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Biometry and Pre-germinating *Manilkara zapota* L. Seed Treatments

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Manilkara zapota L., popularly known as sapoti, has commercial relevance both for fruits, with high prices in the regional markets, and for its wood. Thus, the propagation of this species can be done by seed, however, the germination is slow and uneven. The objective of this work was to study the biometry of the seeds and to evaluate pre-germinative treatments to facilitate the propagation of sapoti. The fruits were from the municipality of Brejão-PE and length, width and thickness were determined in millimeters of each seed and submitted to different pre-germination treatments. The seeds were exposed to the following evaluations: biometry, dormancy overrun, germination tests, germination speed index, root length and aerial part of the seedlings and dry mass of the root and shoot of the seedlings. They have variability regarding their biometry and do not require the adoption of treatments for the breakdown of dormancy, which enables and accelerates the production of seedlings. The treatments aimed at overcoming dormancy of sapoti were not efficient to accelerate the germination of the seeds.

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1. INTRODUCTION

The sapotizeiro (*Manilkara zapota L.*) is a tropical fruit that belongs to the Sapotaceae family, being found naturally in several Brazilian states [1], Its fruits can reach high prices in the regional markets and, with ample possibility of commercialization [2]. The family also arouses interest in the importance of their wood [3].

Sapoti is native to southern Mexico and Central America, where it is found in abundance [4]. The fruits are differentiated by their delicious flavor sweet and slightly astringent, and can be consumed in natura or in the form of sweets, jams and jellies [5].

The sapotizeiro adapts to a wide range of latitude, making it capable of being cultivated from the South of the State of São Paulo to the Amazon Region. It has been adapted in most of Brazil, mainly in the Zona da Mata of Pernambuco, where climatic conditions are favorable to its development and production [6]. It is estimated that in the country most of the production of this fruit occurs in the Northeast, and Pernambuco stands out as one of the largest producers, followed by the states of Bahia, Ceará, Pará and Paraíba [7].

The propagation by seed is the most used for the production of seedlings [1]. Research has shown that sapoti seeds germinate slowly and late because they present dormancy [8]. Seed dormancy is an important stage of plant life, characterized by the temporary absence of germination capacity, but on the other hand it becomes a barrier to agriculture, generating uneven seedling emergence [9] and seedling growth.

Works that portray the physical characteristics of seeds help in the determination of plant patterns in genetic breeding programs, as well as provide subsidies for seed handling and conditioning, standardization of laboratory tests and improvement of seedling production [10].

The biometry of fruits and seeds provides information for the conservation and exploitation of species [11]. Often the commercial planting of fruit trees faces obstacles due to the scarcity of information that allows the cultivation technified. In this way studies on the physical characterization of seeds is significant for the establishment of seedling production techniques [12].

Despite the relevance of the sapoti, there are few studies on the biometric characteristics of the seeds, as well as the way the germination of this species occurs. Seeds of native species show lack of uniformity in the physical and germinative aspects, and must be studied in order to establish selection criteria such as: length, width, thickness and other important characteristics related to germination [13].

The objective of this work was to study the biometry of seeds of *Manilkara zapota L*. and evaluate pre-germination treatments to facilitate the propagation of this.

2. MATERIALS AND METHODS

2.1 Location

The experiment was conducted at the Laboratory of Plant Propagation, belonging to the Agricultural Sciences Center of the Federal University of Alagoas, Rio Largo, AL, Brazil.

2.2 Plant Material

Sapoti fruits were obtained from the municipality of Brejão-PE, harvested directly from the canopy of two trees and taken to the laboratory, where they remained for ten days to facilitate the removal of seeds.

Seed biometry: For the physical characterization, eight replicates of 100 seeds were used, the length (mm), width (mm) and thickness (mm) of each seed were determined using a digital caliper, the measured length from base to apex and thickness measured at the median line of seeds [14].

