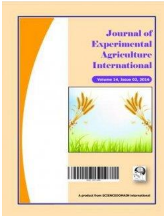

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Morphometry and Physiological Quality of *Moringa oleifera* Seeds in the Function of Their Fruit Position

Bruno Gomes Noronha[1], Márcio Dias Pereira[1*], Andressa Vasconcelos Flores[2], Andréa Celina Ferreira Demartelaere[1] and André Dantas de Medeiros[3]

[1]Universidade Federal do Rio Grande do Norte, Distrito de Jundiaí, 59280000 - Macaíba, RN, Brazil.
[2]Universidade Federal de Santa Catarina, Rodovia Ulysses Gaboardi, 3000, 89520-000 Curitibanos, SC, Brazil.
[3]Universidade Federal de Viçosa, Av. PH. Rolfs s/n, 36570000, Viçosa, MG, Brazil.

Authors' contributions

This work was carried out in collaboration between all authors. Author BGN designed the execution of the experimental activities and part of the bibliographical review author MDP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AVF and ACFD managed and analyzed of the study and managed the literature searches, author ADM performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Seed selection as a function of their position in the fruit and its biometric characteristics has been widely used in multiplying several plant species, since these factors may be related to their physiological quality. Thus, the objective of this work was to evaluate the effect of the position that the seeds occupy in the fruits on the morphometry and the physiological quality of moringa seeds. The fruits were analyzed for their biometric characteristics and divided into three regions, called apical, medial and basal, proceeding to separate the seeds considering their position in the fruits. Three seeds were collected from each fruit region with 200 seeds each, and digital morphometry was evaluated using ImageJ® and physiological quality. Four replicates of 50 seeds obtained in

**Corresponding author: E-mail: marcioagron@yahoo.com.br;*

each region of the fruit were used in a completely randomized design. Data were submitted to ANOVA and the means comparison of the variables was performed by the Tukey test at 5% probability. In the morphometry analysis, a statistical difference for the variables of weight, area, diameter, length, perimeter and circularity was observed. For the physiological quality, it was observed that the germination and vigor tests separated the seeds at two levels, except for the epicotyl/radicle length, which presented higher accuracy. Moringa fruit and seeds presented morphometric variation, where the seeds of the basal region were larger and heavier, while the seeds of the medial and basal regions presented greater germination and vigor than those of the apical part.

Keywords: Storage; vigor; germination; biometry.

1. INTRODUCTION

Moringa (*Moringa oleifera* Lam.) presents great potential of use due to its multiplicity of uses in forestry, food, medicine and industry, mainly for the large amount of lipids in the composition of its seeds, which gives them the ability to produce biofuels [1]. As a result, the demand for seeds and seedlings of this species has considerably increased, especially in regions with drier and hotter climates, since it adapts well from tropical to semiarid conditions [2].

The commercialization of seeds and seedlings quality, not only of moringa but of any kind, is fundamental to guarantee the productivity and good quality of the final product. Thus, selecting and using seeds that may present better performance in the nursery and post-planting seedlings production are very important in this process [3].

Several factors may affect seed quality such as the position of the seeds in the plant or fruit, which may influence the morphology, physical and physiological aspects of seeds [4]. Each fruit or seed of the same plant can be formed under different edaphoclimatic conditions [5], giving the seeds different quality according to their position in the fruit or the plant. In addition, each species presents specific germinative behavior in relation to the location of the seed in the fruit, independent of the botanical or adaptive similarities [6].

The classification of seeds by size and weight has also been used to multiply several plant species as a strategy for selecting quality seeds, since this factor may be related to physiological quality for some species [7].

For Cruz and Carvalho [8], forest species present great variability in relation to seed size and mass. The seeds with larger dimensions may indicate the presence of a satisfactory metabolic supply during their development, having a well-formed embryo and a greater quantity of reserve substances, consequently being more vigorous and guaranteeing a greater possibility of success in seedling formation [9].

Moringa seeds with a higher weight have a higher percentage and germination speed and generate more vigorous seedlings compared to lighter seeds [10]. However, the influence of seed weight on its physiological quality may vary depending on the genetic variability of the species studied [11]. In studying the effect of seed morphology on its physiological quality, Biruel et al. [12] observed that the rounded seeds had higher thickness and better physiological quality when they evaluated *Caesalpinia leiostachya* Benth. Seeds as a function of size and shape. These studies demonstrate the close relationship between fruit and seed morphometry, and the physiological quality of seed lots; however, this relationship is still poorly elucidated.

