity and biometry of fruit and seeds of a natural population of**

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## Resumo

Diversidade fenotípica e biometria de frutos e sementes de uma população natural de *Cenostigma macrophyllum* Tul. Este estudo teve como objetivo caracterizar e estimar a diversidade fenotípica de *Cenostigma macrophyllum* em população natural do Estado do Piauí, Brasil, a partir das características físicas dos frutos e sementes. Os dados foram coletados no município de Floriano-PI. Para a biometria foram avaliadas as variáveis: comprimento do fruto (mm), largura do fruto (mm), peso do fruto (g), número de sementes por fruto, comprimento da semente (mm), largura da semente (mm) e espessura da semente (mm). Os dados biométricos foram analisados por estatística descritiva e os desvios da normalidade dos dados foram confirmados pelo teste de Lilliefors, para uso da correlação não paramétrica de Spearman. A divergência fenotípica observada entre os indivíduos foi baseada em análises de agrupamento (Tocher e UPGMA) e componentes principais. De acordo com os componentes principais e o dendrograma os indivíduos 3 e 11 apresentam maior dissimilaridade. As variáveis relacionadas aos frutos (comprimento, largura e peso) apresentaram correlação positiva significativa, sendo que as mesmas foram as que mais contribuíram para a variação intraespecífica. Os resultados obtidos foram relevantes, pois servirão de subsídios para estudos relacionados ao pré-melhoramento e conservação da espécie.

**Palavras-chave:** Caneleiro; Caracterização; Morfometria.

## Abstract

Phenotypic diversity and biometry of fruit and seeds of a natural population of *Cenostigma macrophyllum* Tul. The aim of this study was to characterize and estimate the phenotypic diversity of *Cenostigma macrophyllum* in a natural population in the state of Piauí (PI), Brazil, from the physical characteristics of the fruit and seeds. The data were collected in the municipality of Floriano, PI. The following variables were evaluated in biometry: fruit length (mm), fruit width (mm), fruit weight (g), number of seeds per sample fruit, seed length (mm), seed width (mm), and seed thickness (mm). The biometric data were analyzed by descriptive statistics, and deviations from normality of the data were confirmed by the Lilliefors test for use of the Spearman non-parametric correlation. The phenotypic divergence observed among the individuals was based on cluster analyses (Tocher and UPGMA) and principal components. According to principal components and the dendrogram, individuals 3 and 11 have the greatest dissimilarity. The variables related to the fruit (length, width, and weight) had significant positive correlation, and these variables were the ones that most contributed to

intraspecific variation. The results obtained were relevant because they will assist studies related to pre-breeding and conservation of the species.

**Keywords:** Caneleiro; Characterization; Morphometry.

## Resumen

Diversidad fenotípica y biometría de frutos y semillas de una población natural de *Cenostigma macrophyllum* Tul. Este estudio tuvo como objetivo caracterizar y estimar la diversidad fenotípica de *Cenostigma macrophyllum* en una población natural del Estado de Piauí, Brasil, a partir de las características físicas de los frutos y semillas. Los datos fueron recolectados en el municipio de Floriano-PI. Para la biometría se evaluaron las variables: largo del fruto (mm), ancho del fruto (mm), peso del fruto (g), número de semillas por fruto, largo de la semilla (mm), ancho de la semilla (mm) y espesor de la semilla (mm). Los datos biométricos se analizaron mediante estadística descriptiva y las desviaciones de los datos normales se confirmaron mediante la prueba de Lilliefors, para utilizar la correlación no paramétrica de Spearman. La divergencia fenotípica observada entre los individuos se basó en el análisis de conglomerados (Tocher y UPGMA) y componentes principales. Según los componentes principales y el dendrograma, los individuos 3 y 11 muestran mayor disimilitud. Las variables relacionadas con frutos (largo, ancho y peso) mostraron una correlación positiva significativa, y fueron las que más contribuyeron a la variación intraespecífica. Los resultados obtenidos fueron relevantes, ya que servirán como subsidios para estudios relacionados con la pre-reproducción y conservación de la especie.

