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Physical Characterization and Dimensional Analysis of Brazil Nut Seeds: Implications for Germination, Post-Harvest and Optimization of Industrial Processing

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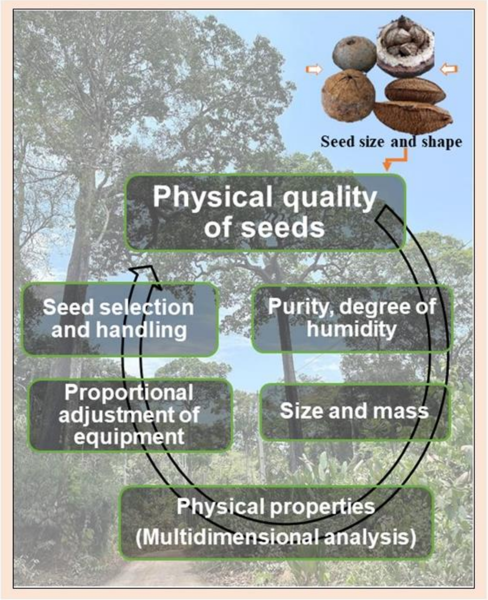
HIGHLIGHTS

- The matrices exhibited seed variability, indicating abiotic and biotic influences on progeny.
- The physical properties of seeds present heterogeneity in size and shape.
- Larger fruits had fewer seeds and consequently the heaviest.
- Analyzing seed physical properties helps tailor processing equipment to size strategies.

Abstract: The seed, the main commercialized non-timber forest product of the Brazil nut tree, still lacks information about its physical characteristics for the promotion of its germination, optimization of post-harvest processes, and industrial processing. Showed that, although each Brazil nut matrix produces fruits and seeds with homogeneous shape and weight, the batches of fruits and seeds from each matrix differ from one another. The PCA showed that most of the variables analyzed interacted with the three geometric dimensions of the seeds, allowing for differentiation between matrices and establishing criteria for seed size classes. To design or use mechanical equipment for the industrial processing of seeds, segregation of matrices with seeds that have similar physical characteristics is essential to the development and/or use of the equipment. The study reveals that each Brazil nut matrix produces fruits and seeds with consistent shape and weight. Despite the within-matrix homogeneity, distinct differences were observed between batches of seeds from each matrix. This suggests that environmental factors or genetic variations might influence the physical characteristics of Brazil nut seeds.

Keywords: Non-timber forest products; post-harvest processing; seed biometrics.

GRAPHICAL ABSTRACT



INTRODUCTION

Brazil nuts are extracted from the fruits of a large tree known in Brazil as Castanheira (*Bertholletia excelsa* Bonpl.), native to the Amazon rainforest and of great economic and environmental importance [1]. Due to the health benefits provided by the consumption of the nut, the Brazil nut tree is known worldwide and sought after in the market, making it one of the main sources of income for thousands of families that inhabit the Amazon rainforest [2]. Its nut is consumed in nature and dehydrated, in addition to its pre-processed products, such as nut milk, oil and the use of almonds in gourmet recipes.

Brazil nuts contain high levels of protein (15%), carbohydrates (9%), lipids (71%) and selenium (Se) (290.5 µg g-1 on average) [3], which makes them a source of potent natural bioactive ingredients used in the preparation of compounds beneficial to human health [4].

Despite being a consolidated product in the market, almost all of the commercialized Brazil nuts still come from the extraction of wild plants that occur naturally in the Amazon rainforest [5]. Plantings of the species for commercial purposes are almost non-existent due to difficulties with propagation.

The main problems associated with the propagation of Brazil nuts include dormancy, which prolongs the time for germination, and high water content, which prevents its storage for a prolonged period. The persistence of these problems stems from a scarcity of studies on the properties and physical parameters of the seeds, as well as deficient knowledge about effective preparation methods before sowing. This demonstrates the importance of investigating and understanding the morphometric characteristics that can favor the proper handling of seeds for post-harvest processing, such as, storage, germination, size segregation and/or industrial processing.

A better understanding of the physical properties of fruits and seeds, for example, could lead to the development of more suitable containers for germination. This is especially important in the case of recalcitrant seeds, whose physical characteristics change with the loss of mass associated with a decrease in water content [6, 7, 8]. This knowledge is also critical for technicians who build sowing, planting, and harvesting machines for different species [9, 10]. According to Landge and coauthors [11], when seed dimensions change, their position, and the volume they occupy in the processing equipment also changes. In the case of agricultural species, a change in the size of the seed added to dosing units of seeders can compromise the efficiency and uniformity of sowing. For forest species, the challenges are even greater due to incipient information about the seed characteristics of numerous species.

Seed dimensions and physical properties are also useful for evaluating seed quality (standardized sizes), genetic variability, and the relationship between three-dimensional aspects and automated industrial processing steps. To advance knowledge on the poorly studied Brazil nut seed, this study examines the physical properties of Brazil nuts through the evaluation of the three-dimensional shape of fruits and seeds collected in forested areas of the southwestern Brazilian Amazon.

MATERIAL AND METHODS

Study area

The study was carried out in the Humaitá Forest Reserve (RFH), a research area belonging to the Federal University of Acre (UFAC) located in the municipality of Porto Acre (9º45'19” S; 67º40'18"W) (Figure 1). The RFH has an area of approximately 2,000 hectares almost completely covered by Open Tropical Forest with palm trees or bamboo in the understory, and to a lesser extent Dense Tropical Forest and Várzea Forest [12]. The relief is softly undulating and intersected by numerous small streams that flood the forested areas in the lowlands during the rainy season.