For the dimensions (length, width and thickness) of seeds, as well as average number of seeds per fruit, the relative frequency [15].

2.3 Determination of Water Content of Seeds

It was determined by the oven method at 105 +/- 3ºC for 24 hours [16], four replications, with 100 g of seeds each.

2.4 Overcoming of Dormancy

The seeds were submitted to the following treatments to overcome dormancy:

- (T1) Mechanical scarification with sandpaper No. 80 on the side opposite the micropyle;
- (T2) witness without scarification;
- (T3) seeds, followed by imbibition in water at 30 °C for 24 hours (in the dark);
- (T4) seeds not impregnated, followed by imbibition in water at 30ºC for 24 hours (in the dark);
- (T5) seeds, followed by soaking in Stimulate® solution at 10 mL.L-1 at 30ºC for 12 hours (in the dark);
- (T6) without impregnation, followed by soaking in Stimulate® solution at 10 $mL.L^{-1}$, at 30ºC for 12 hours (in the dark);

2.5 Germination Tests

Prior to sowing, seeds were disinfected with 2% sodium hypochlorite solution (12.5 mL sodium hypochlorite and 487.5 mL distilled water) for five minutes, washed in running water for four minutes, followed by washing with distilled water for one minute [17]. The test was conducted in germination chambers of type BOD, regulated at a temperature of 30ºC, and the seeds were put to germinate in plastic trays with dimensions of 0,40m in length, 0,40 m of width and 0,11 m of height, containing as substrate sand washed and sterilized in an oven of 105ºC for 2 hours and moistened with distilled water until reaching 60% of its capacity [16].

Evaluations were initiated on the nineteenth day after sowing, extending to the thirty-third day. The germination criterion was that of normal seedlings [16]. Germination was considered to be the germination of the seedlings that showed the appearance of the hypocotyl, being the test closed at 33 days, when there was stabilization of the emergence of the seedlings.

2.6 Index of Speed of Germination (IVG)

The IVG was analyzed together with the germination test, whose normal seedling evaluations were performed daily, at the same time, from the first germination count, 19 days after sowing, followed up to the end of the test, 33 days after sowing and the index was calculated using the formula proposed by Maguire [18].

2.7 Root Lengths and Aerial Part of the Seedlings

At the end of the germination test, the aerial part and the primary root of the seedlings of each subsample were measured with graduated ruler and the results expressed in centimeter.

2.8 Dry Mass of the Root and Aerial Part of the Seedlings

After the measurements, the normal seedlings of each replicate were separated in aerial part and root and packed in paper bags, then placed in an oven at 80ºC, for a period of 24 hours, as recommended by Nakagawa [19]. After this time, the samples were weighed in an analytical balance with an accuracy of 0.0001 g, and the result expressed in g / seedlings.

2.9 Experimental Design

A completely randomized design with six treatments was used, with four replicates of 25 seeds each. Data were submitted to ANAVA, and means were compared by Tukey's test, at 5% probability, with the aid of SISVAR [20].

3. RESULTS AND DISCUSSION

The relative frequency of seeds per fruit is shown in Fig. 1. On average, the number of seeds per fruit is 2.5 seeds, with a minimum of 1 and a maximum of 4 seeds per fruit, ranging from 1.94 to 2.56, corresponding to a higher frequency of 48%. Silva et al. [21] reported that about 97% of the fruits observed had 1 to 3 seeds / fruit of *Eugenia dysenterica* DC. Different results were found by Rebouças et al. [12] working with *Psidium friedrichsthalianum* found that the fruits presented, on average, 72.8 seeds, with a minimum and maximum value of 25 and 131 seeds, thus showing a wide diversity among the species.

The number of seeds per fruit can also be directly influenced by the conditions of the environment in which the matrix tree develops. During flowering, water availability is a relevant factor in population productivity. Thus, the main effect of drought during the flowering phase is the reduction of seed numbers, since the lower water availability promotes decreases in photosynthesis and shortens the period of filling of the seeds causing production losses [22].