**Palabras clave:** Caneleiro; Descripción; Morfometría.

## 1. Introduction

*Cenostigma* is a small genus composed of three species of trees and shrub: *C. macrophyllum tocatinum* Tul., *C. tocatinum* Ducke, and *C. macrophyllum*, and it is considered a genus endemic to Brazil (Alves et al, 2012; Souza & Nascimento, 2018). *C. macrophyllum* is popularly known as caneleiro, canela-velha, and catingueira, has a tree or shrub type growth habit, and is distributed in Caatinga and Cerrado ecosystems in regions of Minas Gerais, west of Bahia, east of Goiás, and Tocantins, and it is relatively common in the central southern part of Piauí (Aguiar et al., 2016; Araújo et al., 2018).

This species is widely used in popular medicine against gastrointestinal diseases. The ethanol extract from its leaves has antinociceptive, anti-inflammatory, antibacterial, antioxidant,

and antiulcerogenic activities (Viana et al., 2013; Santos et al., 2015). It is a relatively important species in Piauí, appearing in phytosociological surveys.

It is used in tree planting and landscaping of squares and parks due to its favorable aspects, such as a straight trunk, rapid growth, leafy canopy, and not very aggressive root system (Moraes & Machado, 2015)

Conservation of genetic resources of plant species is considered a theme of great relevance, the reason for which numerous studies have been performed in quantifying phenotypic diversity and in understanding its magnitude, nature, and distribution among and within populations (Cruz et al., 2012; Costa et al., 2016; Menegatti et al., 2017; Correia et al., 2019).

According to Zuffo et al. (2019), biometric characterization consists of evaluation of morphological features of different parts of the plant, such as fruit and seeds, which contributes to determination of plant patterns in plant breeding programs, and to direct and indirect selection of these characteristics.

Thus, phenotypic characterization performed through biometric evaluation assists in conservation, allowing information for rational and effective use of fruit and seeds, a useful tool for detecting phenotypic variability within populations of the same species and the relations between this variability and environmental factors (Vianna et al., 2017; Carneiro et al., 2018).

In spite of the economic and medicinal importance of caneleiro, there are few studies related to biometric characterization of the fruit and seeds, which constitute an important tool in detection of intraspecific phenotypic variability (Silva et al., 2018).

There are not yet reports in the specialized literature on studies of characterization and phenotypic divergence through morphological descriptors for caneleiro; information dealing with this theme is still scarce. Effective knowledge of phenotypic variability in populations is fundamental for understanding the biology of this species, since characterization of perennial plants has been performed with the assistance of a list of botanical and morphological descriptors.

In light of the above, the aim of this study was to physically characterize the fruit and seeds of caneleiro in native occurring population in the state of Piauí, Brazil, to provide information regarding which phenotypic descriptors are recommended in estimating phenotypic diversity, as well as identifying intraspecific variations.

It is important to note that there are not yet studies on the floristics and structure of the tree community and on the population dynamics of this species for the region.

## 2. Methodology

This research deals with an experimental field study (Pereira et al., 2018). The fruits were collected in an area of naturally occurring vegetation from trees in the city of Floriano, PI, Brazil. Vegetation in the study area is of the sensu stricto Cerrado (Brazilian tropical savanna) type. Ten ripe samples of fruit were collected from the canopy of 22 trees identified as G-1 to G-22, according to the order of collection. Trees were chosen at random in the area at distance of at least 100 m from each other.

Plants were selected based on fruit availability. The geographic coordinates (latitude and longitude) were determined with the assistance of the Global Positioning System (GPS) and botanical material was collected in the reproductive stage, which was identified based on a specialized bibliography. After the fruit and seeds were collected, the material was placed in plastic bags, labeled, and taken to the laboratory of the Universidade Federal do Piauí for morphological characterization and evaluation.

For biometric evaluations of the plants, the following descriptors were proposed and evaluated: fruit length (mm), fruit width (mm), fruit weight (g), number of seeds per sample fruit, seed length (mm), seed width (mm), and seed thickness (mm). The measurements of length, width, and thickness were performed with the aid of a digital caliper and were expressed in millimeters. For measurements of mass, a digital precision balance with expression in grams was used. To effect morphological measurements, 10 repeated measurements were used per individual evaluated.

The following were calculated for each variable: arithmetic mean, standard deviation, coefficient of variation (CV), asymmetry (S), and kurtosis (K). The reference values adopted for the asymmetry coefficient were  $S < 0$ , asymmetric distribution to the left, and  $S > 0$ , asymmetric distribution to the right. For the kurtosis coefficient, the values were  $K > 3$  (leptokurtic), a more “tapered” distribution than normal, and  $K < 3$  (platykurtic), a flatter distribution than normal. The deviations from normality of the biometric data were confirmed by the Lilliefors test (for K samples); after detecting that the data had a curve different from normal ( $P < 0.05$ ), the use of non-parametric statistics was considered. Thus, the Spearman correlation coefficient (rs) and the respective level of significance (p) among the variables was calculated through the t test.