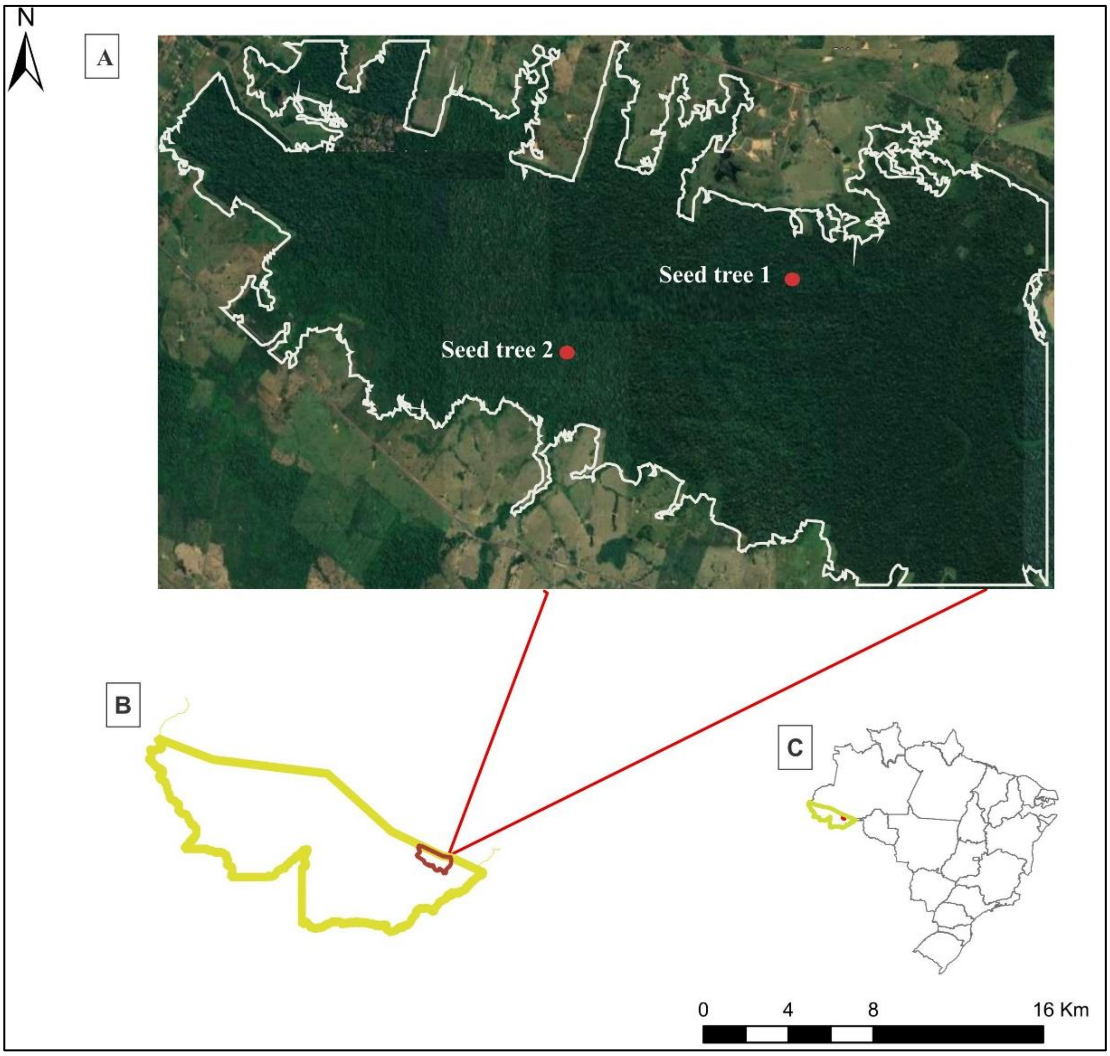


Figure 1. Humaitá Forest Reserve (A, white polygon). Location of the state of Acre and the municipality of Porto Acre (B, yellowish polygon). Map of Brazil with the state of Acre highlighted (C, yellow polygon).

The climate in the region is of the Af type in the Köppen climate classification (Tropical Equatorial), with average annual temperatures between 24 and 26 °C [13]. The average annual evapotranspiration and precipitation are 1728 mm [14] and 2227 mm, respectively. The rains are distributed in two distinct periods: the rainy season, from October to April, when 79.4% of the rains occur, and the dry season, from May to September, with 20.4% of the precipitation [15].

Selection of Brazil nut trees matrices

Two mother trees (Matrix 1 and 2) of *Bertholletia excelsa* located in the primary forest area were selected for study. Each had different characteristics of stem (size and shape) and crown (shape). Matrix 1 had a total height of approximately 32 meters and DBH of 120 cm, a uniform straight trunk, and a well-distributed crown. Matrix 2 was located about 6 km away and measured about 28 meters in height with a DBH of 98 cm, an uneven trunk, and a poorly distributed, skewed crown.

Fruits collection and characterization

The fruits were collected from the forest floor, separately under the canopy of each matrix (10 fruits/matrix, 20 fruits in total), during the dispersal season (December 20, 2022). The fruits were transported to the laboratory of the Instituto Nacional de Pesquisas da Amazônia (INPA) located in Rio Branco, Acre, and individually evaluated for the following characteristics: length (mm), diameter (cm), number of seeds, the total fresh mass of the fruit (g) and fruit thickness (woody mesocarp, mm). Length measurements and weight were recorded with a digital caliper (mm), measuring tape (cm) and digital scale (g), and fruit circumference was measured with measureing tape.

Morphologically, the fruit is an indehiscent pixid, stenocarpic, orbicular to subglobose poricidal capsule that presents a fibrous exocarp, mesocarp and endocarp [16]. The operculum is located in the woody, glabrous, and brownish funiculus. Based on this information, the length of the fruit was determined by measuring from the apex of the poricidal capsule in the opposite direction (funiculus). The diameter was measured in the middle region of the fruit, and the thickness of the mesocarp was measured after the fruits were broken to remove the seeds (Figure 2B).

Seed dimensions and mass

The dimensions and mass of the seeds were measured shortly after their collection due to their recalcitrant characteristic. Externally, the morphology of the stenosperm seeds shows an angular triangular shape, triseriate, with an angled base, margin and apex, but in some situations the base and apex are thinned or not (Figure 2B). It presents two layers of integument, the forehead, outermost, in light brown tones, opaque, rough, glabrous and ligneous, and with fracture lines along its entire length, and the innermost layer, the tegmen, is membranous and darker brown than the forehead. The hilum is depressed (in some situations it may not be visible), subapical, large, oblong, lighter brown than the forehead. The raphe is rigid and protruding, in dark brown tones and homochrome [16].

To obtain the seed individual fresh mass, a heterogeneous sample of 99 units (one unit/seed, random sample) from each matrix was weighed on a precision analytical scale (sensitivity of 0.001g). Afterwards, measurements of the three-dimensional shape of the seeds were made, using a King Tools 150BL digital caliper (sensitivity of 0.01 mm) according to the procedure adopted by Pinheiro and coauthors [17]. The seed length was measured from the hilum to the opposite base, the seed width in the median region of greatest amplitude, and the seed thickness in the narrowest part (Figure 2B).

