

Original research paper

Morphometry and physiological quality of *Moringa oleifera* seeds in the function of their fruit position**ABSTRACT**

Seed selection as function of position in the fruit and its biometric characteristics has been widely used in the multiplication of 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 the separation of 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 each region of the fruit were used in DIC. Data were submitted to ANOVA and the means comparison of the variables was performed by the Tukey test at 5% probability. In the analysis of morphometry, observed a statistical difference for the variables weight, area, diameter, length, perimeter and circularity. 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. The moringa fruits and seeds presented morphometric variation, whereas the seeds of the basal region were larger and heavier, whereas the physiology, 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

The moringa (*Moringa oleifera* Lam.) presents great potential of use, due to its multiplicity of uses forestry, food, medicine and industrial, 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 increased considerably, especially in regions with drier and hotter climate, 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 final product of good quality. Thus, selection and the use of seeds that may present better performance in nursery and post-planting seedlings production are very important in this process [3].

Several factors may affect seed quality, such as the position of 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 strategy for the selection of quality seeds, since for some species this factor may be related to their physiological quality [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 with a greater quantity of reserve substances, consequently, more vigorous, guaranteeing a greater possibility of success in the formation of molt [9].

Moringa seeds with higher weight have 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]. 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 the seeds of *Caesalpinia leiostachya* Benth. 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.

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. MATERIAL AND METHODS

Fruits of 20 moringa trees, distant at least 10 m apart, about 4 m high (on average), were collected at random from the crown of these trees of the same population, in January, 2017. The matrices are located in an experimental plantation, at Agricultural School of Jundiá, near the municipality of Macaíba-RN (5 °53'59.7 "S 35 °21'25.0" W). The region is characterized by the 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 collect, the 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 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.

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

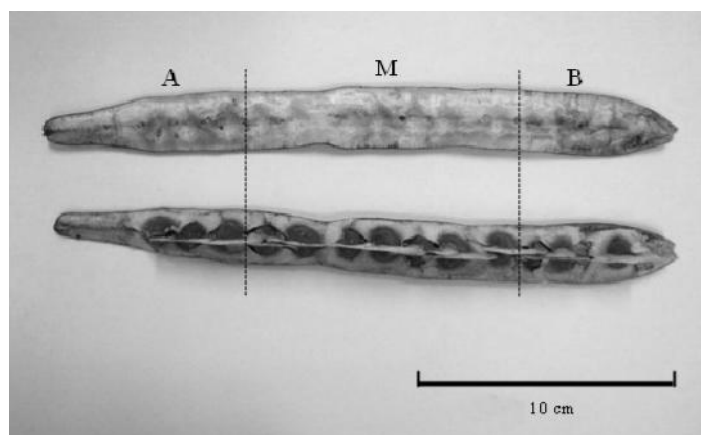


Fig. 1. Moringa capsule evidencing the regions: apical (A), medial (M) and basal (B)

After determination of three region the fruits, seeds of each region were separated. The three seeds were removed from the apical and basal regions, and in the medial region, the three seeds were used, making 200 seeds from each region, adapting the methodology used by Oliveira et al. [15].

For morphometric analysis, the technique of digital image analysis was utilized through the ImageJ® program. In capture of the images seeds, initially wings were removed, and later seeds were arranged in sheets of A4 type paper with the thread facing down in rows of 10 seeds, being 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 maintained the same condition for all selected seeds in the three regions.