Therefore, the objective of this work was to evaluate the effect of the position that the seeds occupy in the fruits on the morphometry and the physiological quality of moringa seeds.

2. MATERIALS AND METHODS

Fruits from 20 moringa trees which were at least 10 m apart and on average 4 m high were collected at random from the crowns of these trees in the same population in January, 2017. The matrices are located in an experimental plantation at the Agricultural School of Jundiaí, near the municipality of Macaíba-RN (5°53'59.7" S 35°21'25.0" W). The region is characterized by a tropical climate classified as Aw according to Köppen and Geiger [13], with an average annual temperature of 25.8°C and 1.134 mm of rainfall.

After collection, fruits with a healthy appearance and a medium color of 7.5 YR 5/6 according to the Munsell color chart [14] were immediately taken to the Laboratory of Analysis and Research in Seeds of the Academic Unit Specialized in Agricultural Sciences of Federal University of Rio Grande do Norte, where the experiment was conducted.

Two hundred (200) closed fruits were measured using with a digital caliper with accuracy of 0.01 mm and a graduated ruler to analyze their length and width data. In addition, the fruits were divided into three regions: apical, medial and basal. Three seeds were counted from tip towards the middle, first defining the apical region of the fruit; then insertion of the peduncle towards the middle, three seeds were counted and the basal region was defined; the remainder was determined as the medial region (Fig. 1). The number of seeds per fruit was also determined in each of the three regions.

After determining the three regions in the fruits, the seeds from each region were separated. Three seeds were removed from the apical and basal regions, and three seeds were used from the medial region, thus totaling 200 seeds from each region, adapting the methodology used by Oliveira et al. [15].

The digital image analysis technique was utilized through the ImageJ® program for morphometric analysis. In capturing the seed images, the wings of the seeds were initially removed, and later seeds were arranged on sheets of A4 type paper with the hilum facing down in rows of 10 seeds, which were then photographed soon after using Samsung N700 photographic equipment at a distance of 30 cm from the seeds. A tripod was used so that all images were obtained with the same distance, and a graduated ruler in centimeters was used as a scale and the same condition was maintained for all selected seeds in the three regions.

The methodology used by Silva et al. [16] was implemented for analyzing the moringa seed images. The image analysis in ImageJ® software was performed by the following steps: Image opening and conversion to the 8-bit (256-bit) grayscale type (Fig. 2A). Scale calibration was then performed, and the reference value for pixels in each image considered in this work was 2400 x 3020 pixels (10.703 pixels/mm). Then, the area of interest for analysis was selected, and in this case, the analysis of each seed was performed using a threshold mask by contrast difference (Fig. 2B). After this procedure, the parameters of area, diameter, width, length, perimeter and circularity were selected (Fig. 2C) and the results were exported to a table (Fig. 2D).

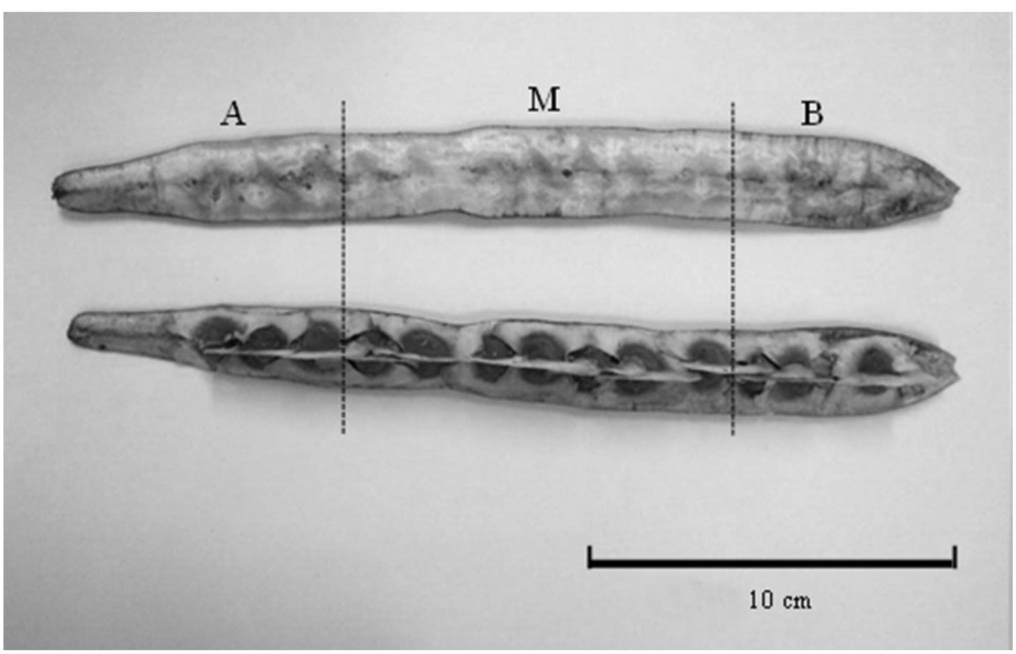

Fig. 1. Moringa capsule evidencing the regions: apical (A), medial (M) and basal (B)