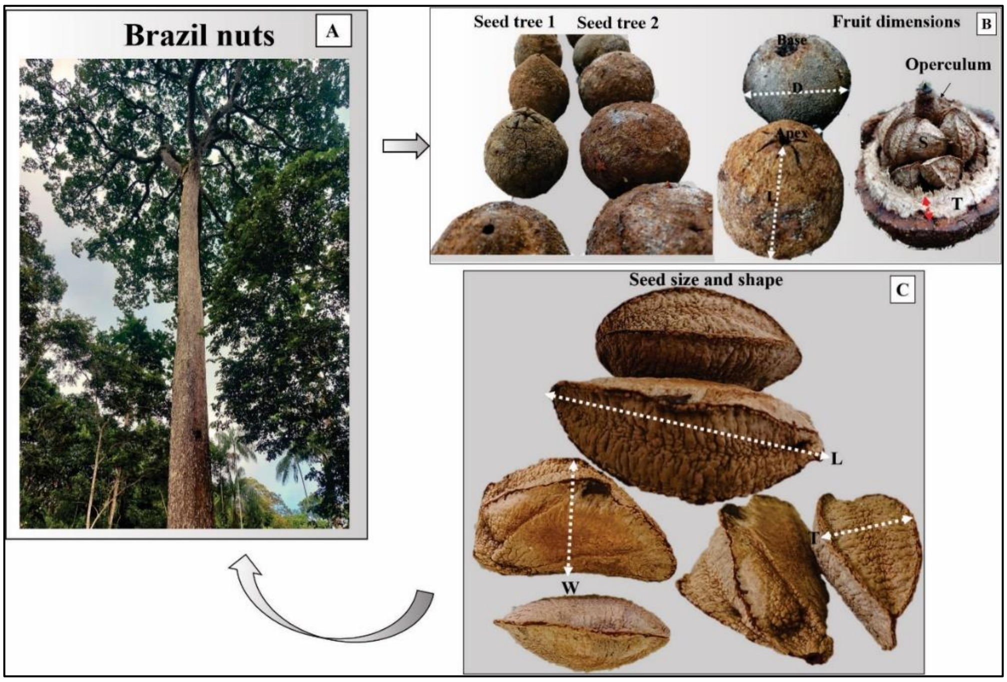


Figure 2. Characteristics of Brazil nut fruits and seeds. (A) Adult matrix; (B) Fruit shape and indication of measurements (L=length, D=diameter); (C) Seed shape measurements (L=length, W=width, T=thickness).

Seeds physical properties

After carrying out the three-dimensional measurements, the physical properties of the seeds were estimated through mathematical calculations: Seed Volume Index (SVI, equation 1, see Vieira and coauthors [18]), Geometric Mean Diameter (GMD, equation 2), Equivalent Mean Diameter (EMD, equation 3), Arithmetic Mean Diameter (AMD, equation 4, see Sahay and Singh [19]), Surface Area (SA, equation 5, see McCabe and coauthors [20]), Seed Sphericity (Ø, equation 6), Aspect Ratio (Ar, equation 7, see Varnamkhasti and coauthors [21]) and Seed Volume (V, equation 8, see Mohsenin [22]).

$$\mathrm{SVI} = \mathrm{length \ x \ width \ x \ thickness} \tag{1}$$

$$\mathrm{GMD} = \mathrm{SVI}^{1/3} \tag{2}$$

$$\mathrm{EMD} = \left[\mathrm{length} \ \frac{(\mathrm{width \ x \ thickness})}{4}\right]^{1/3} \tag{3}$$

$$\mathrm{AMD} = \frac{\mathrm{length} + \mathrm{width} + \mathrm{thickness}}{3} \tag{4}$$

$$\mathrm{SA} = \pi \mathrm{GMD}^2 \tag{5}$$

$$\emptyset = \left[\sqrt[3]{\frac{\mathrm{width \ x \ thickness \ x \ length}}{\mathrm{Length}}}\right] 100 \tag{6}$$

$$\mathrm{Ar} = \left[\frac{\mathrm{width}}{\mathrm{length}}\right] 100 \tag{7}$$

$$\mathrm{V} = \frac{\pi \mathrm{width \ x \ thickness}^2 \mathrm{\ x \ length}^2}{6(2\mathrm{length} - \mathrm{width \ x \ thickness})} \tag{8}$$

Frequency distribution calculations for seed dimensions and mass were defined by pre-established classes. Relative frequency is a way to analyze data through comparison, as it determines the percentage of that data in relation to the total set of collected data. Therefore, these calculations were used only for seed data, defined by the following equation (Equation 9):

$$Rf = \frac{af}{n} \ \mathrm{x} \ 100 \tag{9}$$

Rf = relative frequency; af = absolute frequency; n = total elements

Frequency data were analyzed using a Box or Boxplot diagram to illustrate the seed size dataset according to the defined relative frequency classes.

Data Analysis

The 99 seeds collected from each Brazil nut tree matrix were divided into three subsamples of 33 seeds randomly, and classes size were established using statistical software. Data on morphometric characteristics were explored by calculating mean and amplitude (maximum and minimum = range), coefficient of variation (CV), relative frequency, arithmetic mean, standard deviation and confidence interval to determine the possible association between the phenotypic variation and the analyzed variables. A normality test (Lilliefors test, p value) was performed for a specific distribution function, with known mean and variance. Normality was assessed to determine whether the data set of random variables were well modeled by a normal distribution or not.

All analyzes were processed using R Studio software version 4.2.2 (R Core Team, [23]). To determine and visualize the relationship between the matrices and their physical properties, a principal component analysis (PCA) was performed. The Factoshiny package [24] was used for PCA calculations and graphical visualization. A Pearson's correlation was used to express the relationship between matrices and seed dimensions and mass. Pearson's correlation coefficient (r) was calculated using the Hmisc package [25] and the correlation graphs using the Performance Analytics package [26]. In the graphical analysis, variables without asterisks were not statistically significant, while variables with one, two and three asterisks were statistically significant at 1, 5 and 10%, respectively.

RESULTS

The Brazil nut tree fruits are round, dry, and lignified (woody pixidium, called 'ouriço' in Portuguese), externally very hard, housing several seeds inside. In this study, we found variations in size and shape of fruits and seeds between matrices. Larger fruits house a smaller quantity of seeds (Matrix 1: 98,21 mm of fruit average length, 22 seeds; Matrix 2: 108,12 mm, 18 seeds) (Figure 3). The seeds of larger fruits, however, are bigger (Matrix 1: 6.863,47 ± 1.395,67 mm³; Matrix 2: 11.371 ± 2.729,22 mm³) (Tables 1 and 2). Matrix 1 fruits were smaller but had a greater quantity of seeds of smaller size than matrix 2 fruits. Although the amount of seeds/fruit was smaller in matrix 2, the size of the fruits and seeds was larger. Thus, larger fruits harbor larger seeds in smaller quantities, while smaller fruits produce a larger amount of seeds, but with a smaller size.