Phenotypic diversity among genotypes was evaluated using the average linkage method between groups or the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and Tocher, by means of the simple Euclidean distance matrix. The relative contribution of the traits to phenotypic divergence was quantified, according to the criteria proposed by Singh (1981).

Principal component analysis (PCA) was performed in order to eliminate redundant morphological variables. The data were standardized, and statistical analyses were made using the computational resource Genes 2013.5.1 (Cruz, 2016).

### 3. Results and Discussion

The descriptive analysis for the variables analyzed in the fruit and seeds is presented in Table 1.

**Table 1** - Biometric characteristics of fruit and seeds of *C. macrophyllum*.

Biometric characteristic	n	Minimum	Maximum	Mean	Standard deviation	CV (%)	Asymmetry (S)	Kurtosis (K)
Fruit length (mm)	220	6.18	10.12	7.64	1.07	14.08	0.528	-0.179
Fruit width (mm)	220	1.97	3.42	2.66	0.36	13.80	0.047	0.321
Fruit weight (g)	220	6.06	12.72	8.39	1.20	14.30	0.234	-0.357
Number of seeds per sample fruit	220	2	3	3.09	0.30	10.97	-0.445	-0.342
Seed length (mm)	220	1.52	1.95	1.71	0.13	7.60	-0.144	-0.401
Seed width (mm)	220	0.79	1.35	0.18	1.09	16.03	0.122	-0.937
Seed thickness (mm)	220	0.24	0.35	0.03	0.30	10.45	-0.04	-0.722

\*n= sample size, CV= coefficient of variation. Source: Authors (2020).

There was considerable variation in amplitude (maximum and minimum values) of the biometric characteristics of the fruit and seeds of the caneleiro populations evaluated. The coefficient of variation differed among the variables studied; the values were considered satisfactory ( $CV \leq 20\%$ ), confirming good experimental precision for all the characteristics.

In relation to asymmetry, the variables of number of seeds per sample fruit, seed length, and seed thickness had a negative asymmetry coefficient (asymmetric distribution to the left), indicating that seeds with shorter length and thickness predominate in the sample analyzed. The other characteristics had positive asymmetry coefficients (asymmetric distribution to the right), indicating that fruit of greater length, width, and weight predominate in the sample analyzed. All the characteristics had a kurtosis measurement smaller than that of normal distribution, with  $K < 3$  (platykurtic), indicating dispersion of the data around the mean.

Considering the distribution of the biometric variables of the fruit of the trees analyzed, fruit was identified with weight ranging from 6.06 to 12.72 g, the values for fruit length were from 6.18 to 10.12 mm, and the fruit had from two to three seeds, which shows phenotypic variation between the two populations evaluated. These values are similar to those reported by Faria et al. (2018), who found mean values of 5.26 and 6.66 g for fruit weight, 7.48 and 7.32 mm

for fruit length, and from one to five seeds per fruit in urban and rural areas in the municipality of Teresina, PI, Brazil. This phenotypic similarity indicates that biometric characterization of the fruit can be applied as an important tool in the taxonomy and identification of caneleiro varieties.

The seed length, width, and thickness variables had values ranging from 1.52 to 1.95 mm, 0.79 to 1.35 mm, and 0.24 to 0.35 mm, respectively. According to Pereira et al. (2018), characterization of seeds is of extreme importance since variations in the biometric characteristics of seeds can affect germination capacity and is related to reproductive problems of the species.

We used the Spearman correlation coefficient (rs) to express the degree of association among variables (Table 2). The values obtained for correlation indicate that there was positive and significant association among the variables that involve fruit length, width, and weight. The highest correlation values observed were between fruit width and fruit weight (0.597) and between fruit length and fruit weight (0.552). This result was expected due to the association between length and width for constitution of fruit weight. Thus, selection of plants with wide fruit favors selection of plants that have heavier fruit.

It is important to emphasize that knowledge of the correlation between these variables also assists in the selection process because it allows definition of the effect of the selection carried out for one characteristic on another, as well as indirect selection for characteristics that are difficult to measure (Gonçalves et al., 2013).