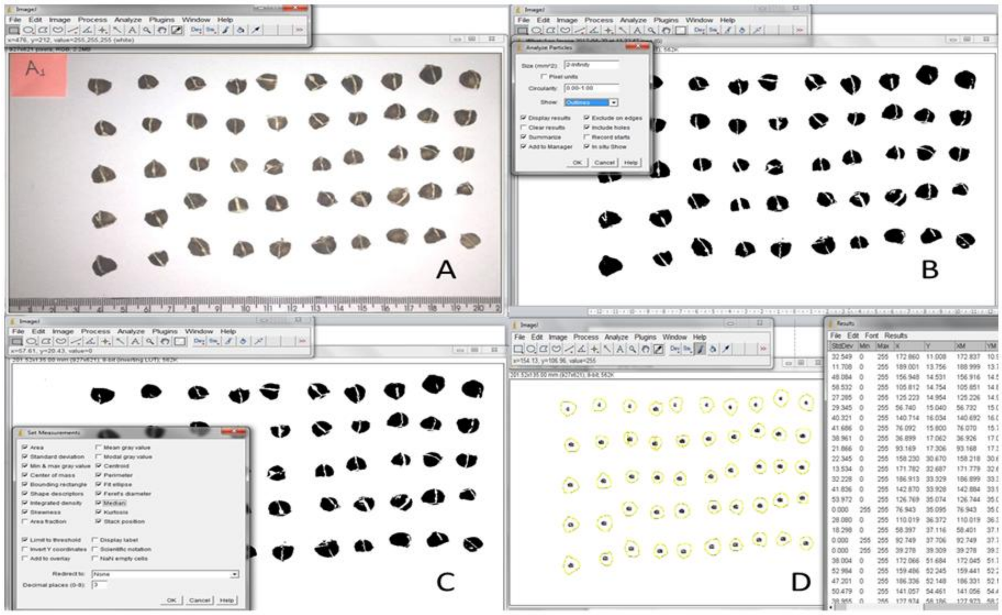

Fig. 2. Digital image analysis of moringa seeds using the ImageJ® program, with image opening and scale definition (A), application of the contrast mask (B), selection of evaluated parameters (C) and obtaining data after processing (D)

After analyzing the images, seeds were also weighed individually using an analytical balance of 0.001 mg. Afterwards, the germination test was carried out using the paper roll method, in which seeds were placed on germitest® paper, and then moistened with distilled water in an amount equivalent to 2.5 times total weight of paper. The rolls were placed in plastic bags so that water did not evaporate and then kept in a BOD (Biochemical Demand Oxygen) chamber at 30ºC, the first normal seedling count being carried out at five days and final count at ten days for the purpose of germination evaluation (%) according to the methodology defined by Bezerra et al. [10]. Normal seedlings were considered as having total length (epicotyl + radicle) of at least four times the size of the seed and without anomalies, as described by Brasil [17] for tree species of hypogeous-cryptocoledonar germination, in this case seedlings having a length equal to or greater than 6 cm.

In addition to the germination test, abnormal seedlings were also considered and computed, which were the seedlings that presented changes in their morphology or were damaged. The percentage of dead seeds was also considered, which were those that did not germinate or were in the process of visible deterioration.

The vigor of seeds was analyzed by the following tests: first counting - conducted in conjunction with the germination test, as previously described, following the recommendations of Bezerra et al. [10], germination speed index (GSI) - also carried out together with the germination test, evaluating the number of seeds germinated daily and calculating the germination rate according to the formula described by Maguire [18]; seedling length - the seedlings were individually measured with the aid of a graduated ruler, according to the methodology defined by Nakagawa et al. [19] and seedling dry mass - determined in a greenhouse, as recommended by Nakagawa et al. [19].

Four replicates of 50 seeds were used for each fruit region (apical, medial and basal) in a completely randomized design. The data were submitted to analysis of variance, and the means were compared for each region using the R program [20] and the Tukey test at 5% probability.