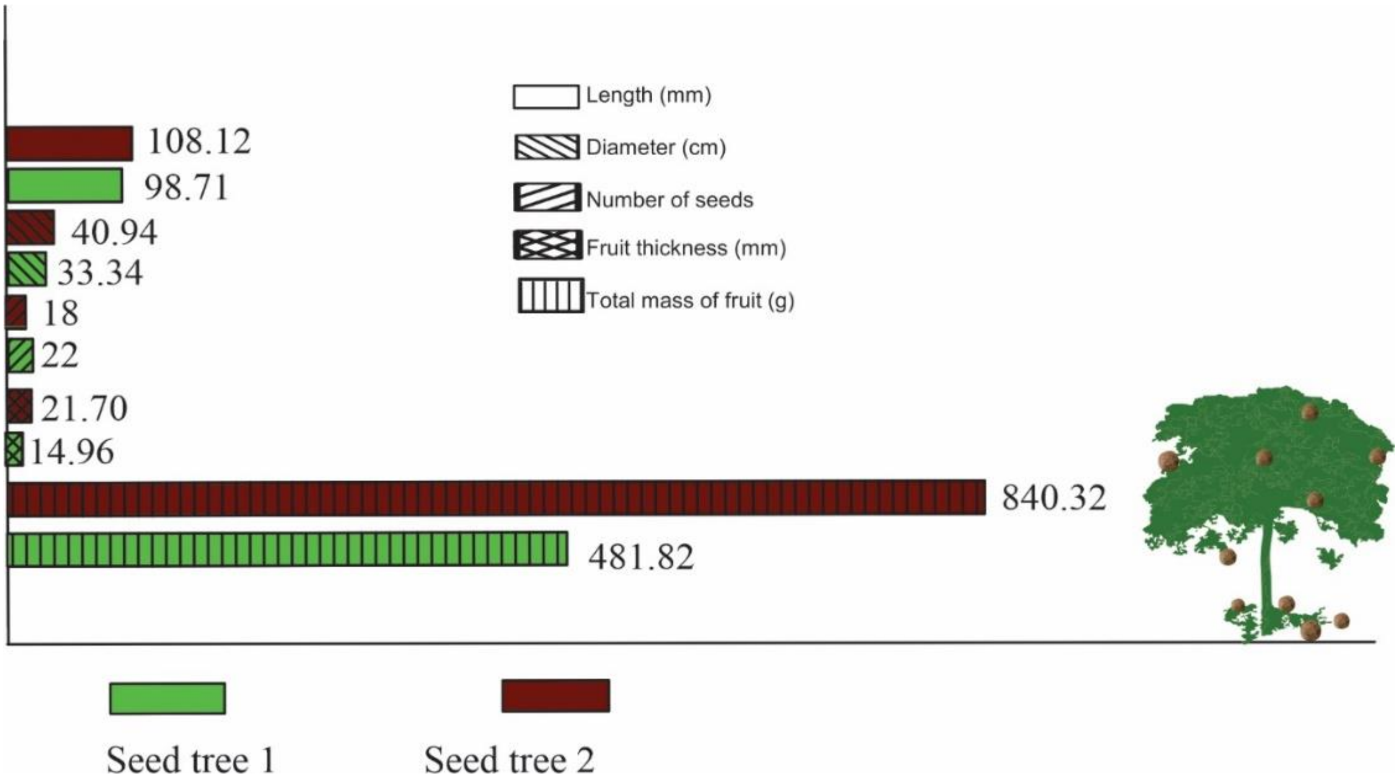


Figure 3. Quantification of mean values and physical aspects of fruits from two Brazil nut matrices.

The fruits average thickness in their median region (after being cut to remove the seeds) was 14.96 mm for matrix 1 and 21.70 mm for matrix 2. The greater the length and diameter, the heavier were the fruits (Figure 3). This shows the differences in phenotypic aspects of each mother plant, which can influence her progeny (seeds). Maternal processes can act on the environmental perceptions (environmental changes), causing variations in their morphophysiological characters, such as fruits and seeds with different dimensions [27, 28].

Physical characteristics of the seeds, as a function of their three-dimensional dimensions (biometry) and some physical properties, is summarized in Tables 1 (matrix 1) and 2 (matrix 2). Results showed relatively low variability for length, width and thickness (CV<13%), with a proportionality to their three-dimensional shape. They present an irregular angular triangular shape, in which the base (where the embryo is located) is more robust and the opposite side is more tapered. However, the sides of the seeds with tegument are thicker than the others, emulating the triangular aspect. When removing the tegument, the kernel becomes more cylindrical.

The fresh seed mass showed a relatively moderate coefficient of variation (CV), not exceeding 20% (Matrix 1: 16.63%; Matrix 2: 19.19%). The fresh mass of the seeds is proportional to their size and because they are recalcitrant, they lose mass quickly. The water content, which influences their total mass, suffers humidity oscillations of loss, or gain to the environment, favoring the process of seed deterioration.

Table 1. Biometric and physical properties and characterization of fresh mass of Brazil nut seeds from matrix 1. Seed volume index (SVI), Geometric mean diameter (GMD), Equivalent mean diameter (EMD), Arithmetic mean diameter (AMD), Surface area (SA), Seed volume (V). Sample number (n = 99).

Properties	**Range**	**Mean ± SD**	**CV (%)**	**± 95% CI**	**Valor *p***
L (mm)	28.30 – 44.90	35.81±2.73	7.61	35.27 – 36.34	nd
W (mm)	18.00 – 28.10	22.64±2.05	9.08	22.23 – 23.04	nd
T (mm)	10.10 – 19.60	16.06±1.85	11.49	15.69 – 16.42	nd
M (g)	1.90 – 7.90	5.69±0.95	16.63	5.50 – 5.87	*
SVI	6860.09 – 22030.40	13108.26±2665.55	20.33	12583.20 – 13633.30	nd
GMD (mm)	19.00 – 28.03	23.47±1.60	6.80	23.51 – 23.78	nd
EMD (mm)	6.22 – 7.88	7.02±0.31	4.42	6.95 – 7.01	nd
AMD (mm)	20.70 – 29.50	24.70±1.65	6.63	24.51 – 25.16	nd
SA (mm^2)	1134.24 – 2468.87	1738.66±235.64	13.55	1692.24 – 1785.08	nd
V (mm^3)	3591.94 – 11535.09	6863.47±1395.67	20.33	6588.54 – 7138.39	nd
Ø (%)	55.71 – 74.27	65.66±3.41	5.20	64.66 – 66.33	nd
Ar	50.27 –78.03	63.32±4.89	7.73	62.35 – 64.28	nd

Where: L= length; W = width; T = thickness; M = mass; Ø = sphericity; Ar = aspect ratio; CV = coefficient of variation; CI = confidence interval; SD= standard deviation; nd = normal distribution. *Does not follow a normal distribution at the level of p < 0.05; **does not follow a normal distribution at the level of $p < 0.01$.

Table 2. Biometric and physical properties and characterization of fresh mass of Brazil nut seeds from matrix 2. Seed volume index (SVI), Geometric mean diameter (GMD), Equivalent mean diameter (EMD), Arithmetic mean diameter (AMD), Surface area (SA), Seed volume (V). Sample number (n = 99).