**Table 2** - Spearman correlation (rs) between the biometric variables of the fruit and seeds of *C. macrophyllum*.

Correlation	rs
Fruit length x Fruit width	0.497**
Fruit length x Fruit weight	0.552**
Fruit length x Number of seeds per sample fruit	0.278 <sup>ns</sup>
Fruit length x Seed length	0.134 <sup>ns</sup>
Fruit length x Seed width	-0.375 <sup>ns</sup>
Fruit length x Seed thickness	0.217 <sup>ns</sup>
Fruit width x Fruit weight	0.597**
Fruit width x Number of seeds per sample fruit	0.068 <sup>ns</sup>
Fruit width x Seed length	0.227 <sup>ns</sup>
Fruit width x Seed width	0.0175 <sup>ns</sup>
Fruit width x Seed thickness	0.390 <sup>ns</sup>
Fruit weight x Number of seeds per sample fruit	0.167 <sup>ns</sup>
Fruit weight x Seed length	-0.0016 <sup>ns</sup>
Fruit weight x Seed width	-0.0513 <sup>ns</sup>
Fruit weight x Seed thickness	0.4093*
Number of seeds per sample fruit x Seed length	-0.0208 <sup>ns</sup>
Number of seeds per sample fruit x Seed width	-0.110 <sup>ns</sup>
Number of seeds per sample fruit x Seed thickness	0.282 <sup>ns</sup>
Seed length x Seed width	0.325 <sup>ns</sup>
Seed length x Seed thickness	0.209 <sup>ns</sup>
Seed width x Seed thickness	0.084 <sup>ns</sup>

\*\* = P <0.01; \* = P <0.05; ns = not significant. . Source: Authors (2020)



According to the method of Singh (1981), the fruit weight, length, and width descriptors were the characteristics that most contributed to intraspecific phenotypic divergence, whereas the characteristics related to seed biometry – seed length, width, and thickness – were the descriptors that made the lowest effective contribution to phenotypic diversity (Table 3).

**Table 3** - Relative contribution of the morphological variables evaluated in *C. macrophyllum* through the method of Singh (1981).

Characteristic	Relative contribution (%)
Fruit length	21.85
Fruit width	25.93
Fruit weight	51.23
Number of seeds per pod	0.869
Seed length	0.0996
Seed width	0.0148
Seed thickness	0.0001

Source: Authors (2020).

The variation observed from biometry of the fruit and seeds of caneleiro was possibly enhanced by environmental factors, especially by water availability, which is an essential factor for formation of fruit and seeds, as well as the phenotypic variability of the population (Zuffo et al., 2014; Lucio et al., 2019).

Consequently, studies with this focus allow selection of characteristics of interest that meet commercial needs and the needs of programs for recovery of degraded areas and landscaping with seeds that promote the establishment of vigorous plants and meet the requirements of the desired phenotypic variability (Correia et al., 2019).

In spite of being characteristic of each species, the number of seeds formed by the fruit can be affected by environmental factors, while the morphometric characteristics of the seeds can vary within the same population, due to phenotypic diversity (Freire et al., 2015). In addition to the study of the characteristics intrinsic to fruit and seeds, it is also necessary to evaluate the association among these traits, since that allows determination of the degree of the effect of one characteristic on another, and allows indirect selection (Zuffo et al., 2016).

The Tocher optimization method (Table 4) formed three groups. Group I gathered a larger number of trees (90.9%). The other groups formed are constituted by only one individual. From analysis of the intergroup distances, the greatest phenotypic dissimilarity was found between groups II and III (8.03).



**Table 4** - Clustering by the Tocher method based on dissimilarity estimated by the mean Euclidean distance in relation to the biometric characteristics of the fruit and seeds evaluated in 22 *C. macrophyllum* trees collected in Floriano, Piauí, Brazil.