3. RESULTS AND DISCUSSION

The moringa fruits presented simple, dry, loculicidal capsules, dehiscent, presenting three leaflets, with the most distal part of the base of the fruits of sharp shape opening longitudinally (Fig. 3). This type of fruit was also described by

Ramos et al. [21] for the species. In the fruit are the seeds attached to parietal placentas along their length, so that the wings are fitted to each other (Fig. 3B).

The fruits presented an average of 31 cm in length, with a minimum value of 21 cm and a maximum of 42 cm (Table 1). Variations in fruit size may be promoted by environmental factors during flowering and development, but may also represent an indication of high population genetic variability. In addition, continuous flowering facilitates producing fruits of various ages at the same time, so it is possible to observe fruits of various sizes depending on the competition for assimilated at the time it was formed.

There is variation in relation to average diameter of the fruit parts, whereas for the apical region this value is 4.52 cm, while the values observed in the medial and basal regions were 7.55 and 7.03 cm, respectively (Table 1). This occurs because the fruits have a tapered shape in the apical region (Fig. 3A), reducing the diameter of this portion of the capsules. In general, fruit did not show large variations in diameter, the largest of which was observed in the apical region of fruit, in which the difference between the smallest and largest diameter was 3 cm. Continuous flowering, as occurs with moringa, enables producing fruits of various ages at the same time, so it is possible to observe fruits at different stages of ripening and development, or even with different development at the same maturation stage, provoking small variations in the parts of fruits that are in different stages or formation stages [15].

The fruits have an average of 13.80 seeds in their interior (Table 1). When comparing these values with those obtained in works of other authors such as Ramos et al. [21] and Bezerra et al. [10], it is possible to verify that the length of the fruit obtained in this study (31 cm) differs little from what was described by most of these authors (28 cm), which explains the fact that the quantity of seeds per fruit found in the present study corroborate the results described by those studies, ranging from 8 to 20 seeds per fruit. Therefore, it is observed that there is a great variation in the quantity of seeds per fruit of this species, which is directly related to the great variation of fruit sizes, as observed in Fig. 3.

In relation to seeds, they presented three equidistant wings and a triangular shape (Fig. 4A), characteristics that are related to anemocoria [22] (Fig. 4B). The seeds have a brown color and globose shape, slightly angulated on the opposite side of the hilum; which is small, linear and not slightly protruding (Fig. 4C).

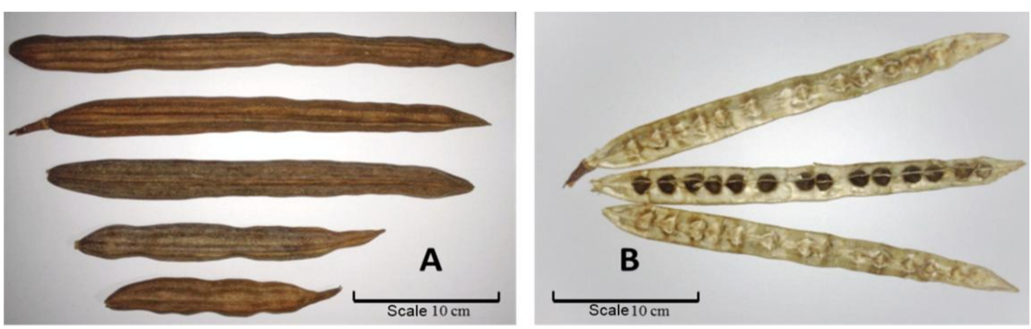

Fig. 3. Moringa capsules evidencing its external (A) and internal section, with the leaflets and seed arrangement longitudinally (B)

Table 1. Descriptive statistics for the length (L), diameter (D) for parts (apical, medial and basal) and quantity (Q) in *M. oleifera* fruits

	L (cm)	D (cm)			Q
		Apical	Medial	Basal	
Average	31.28	4.52	7.55	7.03	13.8
Minimum	17	3	7	5	5
Maximum	42	6	8	9	20
Standard deviation	6.90	1.10	0.49	1,29	3.85
C.V.%	22.06	24.36	6.59	18.32	27.71