Properties	**Range**	**Mean ± SD**	**CV (%)**	**± 95% CI**	**Valor *p***
L (mm)	17.90 – 55.50	45.74 ± 5.16	11.29	44.71 – 46.75	nd
W (mm)	18.60 – 36.30	25.87 ± 2.60	10.04	25.36 – 26.38	nd
T (mm)	12.30 – 22.70	18.17 ± 2.19	12.08	17.73 – 16.42	nd
M (g)	4.30 – 14.20	9.32 ± 1.75	19.19	8.96 – 9.66	*
SVI	6895.08 – 33287.36	21718.13 ± 5213.12	24.00	20691.20 – 22745.00	nd
GMD (mm)	19.03 – 32.17	27.71 ± 2.39	8.29	27.71 – 28.16	nd
EMD (mm)	5.61 – 32.17	7.94 ± 0.47	5.88	7.84 – 8.03	nd
AMD (mm)	19.10 – 34.83	29.93 ± 2.52	8.43	29.92 – 30.42	nd
SA (mm^2)	1138.09 – 3250.89	2429.51 ± 394.75	16.25	2351.75 – 2507.27	nd
V (mm^3)	3610.26 – 17429.22	11371.59 ± 2729.22	24.00	10833.90 – 11371.26	nd
Ø (%)	51.78 – 106.33	61.12 ± 6.25	10.23	59.88 – 62.34	**
Ar	44.93 – 119.55	57.22 ± 8.69	15.19	55.51 – 58.93	**

Where: L= length; W = width; T = thickness; M = mass; Ø = sphericity; Ar = aspect ratio; CV = coefficient of variation; CI = confidence interval; SD= standard deviation; nd = normal distribution. *Does not follow a normal distribution at the level of p < 0.05; **does not follow a normal distribution at the level of $p < 0.01$.

Seed shape is vital to the analysis and prediction of their tolerance behavior to reduced water content after harvest. The physical properties of seeds described through mathematical equations based on their three-dimensional linear measurements (Tables 1 and 2) elucidate their geometric variation and morphological aspects that allow processors to adjust and regulate post-harvest machines and are also essential for electrostatic separation and automated classification by size.

In matrix 1, the mean seed volume index (SVI) and mean seed volume (V mm^3) presented the highest coefficient of variation (CV), with 20.33 and 24%, respectively. All other variables presented CVs lower than 20%. The variables with the lowest CVs were arithmetic mean diameter (AMD mm), seed sphericity (Ø %), equivalent mean diameter (EMD mm), and geometric mean diameter (GMD mm) with CVs of 4.42, 5.20, 6.63 and 6.80%, respectively.

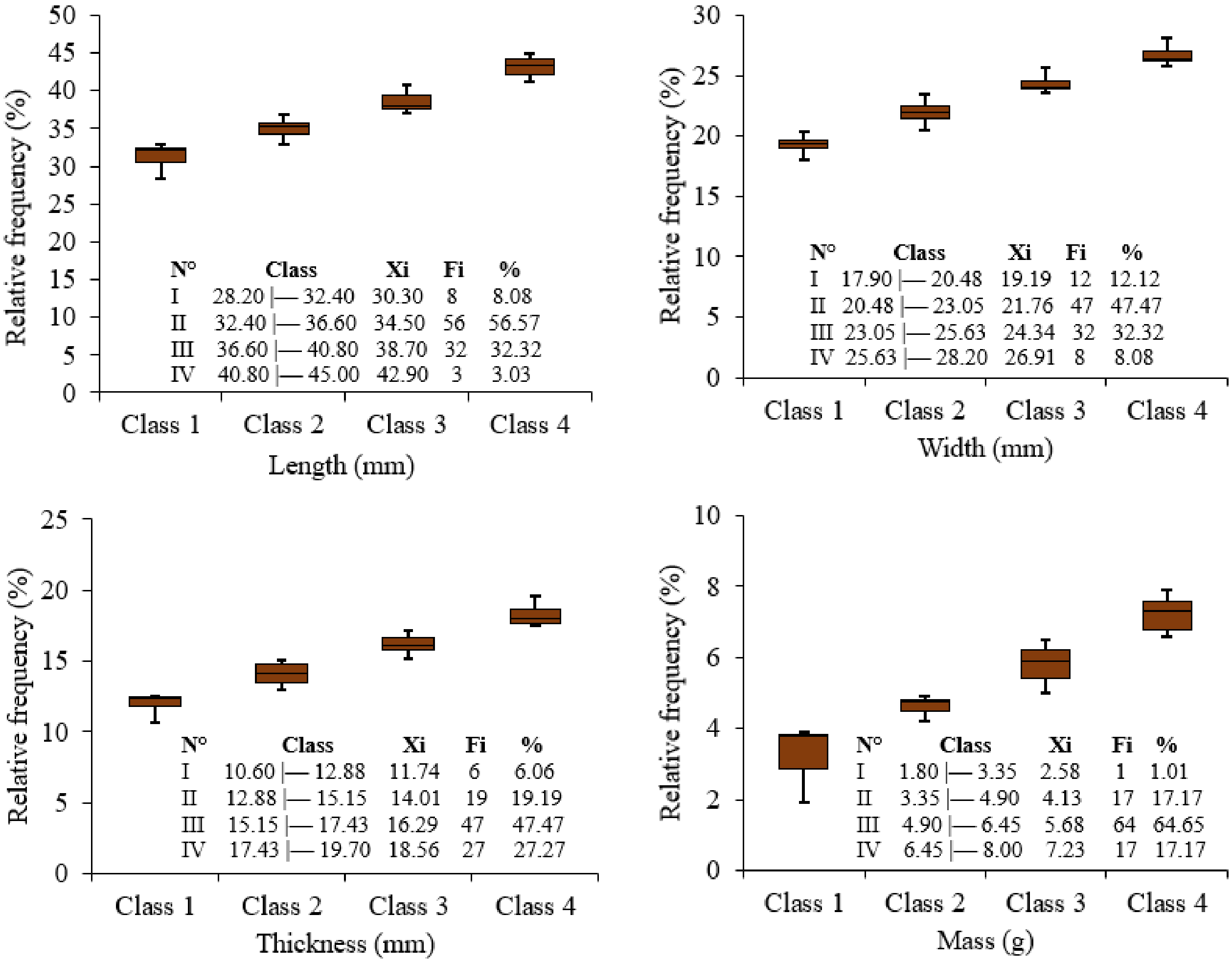
In matrix 2, the greater the three-dimensional seed measurements, the greater the geometric mean diameter (GMD mm) (CV = 8.29%), equivalent mean diameter (EMD mm) (CV= 5.88%) and arithmetic mean diameter (AMD mm) (CV = 8.34%). Surface area (SA mm²) (CV = 16.25%), seed sphericity (Ø) (CV = 10.23%), and seed aspect ratio (Ar) (CV = 15.19%) (Table 2) showed higher CV values because the longer the seed length, the thinner its thickness.