For the number of seeds per fruit there was positive asymmetric behavior, with the curve displacement located to the left of the graph (right asymmetry), with a predominance of the number of seeds per fruit below the mean value, predominating between 1.94 and 2.56 mm (48%) (Fig. 1).

Fig. 2A shows the values for seed length of *M. zapota*, where about 68% are in the range of 19.52 to 21.95 mm. The highest percentage of the relative frequency of seed width (48%) is in the range of 13.61 to 14.48 mm (Fig. 2B). For thickness, the highest percentage (54%) was found in the range of 6.11 to 6.66 mm (Fig. 2C). As observed in the fruits, the seeds did not show uniformity in size, with variation in length, width and thickness.

During the physical characterization of fruits and seeds of *Manilkara salzmannii*, Reyes et al. [4] found the dimensions of 10.6 to 15.4 mm in length, 7.4 to 9.7 mm in width and 4 to 5.4 mm in thickness, and a number of 1 to 4 seeds per fruit. Almeida and Martins [23] studying the characterization of seeds of two species of passion fruit, *Passiflora edulis* Sims and *Passiflora cincinnata* Mast, found that the mean length (mm), width (mm) and thickness (mm) of the seed for *P. edulis* Sims (mm) was 6.07, width (mm) with 3.54 and thickness (mm) was 2.54, presenting distinct characteristics, which facilitates the identification of the two species.

The length of the seeds of *M. zapota* L. presented higher variance and standard deviation in relation to the width and thickness, indicating a high variation for this characteristic studied.

The vigorous seeds provide greater transfer of dry mass of their reserve tissues to the embryonic axis, in the germination phase, giving rise to seedlings with greater weight, due to the greater accumulation of mass [19]. When the means of length, width and number of seeds were analyzed, differences were obtained between them.

Seed size, along with vigor, germination, dry mass content and water content, are parameters indicative of physiological quality [24], used in this research. For soybean seeds (*Glycine max* L.), Crookston [25] found that reducing seed size as a consequence of moisture loss is the most accurate indicator for physiological quality.

In Table 1 were expressed the data referring to the percentage of (G) and germination speed index (IVG) of seeds of *M. zapota*. The lowest G and IVG were verified in the treatments where the seeds were submitted to scarification, with water immersion and Stimulate® (T3 and T5, respectively), without germination when the seeds were scarified only (T1 control with mechanical scarification with sandpaper for wood 80, opposite the micropyle). When seeds were submitted to T4 (non-scarified seeds, followed by soaking in water for 24 hours in the dark) and T6 (uncured seeds, followed by soaking in Stimulate® solution at 10 $mL.L^{-1}$ at a temperature of 30°C for 12 hours in the dark), a reduction in germination and a rate of germination rate was observed, not statistically different from each other.

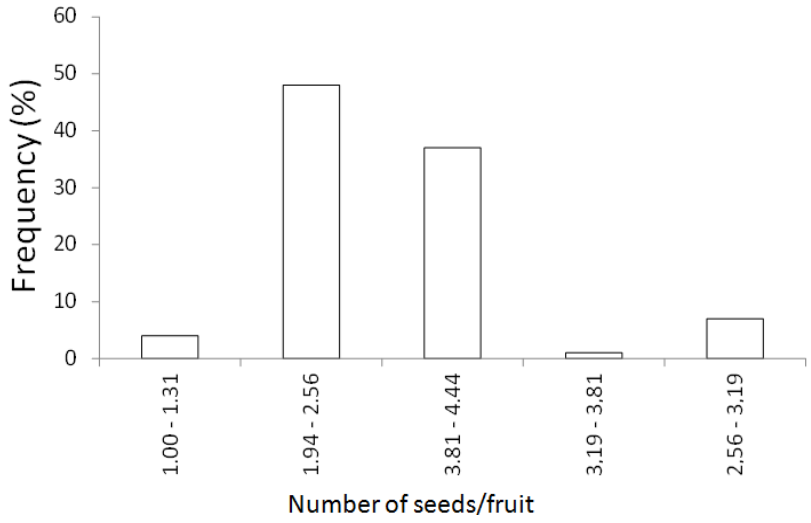

Fig. 1. Relative frequency for the number of seeds/fruit of *Manilkara zapota L*. (UFAL/CECA, 2019)