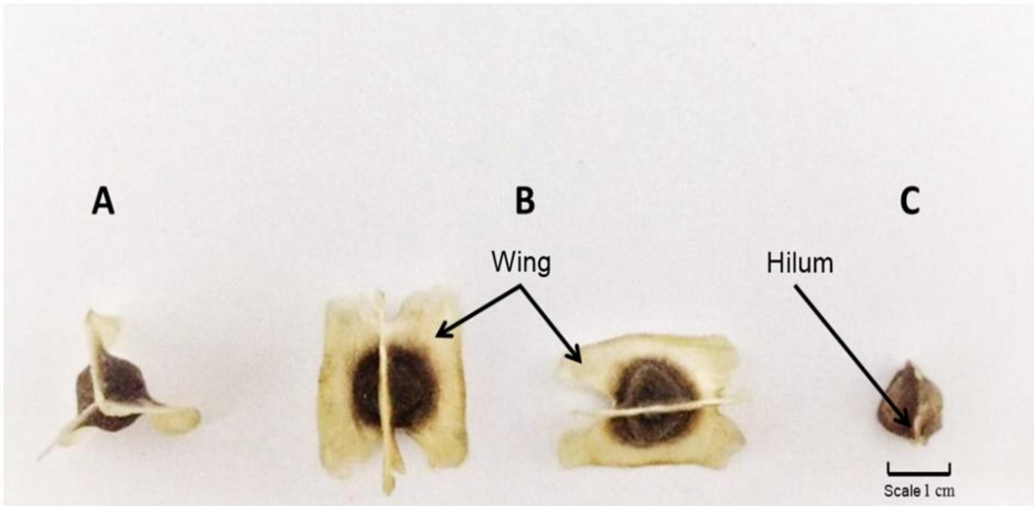

Fig. 4. Moringa seeds in frontal section (A), lateral, showing the seminal wings (B) and frontally after manual removal of the wings highlighting the wire with arrow (C)

The seeds located in the apical part of the fruit were smaller and lighter (Table 2), which probably occurs due to the lower number of cells and low capacity to compete for photoassimilates. These decrease the seed size of the fruit apex may also be explained by presence of developing seeds inhibiting the subsequent set of seeds subsequent to these, affecting development. This inhibition, besides being caused by the competition for available assimilates, may also occur as a function of the regulators of the fruit in development, or by a combination of competition and dominance between the fruits and seeds [23].

The seeds of apical region are smaller than those formed in the medial region, which in turn are smaller than those of the basal region. A statistical difference in the results of the morphometric analysis was observed when the weight, area, diameter, length, perimeter and roundness of the seeds were evaluated (Table 2). In this way it is possible to identify a difference in seed formation in relation to source-drain and its position in the fruit.

The sucrose/monosaccharide ratio functions as a physiological marker of the developing tissues, so seeds closer to the source-drain are more likely to satisfactorily accumulate reserves than those more distant [24]. The same pattern was observed for the moringa seeds, in which the most distal seeds were those that presented smaller size and weight (Table 2).

Table 2. Weight (mg), area (mm²), diameter (mm), length (mm), perimeter (mm) and roundness (mm) of *M. oleifera* seed in function the fruit position

Fruit position	Weight (mg)	Area (mm²)	Diameter (mm)
Apical	186.86 c[1]	7.30 c	4.52 b
Medial	247.87 b	8.34 b	7.03 a
Basal	292.72 a	10.87 a	7.54 a
F	190.09*	85.22 *	108.15*
C.V.%	3.8	4.5	4.8
	Lenght (mm)	**Perimeter (mm)**	**Roundness**
Apical	13.105 b	35.39 b	0.743 b
Medial	13.346 b	35.96 b	0.785 ab
Basal	18.928 a	41.72 a	0.807 a
F	17.579 *	43.21 *	7.90 *
C.V.%	10.39	2.83	2.95

*[1]The averages followed by the same letter in the column do not differ statistically from each other by the Tukey test (*P < 0.05)*

Table 3. Normal seedlings (NS), first count (FC), germination speed index (GSI), abnormal seedlings (AS), dead seeds (DS), mean aerial (MA) and root (MR) length, dry matter (DM) of moringa seedlings as a function of fruit position

Fruit position	NS (%)	FC (%)	GSI	AS (%)
Apical	48.2 b[1]	30.2 b	1.95 b	6.3 a
Medial	88.5 a	75.5 a	4.35 a	1.0 b
Basal	88.2 a	76.2 a	4.60 a	0.4 b
F	15.71*	11.80*	21.80*	16.80*
C.V.%	16.57	10.45	12.15	10.62
	DS (%)	**MA (cm)**	**MR (cm)**	**DM (g)**
Apical	45.3 a	3.5 b	3.1 b	0.176 b
Medial	10.3 b	5.9 a	8.8 a	0.289 a
Basal	11.3 b	5.9 a	8.1 a	0.284 a
F	11.80*	10.93*	22.16*	86.38*
C.V.%	14.56	8.05	20.1	5.47

*[1]ssThe averages followed by the same letter in the column do not differ statistically from each other by the Tukey test (*P < 0.05)*

For germination (Table 3), it was observed that the apical region seeds obtained 48.2% of normal seedlings, whereas those of the medial and basal regions presented 88% of normal seedlings, and no significant differences were identified between the latter two. This pattern follows the other tests such as first count and germination speed index (Table 3).