The relative frequency distribution of the seeds in relation to their length, width, thickness and mass were distributed into four classes (Figure 4 and 5). For length, class II represented the highest relative frequency for matrix 1 (56.57%; 32.40 |- 36.60 mm) and class IV for matrix 2 (52.53%; 46.15 |- 55.60 mm). For width, in both matrices, higher relative frequencies were observed for class II, with 47.47% (20.48 |- 23.05 mm) and 60.61% (22.98 |- 27.45 mm).

Regarding seed thickness, the most frequent interval for matrix 1 was class III (47.47%; 5.15 |- 17.43 mm). In matrix 2, classes II and III were similar, with 35.35% of observations and variations of 14.85 |- 17.50 mm and 17.50 |- 20.15 mm, respectively. The fresh mass in matrix 1 showed highest frequency in class III (64.65%; 4.90 |- 6.45 g), while in matrix 2 class II had the highest frequency, with 45.45% and 6.73 |- 9.25 g.

The information in Figures 4 and 5 complements the distribution of seed sizes and fresh mass. The characteristics of the variance of each dimension evaluated and the seed mass show similar values for the characteristics of sizes and fresh mass, being a symmetrical distribution.

The results of the upper quartiles offer smaller distances between the data dispersions, characterizing seeds with similar sizes for each matrix or proportional to their morphology. We observed high similarity of the quartiles and the upper and lower limits, respectively, with the error bars, which are well proportioned and denote that the seeds present similar characteristics in size and shape.



N°	Class	Xi	Fi	%
I	28.20 |— 32.40	30.30	8	8.08
II	32.40 |— 36.60	34.50	56	56.57
III	36.60 |— 40.80	38.70	32	32.32
IV	40.80 |— 45.00	42.90	3	3.03

N°	Class	Xi	Fi	%
I	17.90 |— 20.48	19.19	12	12.12
II	20.48 |— 23.05	21.76	47	47.47
III	23.05 |— 25.63	24.34	32	32.32
IV	25.63 |— 28.20	26.91	8	8.08

N°	Class	Xi	Fi	%
I	10.60 |— 12.88	11.74	6	6.06
II	12.88 |— 15.15	14.01	19	19.19
III	15.15 |— 17.43	16.29	47	47.47
IV	17.43 |— 19.70	18.56	27	27.27

N°	Class	Xi	Fi	%
I	1.80 |— 3.35	2.58	1	1.01
II	3.35 |— 4.90	4.13	17	17.17
III	4.90 |— 6.45	5.68	64	64.65
IV	6.45 |— 8.00	7.23	17	17.17

Figure 4. Boxplot of frequency distribution of Brazil nut seed size variables in matrix 1: length (mm), width (mm), thickness (mm) and mass (g). Where: N° = number of classes; Xi = midpoint; Fi = absolute frequency; % = percentage. |—, this symbol shows the interval in which the first number is included in the class, but the limit number is not.

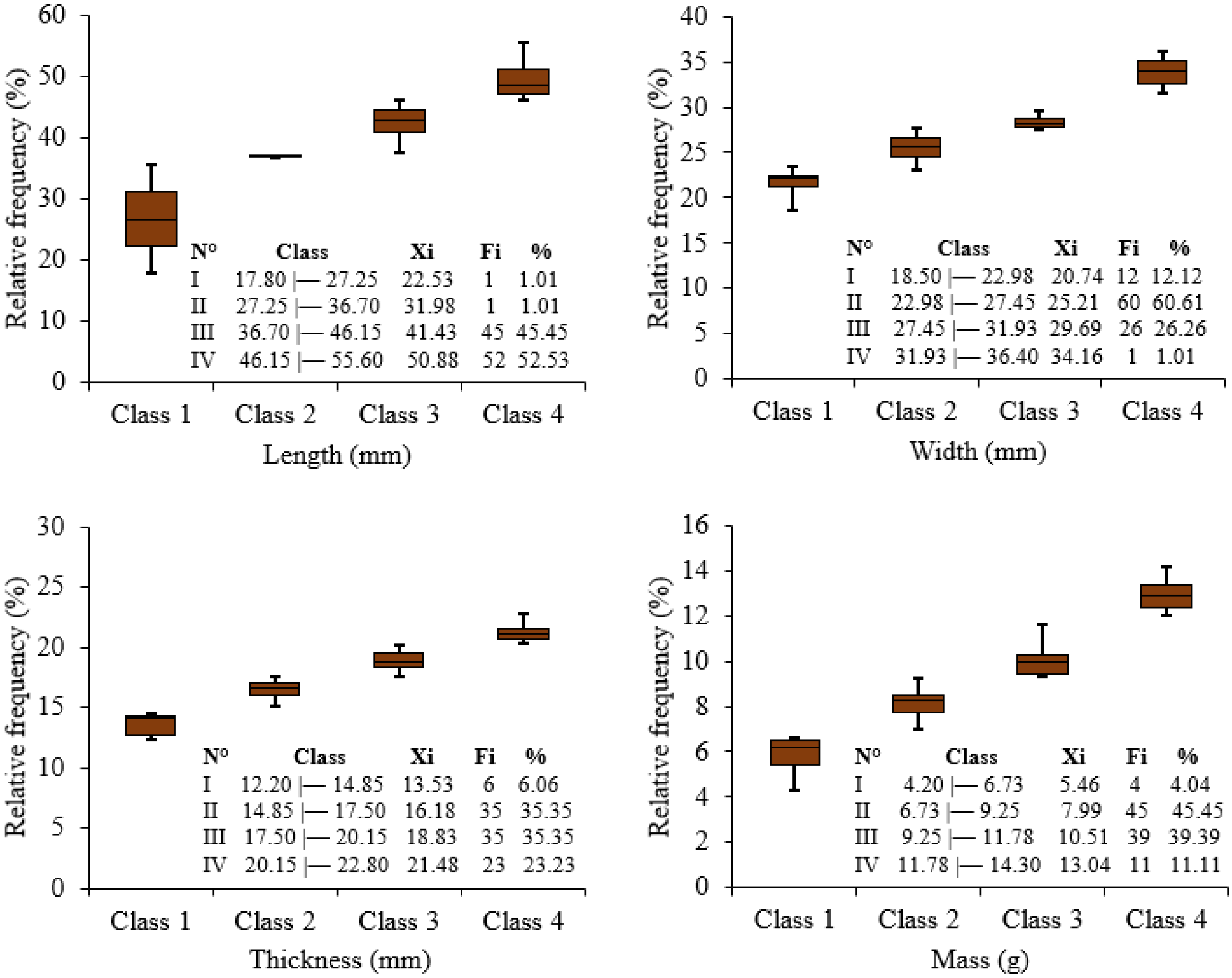


Figure 5. Boxplot of frequency distribution of Brazil nut seed size variables in matrix 2: length (mm), width (mm), thickness (mm) and mass (g). Where: N° = number of classes; Xi = midpoint; Fi = absolute frequency; % = percentage. |—, this symbol shows the interval in which the first number is included in the class, but the limit number is not.