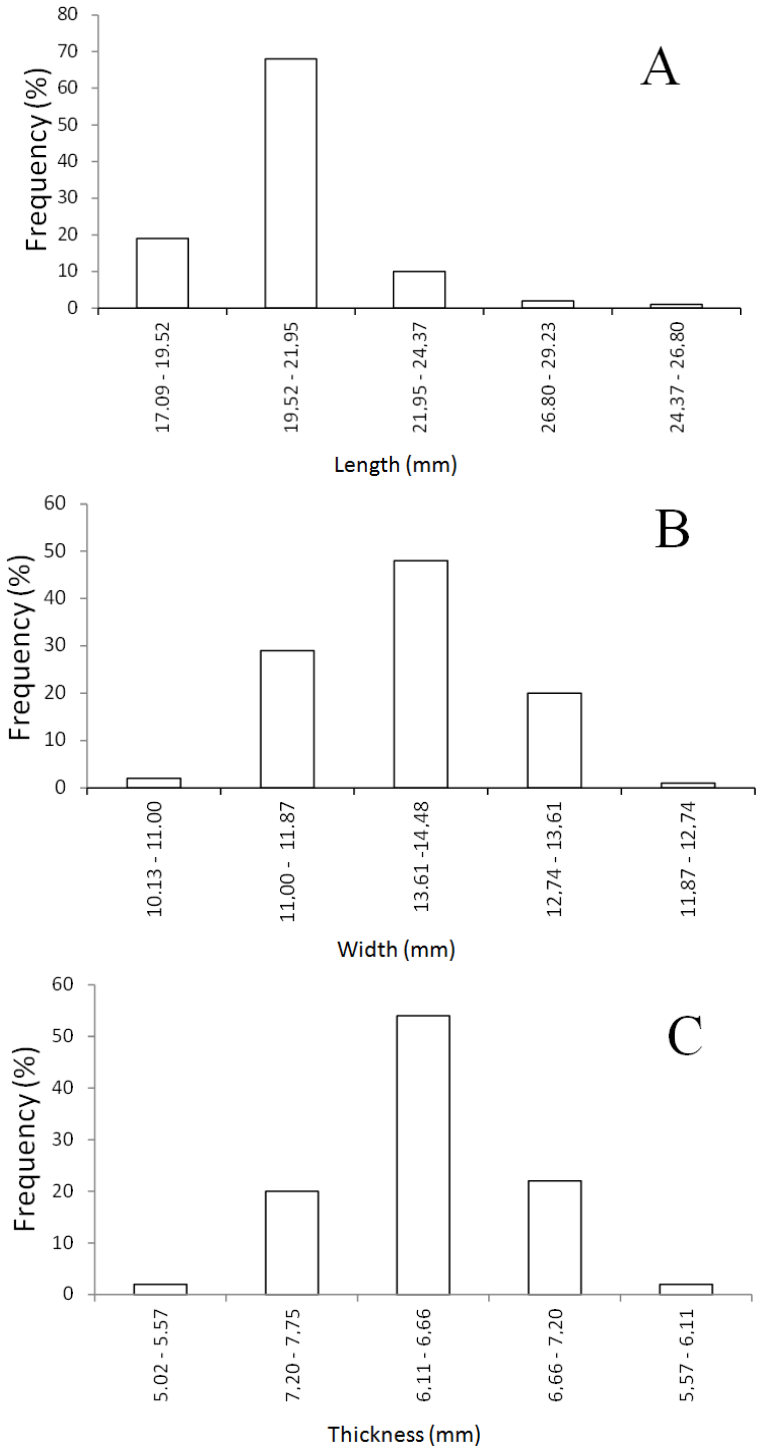

Fig. 2. Relative frequency related to the length mm (A), width mm (B) and thickness mm (C) of seeds of *Manilkara zapota* L. (UFAL/CECA, 2019)