Group	Samples
I	G-5, G-20, G-6, G-14, G-12, G-7, G-19, G-16, G-1, G-21, G-15, G-10, G-22, G-17, G-4, G-9, G-13, G-2, G-18, G-3
II	G-8
III	G-11

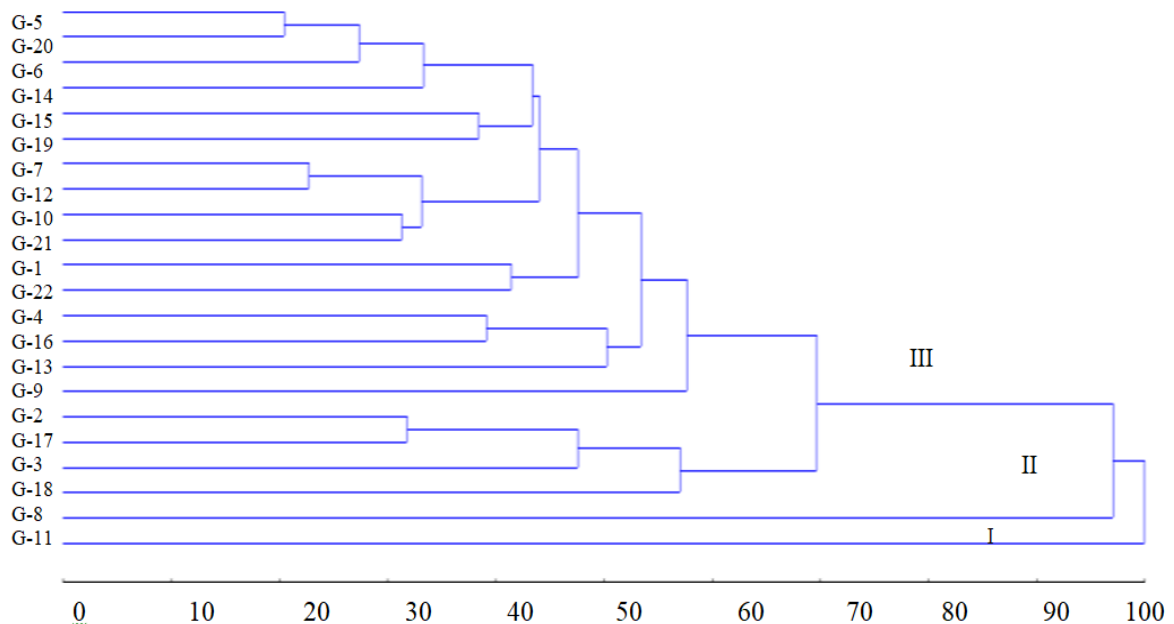
Source: Authors (2020).

Based on the measurements of dissimilarity, we applied the UPGMA technique, which consists of clustering to discriminate individuals and separate them into groups, analyzing the set of features inherent to each individual and classifying them in such a way that there is homogeneity within the group and heterogeneity among groups (Cruz et al., 2012; Rosa et al., 2019).

The consistency of the clustering pattern, as well as the ability of the dendrogram to reproduce the dissimilarity matrices was confirmed from a cophenetic correlation coefficient of 0.823. According to Rohlf (1970), cophenetic correlation values lower than 0.70 indicate unsuitability of the clustering method.

According to the dendrogram that evaluated the similarity between individuals regarding the biometric characteristics of the fruit and seeds, the greatest similarity was observed between individuals 5 and 20, with maximum dissimilarity between plants 3 and 11 (Figure 1). Allowing for high adjustment of the matrices and bidimensional representation, at least three groups of phenotypic diversity were formed. Allocation of the genotypes among the groups of diversity occurred in the following manner: groups I and II are composed of only one individual each, whereas group III is composed of 20 individuals.

**Figure 1** - Dendrogram obtained by the UPGMA method from the dissimilarity measures among 22 *C. macrophyllum* trees based on mean Euclidean distance.



Source: Authors (2020).

The aim of performing principal component analysis was to reveal the tendencies in variation of the plants analyzed within the entire set of morphological descriptors that were proposed to estimate phenotypic diversity.

From the mean values of the seven descriptors, values for variance, percentage of variance, and accumulated variance of each principal component were provided (Table 5). The first principal component was responsible for 35.46% of the total variation, while the two principal components accumulated 57.25% of the total variance. According to the criterion of Kaiser (1960), we selected the first three principal components, since eigenvalues greater than 1.0 were obtained. Thus, this method proved to be effective in evaluation of phenotypic diversity in caneleiro by phenotypic markers.