The principal component analysis (PCA) performed showed that most of the variables analyzed presented a relevant degree of interaction in the three-dimensional geometric dimensions of the seeds for matrix differentiation and establishment of seed size classification. The PCA and correlation analysis were performed to establish the relationship between the morphophysiological classification and the physical properties of the two matrices as a function of size variation of their progenies.

The first and second component of the PCA analysis explained 17.25 and 69.37% (Matrix 1) and 20.05 and 68.45% (Matrix 2), respectively, of the variation in seed physical properties (Figure 6A and C). All variables in matrices 1 and 2, with the exception of aspect area and seed sphericity in matrix 2, showed high quality of representation.

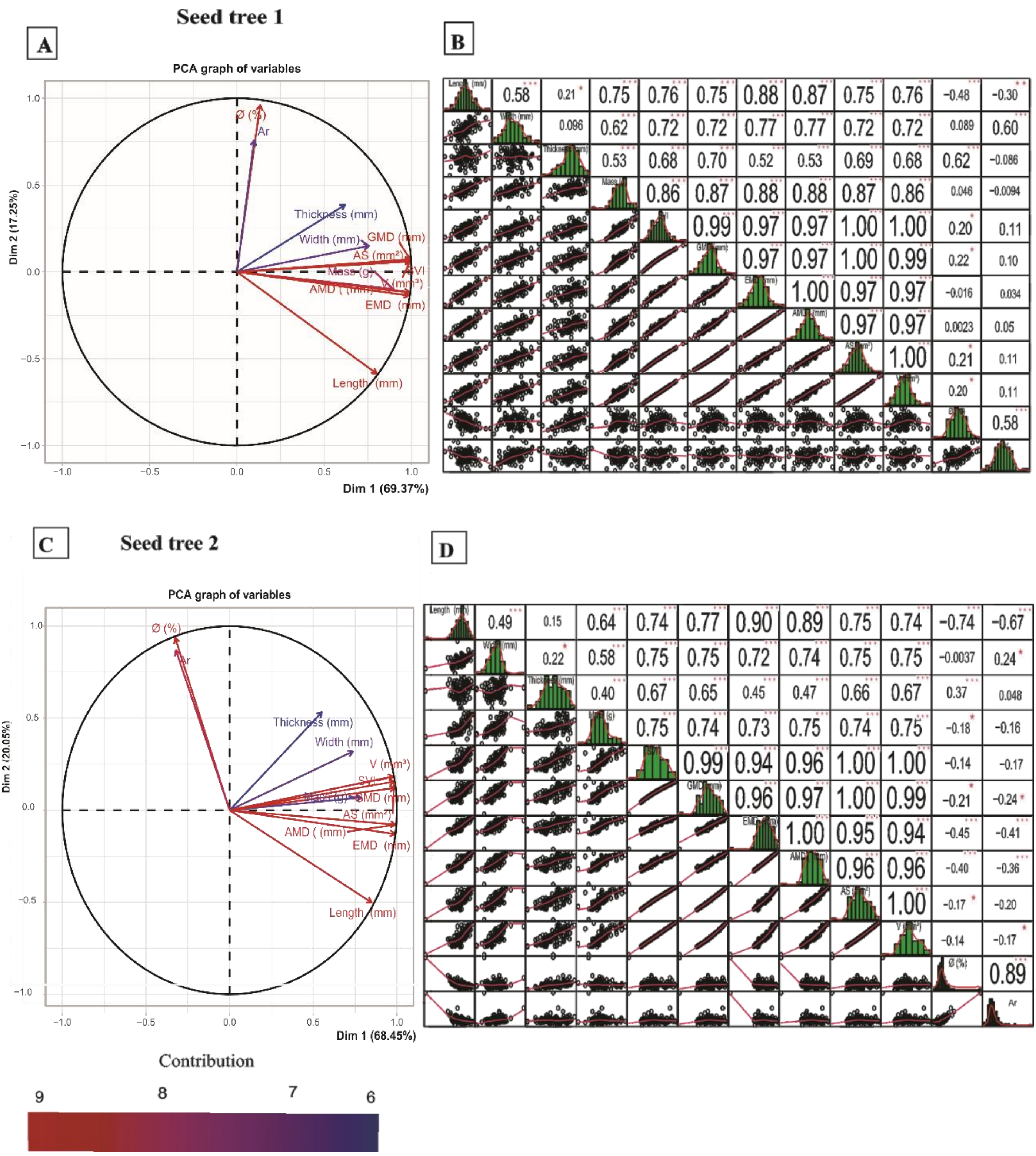


Figure 6. Distribution of elements of physical properties of Brazil nut seeds in principal component analysis (PCA) and Pearson correlation (r). The asterisk (*) represents the level of significance: $*p < 0.01$; $**p < 0.05$; $***p < 0.001$; one, two and three asterisks mean that the corresponding variable is significant at the 1%, 5% and 10% levels, respectively).

Regarding the correlation analyses (Figure 6B, D), data from seed evaluations of matrix 1 showed positive and strong correlations between characters M, SVI, GMD, EMD, AMD, SA and V, with r-values ranging between 0.86 and 1.00 at the 10% significance level. Data from seed matrix 2 showed strong positive correlations between SVI, GMD, EMD, AMD, SA, and V, with r-values ranging from 0.94 to 1.00 at the 10% significance level.

Moderate correlations in the data of seeds from the matrix 1, with r-values ranging between 0.52 and 0.77, occurred between variables L, W and T and all the others, except in the interactions with strong correlations between variables L x EMD (r = 0.88) and L x AMD (r = 0.87), all at the 10% significance level. Seed evaluation data from the matrix 2 showed moderate correlations between variables L, W, T and M and all the others, with r-values ranging between 0.58 and 0.77 at the 10% significance level.

Weak correlations in the seed evaluation data from matrix 1 (r < 0.50) were observed between the variables L x T, L x Ø and L x Ar. In the case of matrix 2, weak correlations occurred in the interactions between the variables L x W, W x T, W x Ar, T x M, T x EMD, T x AMD, and T x Ø. Overall, correlations of seed data from the two matrices showed very weak correlations (r-values ranging from 0.17 to -0.48) or nonexistent in the interactions between variables Ø and Ar and all other variables.

Pearson's correlation matrix (r) and the PCA analysis, revealed the close correlation of the variables analyzed and their interdependence, providing clues to the differences between the matrices regarding the three-dimensional shapes and the spatial dimensions (physical properties) of the Brazil nut seeds and highlighting which variables had the greatest contribution to the data variation.