The highest percentages of germination and rate of germination were from seeds of treatment T2 (control), with 80% of germination and IVG of 0.905, respectively. Thus, it can be inferred that the seeds of *Manilkara zapota* do not present dormancy. Similar results were found by

Marubayashi et al. [26] working with dormancy overcoming in *Mauritia flexuosa* L. seeds, and affirmed that the mechanical scarification of seeds without or with water soaking increased seed mortality.

Water is essential for germination to begin, and some care must be taken because it may be responsible for the death of the embryo if it is supplied in high quantity. The water content of the sapoti seeds was 35%, which probably caused damage to the physiological quality of the seeds when submitted to water soaking and Stimulate®. The imbibition damage will be proportional to the water potential difference between the seed and the medium, thus, the already damaged seed has less energy available for the germination process, resulting in less vigor [27].

Phytohormones (Stimulate®) promote seed germination in several plant species, and for this purpose, Richard et al. [28] used gibberellic acid to break dormancy of *Passilflora nitida* KUNTH, where they concluded that plant hormones optimized the germination process (86% of germinated seeds). Differently from that observed in the present work with T5 treatments (scarified seeds, followed by soaking in Stimulate® solution at 10 $mL.L^{-1}$, at 30ºC for 12 hours in the dark) and T6 (uncured seeds, followed of soaking in Stimulate® solution at 10 $mL.L^{-1}$, at 30ºC for 12 h in the dark), in which the phytohormones did not favor the germination and development of the seedlings.

Regarding the root length and aerial part of the seedlings (Table 2), the highest results were 4.56 and 6.77 cm, respectively, from seeds of treatment T2 (control). The lowest values of shoot length and primary root of T3 (scarification seeds, followed by soaking in water at 30ºC for 24 hours in the dark). The treatments T3 (scarification seeds, followed by soaking in water

Table 1. Germination and seed germination rate index of *Manilkara zapota* L. submitted to treatments to overcome dormancy. (UFAL/CECA, 2019)

Treatments	Germination (%)	IVG
1	0 d	0.000 d
2	80 a	0.905 a
3	22 c	0.223 c
4	61 b	0.630 b
5	17 c	0.198 c
6	60 b	0.624 b

T1 (mechanical scarification with sandpaper No. 80 on opposite side to micropyle); T2 (control); T3 (scarified seeds, followed by soaking in water at 30 ° for 24 hours in the dark); T4 (seeds not scarified, followed by imbibition in water for 24 hours in the dark); T5 (scarified seeds, followed by soaking in Stimulate® solution at 10 $mL.L^{-1}$, at 30 ºC for 12 hours in the dark); T6 (uncured seeds, followed by soaking in Stimulate® solution at 10 $mL.L^{-1}$, at 30 ºC for 12 hours in the dark). Means followed by the same lowercase letter in the column do not differ by a 5% probability by the Tukey test

Table 2. Length of the root and aerial part of the seedlings of *Manilkara zapota* L. submitted to treatments to overcome dormancy (UFAL/CECA, 2019)

Treatment	Root length (cm)	Length of aerial part (cm)
1	0.00 d	0.00 d
2	4.56 a	6.77 a
3	0.61 c	1.25 c
4	2.53 b	4.69 b
5	0.53 c	0.99 c
6	2.65 b	4.57 b