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

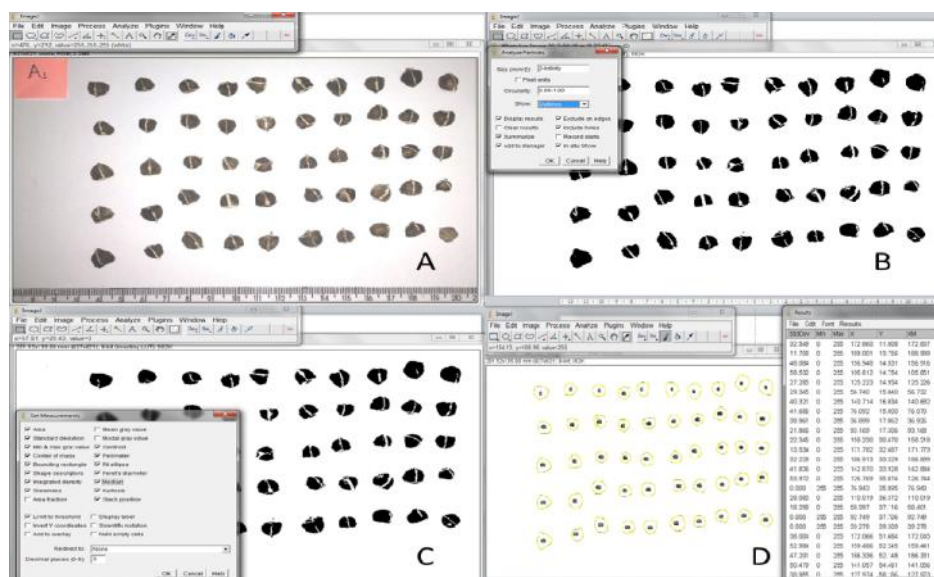


Fig. 2. Digital image analysis of moringa seeds using the ImageJ® program, with image opening and scale definition (A), application of the contrast mask (B), selection of evaluated parameters (C) and obtaining data after processing (D)

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 paper roll method, in which seeds were placed on germitest® type-paper, 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 chamber at 30 °C, 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-cryptocoleonar germination, in this case considered seedlings having a length equal to or greater than 6 cm.

In addition, the germination test, abnormal seedlings were also considered and computed, which were the seedlings that presented changes in their morphology or were damaged. It was also determined the percentage of dead seeds, 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 (IVG) - also carried out together with the germination test, evaluating the number of seeds germinated daily and calculating the rate of germination according to the formula described by Maguire [18]; length of seedlings - the seedlings were individually measured with the aid of graduated ruler, according to the methodology defined by Nakagawa et al. [19] and dry mass of seedlings - determined in 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 averages were compared for each region, using the R program [20], using Tukey test at 5% probability.

3. RESULTS AND DISCUSSION

The moringa fruits presented simple, dry, loculicidal capsule type, dehiscent, presenting three leaflets, being the most distal part of the base of the fruits of sharp shape, that open 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).



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

The fruits presented, on average, 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 the production of 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.

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

	L (cm)	D (cm)			Q
		Apical	Medial	Basal	
Average	31.28	4.52	7.55	7.03	13.8
Minimum	21	3	7	6	8
Maximum	42	6	8	8	20
Standard deviation	6.37	1.13	0.5	0.82	3.87
C.V.%	40.53	1.29	0.25	0.67	14.96

There is variation in relation to average diameter of the fruit parts, whereas for apical region this value is 4.52 cm, while in the medial and basal regions, the values observed 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, allows the production of fruits 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 stage of maturation, provoking small variations in the parts of fruits that are in different stages or stages of formation [15].

The fruits have, in their interior, on average, 13.80 seeds (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 with the results described in those researches, ranging from 8 to 20 seeds per fruit. It is observed, therefore, 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, these presented three equidistant wings and triangular shape (Fig. 4A), characteristics that are related to anemocoria [22] (Fig. 4B). The seeds are medium brown and globose, slightly angulated on the opposite side of the thread; which is small, linear and not slightly protruding (Fig. 4C).