The lower germination percentage in the distal position may have occurred due to the disadvantage of seeds located in this position in relation to the competition for nutritional resources. According to Bewley et al. [25], the seed located closest to the peduncle of the fruit (source of nutritional resource) is nutritionally favored in comparison to other seeds, with this competition being more intense in elongated fruits such as pods of legumes and elongated capsules, in which the linear relationship arrangement of seeds may decrease the flow of nutritional resources from one seed to another. Thus, according to Mendonça et al. [26], competition for resources may favor the nearest embryo.

The number of dead seeds (Table 3) observed in the apical region is higher than in the other two regions, reaching close to half (45.3%), whereas the percentage for the medial and basal regions is between 10.3 and 11.3%, respectively.

Several factors may be associated with seed death, from genetic characteristics to the mobilization of reserves [5]. In general, carbohydrates, lipids and proteins are the substances stored in larger proportions and have variable contents in the seeds of several forest species [27]. In a study conducted by Borek et al. [28] with *Lupinus luteus* seeds, it was observed that the lipids accumulate during the process of physiological maturation and are hydrolyzed during the germination phase to release fatty acids, which in turn are broken and release energy to the new plant.

These authors also state that the presence of sugars or starch during the germination stage and in the seedling development stage affect lipid mobilization. Seed-killing issues may be related to malformation and deficiency in the mobilization of reserves [5], since in this experiment, the apical region seeds (smaller and lighter) were the ones with the worst performance and presented higher number of dead seeds in relation to the seeds formed in the medial and basal regions.

In relation to the shoot/root length, it was efficient in characterizing more vigorous seeds, distributing them in two levels of quality (Table 3), coinciding with the differentiation of seed diameters in the three fruit regions (Table 2), thus indicating that the development of the moringa seedling is directly associated to the size of the seeds.

Considering that moringa is a native species to tropical regions and exposed to drought [29], the low ratio between shoot/root system may consist of a mechanism of resistance to water deficit during the seedling establishment, since a more extensive and deep root system can guarantee greater efficiency in water absorption [30]. This behavior suggests that smaller and lighter seeds also generate smaller seedlings, mainly due to the amount of assimilates and their availability in the seedling establishment, as occurred in this research and corroborating the results obtained by Ramos et al. [21] and Bezerra et al. [10].

Large and well-formed seeds present less restriction under natural conditions in seedling establishment, which gives them greater adaptive advantages [31]. This condition results from the relationship between seed size and seedling size, which affects its initial establishment, called the "reserve size effect" [32].

In species with heterogenous fruiting such as *M. oleifera*, fruits are found in different maturity stages and distributed unevenly in the branches, and therefore the position in which the fruits are arranged in the plant can be related to different stages of development and maturity of fruits and seeds. In addition, different amounts and quality of light affect the leaves under different strata of the canopy, which conditions the production and translocation of photoassimilates in a differentiated way between the crown and branches [33].

This pattern of partial nutrient allocation composed of high concentrations of proteins, lipids, and carbohydrates (among others) is distributed in pulses in order to meet the needs of the plant in different stages, as it requests resources to finalize the development of fruits and seeds [34].

In this way, the position that the fruits occupy in the crown and in the branches can be related to the differential development of fruits and the chemistry, biochemistry, physical and physiological quality of seeds, as well as the ideal maturation stage, thus resulting in obtaining more vigorous plants with high potential for use in various industrial and medicinal purposes [33].

These results lead to an important reflection from the practical point of view, as the position of the seed in the fruit interferes in its germinative performance, thus in the case of commercializing seeds of this species, it is recommended to separate these seeds by position and size, getting higher value from those in benefited positions as to filling, basal and median regions of the fruits.

4. CONCLUSION

Moringa fruits and seeds presented morphometric variation, with the seeds of the basal region being larger and heavier, while the seeds of the medial and basal regions presented greater germination and vigor than those of the apical part.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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