T1 (mechanical scarification with sanding paper for wood nº 80, on the side opposite to micropyle); T2 (control); T3 (scarified seeds, followed by soaking in water at 30º for 24 hours in the dark); T4 (seeds not scarified, followed by imbibition in water for 24 hours in the dark); T5 (scarified seeds, followed by soaking in Stimulate® solution at 10 $mL.L^{-1}$, at 30ºC for 12 hours in the dark); T6 (uncured seeds, followed by soaking in Stimulate® solution at 10 $mL.L^{-1}$, at 30ºC for 12 hours in the dark). Means followed by the same lowercase letter in the column do not differ by a 5% probability by the Tukey test

Table 3. Dry mass of the root and aerial part of the seedlings of *Manilkara zapota* L. submitted to treatments to overcome dormancy. (UFAL/CECA, 2019)

Treatment	Dry mass from the root (g)	Dry mass of the aerial part (g)
1	0.0000 d	0.0000 d
2	0.0194 a	0.1003 a
3	0.0025 c	0.0159 c
4	0.0111 b	0.0691 b
5	0.0021 c	0.0050 c
6	0.0102 b	0.0740 ab

T1 (mechanical scarification with sanding paper for wood nº 80, on the side opposite to micropyle); T2 (control); T3 (scarified seeds, followed by soaking in water at 30° for 24 hours in the dark); T4 (seeds not scarified, followed by imbibition in water for 24 hours in the dark); T5 (scarified seeds, followed by soaking in Stimulate® solution at 10 mL.L^{-1}, at 30ºC for 12 hours in the dark); T6 (uncured seeds, followed by soaking in Stimulate® solution at 10 mL.L-1, at 30ºC for 12 hours in the dark). Means followed by the same lowercase letter in the column do not differ by a 5% probability by the Tukey test

at 30º for 24 hours in the dark) and T5 (scarified seeds), followed by soaking in Stimulate® solution at 10 mL.L^{-1}, at a temperature of 30ºC, for 12 hours in the dark) did not differ statistically from each other, however, being statistically different from the other treatments.

In the treatments in which the seeds were submitted to scarification, the lowest root and shoot lengths of the seedlings were observed, which reinforces the evidence of damage caused to the seed. Contrary results were observed by Passos et al. [29], working with seeds of *Morinda citrilifolia* L., in which the best results, as regards root length, were represented by seeds that underwent emergence.

When evaluating the root and shoot dry mass (Table 3), it was observed that the T2 treatment (control) provided the highest results (0.0194 and 0.1003) and T1 treatment (mechanical scarification with sandpaper No. 80) the smallest contents.

The treatments T3 (scarified seeds, followed by soaking in water at 30º for 24 hours in the dark) and T5 (scarified seeds, followed by soaking in Stimulate® solution at 10 mL.L^{-1}, at a temperature of 30ºC, for 12 hours in the dark), did not differ in dry mass of root and aerial part of the seedlings. For the dry matter variable of the aerial part, the results of T6 treatments (uncured seeds, followed by soaking in Stimulate® solution at 10 mL.L^{-1}, at 30ºC for 12 hours in the dark) was statistically similar to the treatment T4 (non-scarified seeds, followed by soaking in water for 24 hours in the dark) and T2 (control).

Guedes et al. [30] observed that in the treatment with scarification in sandpaper No. 80, the highest dry mass content of seedlings from *Opuntia fícus-indica* Mill. Seeds was obtained, differing from the results found in the present study. In seeds of *Spondias lutea* L., Sarmento [31], affirmed that the scarified seeds produced plants with higher dry mass of roots and leaves. According to Ramos et al. [32], the aerial and root dry mass is important in the evaluation of the development of the plants, ensuring the establishment of the same in the field.

It is observed that the use of scarification with sandpaper No. 80 was harmful in the physiological behavior of the seeds, which can be attributed to the fact that the seeds do not present dormancy.

4. CONCLUSIONS

The seeds of *Manilkara zapota* L. show great variation in the size and number of seeds per fruit.

For the seeds under study it is not necessary to adopt pre-germination treatments, being recommended the use of seeds without treatments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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