DISCUSSION

In this study, the two parent plants were located at a distance of more than 6 km, suggesting no genetic drift or parental crossing between the two trees. Thus, one possible explanation for the smaller fruits produced by matrix 1 is related to the loss of vigor of producing plants as they age [29]. Another possibility is that Brazil nut trees vary significantly in their reproductive biology. In their study of Brazil nut ecophysiology, Da Costa and coauthors [30] observed the physiological plasticity of Brazil nut trees in response to abiotic stresses and that the availability of some resources (e.g., light, water, nutrients) and environmental variation can influence population structure, tree growth, and fruit production.

Brazil nut fruits represent an indehiscent pyxidium that requires a dispersal agent and can take up to a year to reach maturity and detach from the mother plant. This long production process demands a high energy expenditure by the plant, so that an individual tree’s vigor and environmental conditions can affect fruit development during the long process of maturation.

Another contributing factor is that, over time, the trees stop emitting or lose part of their existing adventitious roots that help allocate nutrients to the plant. This decrease in nutrient availability may reduce the production of larger fruits and seeds over the years. In general, the taller and larger the diameter at breast height (DBH) of the tree, the older it is, resulting in a reduction in the quantity and size of fruits and, consequently, in commercial production.

The matrices described in the present study are located within a forest fragment, and previous studies have shown that degradation, fragmentation, and isolation of trees of the same species can negatively affect fruit production [e.g., 31, 32, 33, 34]. The sanitary and parasitic conditions of the matrices can also contribute to a drop in fruit production. Brandão and coauthors [35] emphasized in their studies in the Brazilian Amazon region that maximum temperature stood out as the climatic variable with the greatest influence on the annual variation in Brazil nut production, also affecting the size and shape of the fruits and seeds.

Brazil nut seeds have an irregular format and in its three-dimensional geometry with the tegument (shell), one side is narrower, both in the horizontal or vertical direction, according to the handler position. Thus, morphological differences in relation to the protective tegument are visible. According to Borella and coauthors [36], the seed (kernel) consists of two teguments, the outer testa, or shell, a lignified and resistant layer and the inner, tegument, a thin and membranous layer. The seed tegument presents striations in the longitudinal direction, which confer greater resistance to the force exerted to open the seed [16].

The success of seed processing for selection by size depends on the knowledge its physical and morphological properties, in addition to the choice of adequate drying and processing equipment. Industrial processing, including kernel peeling, milling, heat conduction and dryer selection, depends on the geometric properties of the material that will be processed [37, 38, 39].

Analysis of the seed data indicated that most of the variables presented a normal distribution (cite table or figure). For forest species, this type of information is rare, because the variations in the shape of the seeds are heterogeneous and, somehow, the presence of nodules, folds or deformations characteristic of the seeds, cause non-significant changes, contributing to a high coefficient of variation. That is why Ferreira and Patino [40], consider it a mistake to think that if the p-value is non-significant (greater than 5%), the new treatment has no effect. The p-value indicates the probability of observing a difference as large as or larger than what was observed under the data distribution. However, if the new treatment has a smaller effect size, a study with a small sample size may not have enough power to detect it.

In a study with lemon seeds, Benestante and coauthors [41] reported no significant changes in observed seed thickness ($p > 0.05$) and attributed this to the naturally deformed shapes of those seeds. However, the measurement technique used may not have adequately quantified the changes. In the case of Brazil nuts, the shape of the seed tegument contributes to the irregularities in seed dimensions.

The fresh mass of seeds from both matrices was asymmetric with p-value>0.05. Seed mass was not proportional to seed size and/or dimension, as larger measurements of width, thickness and length were not associated with increased mass. However, during storage seeds lose dry mass due to the rapid deterioration that occurs as water content falls. Because they are recalcitrant, viability decreases with the reduction of mass through moisture loss.

The frequency of distribution of the seeds' dimensions and fresh mass allowed us to observe the percentage of variation found in the seeds through the established classes. The length variable, which showed low oscillations in its measurements, stood out in classes III and IV of both matrices. The width and thickness in both matrices showed oscillations between the classes and were not proportional to their longitudinal linear distribution.

Engineering properties are of great importance to harvesting and handling seeds and to the mechanical design and product processing equipment [42]. The linear dimensions and shape of seeds determine the effective separation of unwanted materials and influence the design and construction of sorting devices [43]. Based on the differences in size and shape, seeds from the matrix 1 would flow or roll through chutes and hoppers more easily than seeds from matrix 2. Brazil nut seeds are not spherical, and their large surface area suggests that they would have more difficulty rolling on a surface with a shallow or even flat slope angle.

Brazil nut seeds have a more rounded shape in the region opposite the hilum, which makes it difficult to remove the tegument without causing damage to the kernel. This condition makes it more difficult to break seed dormancy, since one of the most effective methods is to immerse the seeds in water for 72 hours and then remove the coat. If during the removal of the tegument the kernel is damaged, germination sacrificed, with total loss of the seed.

Knowledge about the physical properties of seeds is crucial for the proper design and operation of seed machines and equipment for drying, shelling, storage, oil extraction [44], or Brazil nut milk preparation. Few studies report on the physical properties of Brazil nut seeds or grading options to increase their homogeneity. Studies that characterize the biometric qualities of fruits and seeds of this species [36, 45] show that the variability may be associated with environmental, fertilization and even pollination conditions of the matrices.

Information on the variations and correlations between seed physical properties is essential for designing and modeling seed processing operations [46]. The Principal Component Analysis (PCA) and Pearson correlation showed that the physical properties and fresh mass of seeds from two Brazil nut matrices were correlated highlighting the importance of these parameters.

The seed fresh mass correlated moderately with seed length, width, and thickness, indicating that this may be a species-specific trait in which increases in one trait tend to be associated with another and vice-versa. For the other variables, however, the correlation was weak. The moderate correlation of mass with the other characteristics suggests no need to perform selection by dry mass.

The seed is currently the main commercialized non-timber forest product from the Brazil nut. In the present study, it was found that larger fruits, although containing a smaller amount of seeds, produce seeds of greater size and weight. This information denotes the importance of knowing the physical properties of agricultural (agroextractive) products to predict the quality of the raw material before, during, and after processing [8].

CONCLUSION

The study reveals that each Brazil nut matrix produces fruits and seeds with consistent shape and weight. Despite the within-matrix homogeneity, distinct differences were observed between batches of seeds from each matrix. This suggests that environmental factors or genetic variations might influence the physical characteristics of Brazil nut seeds.

The study highlights the need for further research to explore the underlying factors contributing to the observed differences between matrices. Understanding the environmental and genetic influences on seed characteristics could provide valuable insights for sustainable harvesting and processing practices.

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