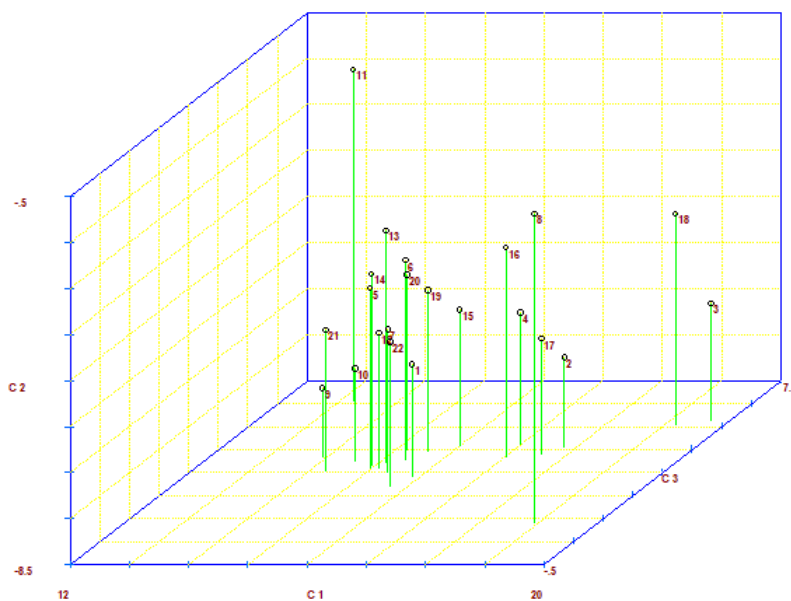
The traits that most contributed to the variability of the first three components were fruit weight, width, and length, and they were the same descriptors indicated by the Singh method. The traits of greatest discriminatory effect for the principal components PC1, PC2, and PC3 make a direct contribution to important correlated traits (Table 5). The biplot diagram in which principal components 1, 2, and 3 were plotted shows that plants 3 and 11 are the most divergent (Figure 2).

**Table 5** - Estimate of the eigenvalues (EV) associated with the principal components and their percentage of variance [EV(%)] and accumulated variance (% Accumulated) of the biometric variables of the fruit and seeds from 22 *C. macrophyllum* trees collected in Floriano, Piauí, Brazil.

Principal component	EV	EV(%)	% Accumulated
PC1	2.482	35.46	35.46
PC2	1.525	21.79	57.25
PC3	1.288	18.40	75.65
PC4	0.712	10.18	85.83
PC5	0.495	7.084	92.91
PC6	0.295	4.214	97.128
PC7	0.199	2.854	100.00

Source: Authors (2020).

**Figure 2** - Biplot diagram comparing the 22 *C. macrophyllum* trees, collected in Floriano, Piauí, Brazil.



Source: Authors (2020).

Understanding regarding estimates of phenotypic parameters, as for example, heritability, allow inferences to be made regarding phenotypic variance and more efficient strategies to be directed to plant breeding programs (Baldissera et al., 2014). Thus, we consider the variables of greatest discriminatory power among the three principal components (PC1, PC2, and PC3) as the

most important variables for discrimination among natural populations of caneleiro. Principal component analysis therefore allowed the variables that explain little of the variation among the population, related to the width and thickness of the seeds, to be discarded.

That way, all the multivariate analyses used – the Tocher method, the UPGMA method, and principal components – showed similarities in the association and in the clustering of the trees in the natural population evaluated, indicating that the morphological descriptors that were proposed for biometric evaluation in *C. macrophyllum* are efficient in the study of phenotypic diversity of this native plant. Diverse studies of phenotypic diversity in natural populations have shown that both principal component analysis and cluster methods are similar in clustering of the plants, which has contributed to knowledge of the phenotypic patterns of variation and divergence of species (Costa et al., 2016; Vianna et al., 2017; Menegatti et al., 2017; Rosa et al., 2019; Carneiro et al., 2019).

#### 4. Final Considerations

Studies of fruit and seed biometry supply additional information relevant for conservation of species. Thus, as studies on *C. macrophyllum* are still few, more information can support programs for genetic conservation of natural populations and for raising species. From the data obtained in the present study, it can be inferred that the variables related to fruit exhibited significant positive correlation, and these variables were the ones that most contributed to intraspecific variation.

Furthermore, the biometric characteristics evaluated are efficient in estimating the phenotypic divergence of the natural population of caneleiro. Of the morphological variables evaluated, three exhibit a greater contribution to total variation, namely, fruit weight, width, and length; furthermore, a positive correlation predominates among them. The results obtained in this study are relevant because they will serve as a basis for future studies involving pre-breeding, propagation, and genetic conservation of the species.

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