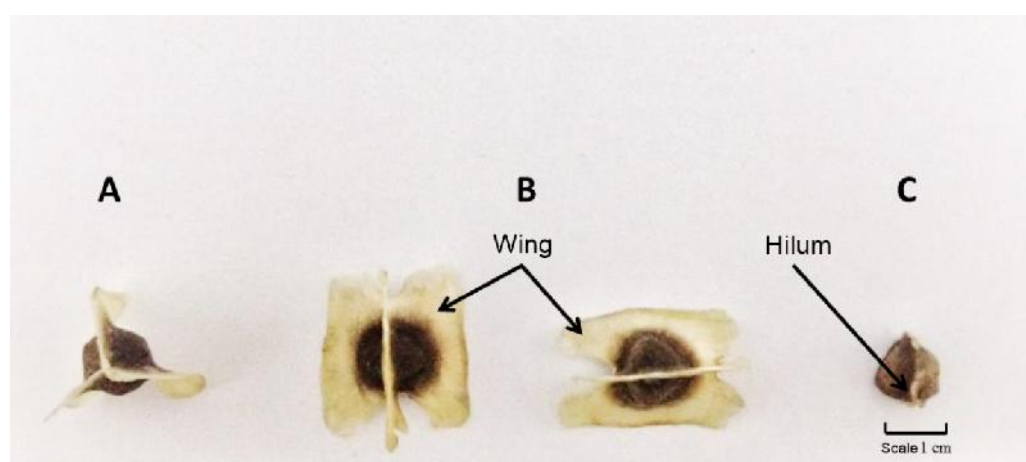


Fig. 4. Moringa seeds in frontal section (A), lateral, showing the seminal wings (B) and frontally after manual removal of the wings highlighting the wire with arrow (C)

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 decreases in 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 the regulators of the fruit in development, or by a combination of competition and dominance between the fruits and seeds [23].

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

Fruit position	Weight (mg)	Area (mm ²)	Diameter (mm)
Apical	186.86 c ¹	7.30 c	4.52 c
Medial	247.87 b	8.34 b	7.03 b
Basal	292.72 a	10.87 a	7.54 a
F	266.41*	85.79 *	142.58*
DMS	10.82	6.61	0.14
C.V.%	19	2.24	1.34

	Lenght (mm)	Perimeter (mm)	Circularity
Apical	13.105 b	3.539 b	0.743 b
Medial	13.346 b	3.595 b	0.785 a
Basal	18.928 a	4.172 a	0.807 a
F	14.85 *	39.56 *	9.34 *
DMS	2.88	1.85	0.35
C.V.%	5.65	1.47	1.35

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

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. It was observed a statistical difference in the results of the morphometric analysis, when the weight, area, diameter, length, perimeter and circularity of the seeds were evaluated (Table 2). In this way it is possible to identify 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 accumulate reserves satisfactorily than those more distant [24]. For the moringa seeds, the same pattern was observed, in which the most distal seeds were those that presented smaller size and weight (Table 2).

For germination (Table 3) it was observed that the seeds of the apical region obtained 42.2% 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 for the other tests as first count and germination speed index (Table 3).

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	19.5 b	6.3 a
Medial	88.5 a	75.5 a	43.5 a	1.0 b
Basal	88.2 a	76.2 a	46.0 a	0.4 b
F	15.71*	11.80*	21.80*	16.80*
DMS	1.04	2.32	3.08	0.54
C.V.%	16.57	10.45	12.15	10.62
	DS (%)	MA (cm)	MR (cm)	DM (g)
Apical	45.3 a	3.6 b	3.1 c	0.174 c
Medial	10.3 b	6.0 a	6.9 b	0.295 b
Basal	11.3 b	6.4 a	7.8 a	0.314 a
F	11.80*	10.93*	10.72*	11.80*
DMS	1.81	0.42	0.73	0.01
C.V.%	14.56	8.05	6.16	9.38

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

The lower percentage of germination 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 [25], the seed located closest to the peduncle of the fruit (source of nutritional resource) is favored nutritionally in comparison to other seeds, this competition being more intense in elongated fruits, such as pods of legumes and elongated capsules, in which the arrangement linear relationship of seeds may decrease the flow of nutritional resources from one seed to another. Thus, competition for resources, according to Mendonça et al. [26], 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 for the medial and basal regions the percentage 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 are accumulated 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 at the stage of seedling development, affects the mobilization of lipids. Seed-killing issues may be related to malformation and deficiency in the mobilization of reserves [5], since in this experiment, the seeds of the apical region (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 aerial/root length, it was more efficient in the characterization of more vigorous seeds, distributing them in three levels of quality (Table 3), coinciding with the differentiation of the seed sizes in the three regions of the fruit (Table 2), indicating that the development of the moringa seedling is directly associated with the size of the seeds.

Considering that moringa is a species native to tropical regions and exposed to drought [29], the low ratio between aerial part/root system may consist of a mechanism of resistance to water deficit during the establishment of the seedling, 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 establishment of the seedling, as happened in this research and corroborating with 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].

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

4. CONCLUSION

The moringa fruits and seeds presented morphometric variation, whereas the seeds of the basal region were larger and heavier, whereas the physiology, the seeds of the medial and basal regions presented greater germination and vigor than those of the apical part.

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