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Physical and physiological quality of mangaba seeds obtained by different processing methods[1]

Qualidade física e fisiológica de sementes de mangaba obtidas por diferentes métodos de beneficiamento

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HIGHLIGHTS:
The seeds obtained from the fruit pulp industry have a physiological quality for seedling propagation.
There is a high potential for reducing fruit pulp industry residues by using these seeds for propagation.
Higher pixel density is observed for full seeds with an efficiency of forming normal seedlings.

ABSTRACT: The beneficiation of seeds from a native species is often a time-consuming activity and it requires manual processing. A possible alternative are seeds from mechanical processing in the fruit pulp industry. Therefore, the objective of the study was to evaluate the effect of manual and mechanical processing on the physical and physiological quality of mangaba seeds. The water concentration, the weight of 1,000 seeds, mechanical damage by radiographic images, electrical conductivity, the percentage of germination, the first germination count, and the length and dry mass of seedlings were all evaluated. The radiographic images allowed for classification as to the development of the embryos and the occurrences of damage. There were no differences in water concentration, germination, the first germination count, and the length of the seedlings. The mechanically processed seeds showed lower electrical conductivity and seedlings with a greater dry mass. The processing methods used did not affect the physiological potential of the mangaba seeds.

Key words: *Hancornia speciosa*, germination, X-ray, vigor

RESUMO: O beneficiamento de sementes de espécies nativas normalmente é um processo demorado e requer método manual. Uma possível alternativa são sementes oriundas do beneficiamento mecânico na indústria de polpas de frutas. Portanto, objetivou-se avaliar o efeito do beneficiamento manual e mecânico na qualidade física e fisiológica de sementes de mangaba. Avaliou-se concentração de água, peso de 1.000 sementes, danos mecânicos por imagens radiográficas, condutividade elétrica, germinação, primeira contagem da germinação, comprimento e massa de matéria seca de plântulas. As imagens radiográficas permitiram a classificação quanto ao desenvolvimento do embrião e a ocorrência de danos. Não houve diferenças para concentração de água, porcentagem de germinação, primeira contagem e comprimento de plântulas. Sementes beneficiadas mecanicamente apresentaram menor condutividade elétrica e suas plântulas maior massa seca. Os métodos de beneficiamento utilizados não afetam o potencial fisiológico das sementes de mangaba.

Palavras-chave: *Hancornia speciosa*, germinação, raios X, vigor

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Introduction

The mangaba tree (*Hancornia speciosa* Gomes) belongs to the family Apocynaceae and it is present in some South American countries (Peru, Bolivia, Paraguay, and Brazil). Its fruit, the mangaba, has a high concentration of vitamin C, iron, zinc, potassium, carotenoids, phenolic compounds, and antioxidants (Cardoso et al., 2014; Lima et al., 2013).

In 2019, the mangaba production in Brazil corresponded to 1749 tons. The Northeast region was responsible for 93.25% of the national production, especially the States of Paraíba (735 t), Sergipe (381 t), and Bahia (232 t) (IBGE, 2019).

The mangaba tree is a medicinal species, with an antimicrobial and antioxidant potential (Santos et al., 2016). It is also antihypertensive (Silva et al., 2016), antidiabetic (Pereira et al., 2015), antimutagenic and anticancer (Lima et al., 2015), together with a healing activity (Geller et al., 2015), and anti-inflammatory actions (Torres-Rêgo et al., 2016). This plant is part of the official list of priority species for research development in Brazil (Silva Junior et al., 2018).

The seeds of this species are recalcitrant and are most usually obtained from manual processing, which is a time-consuming process. In the case of the forest species seeds, any processing is considered minimal because only the impurities are eliminated. Full knowledge and an improvement of this operation are both crucial for obtaining pure seeds with a greater germinating potential, thus enabling the formation of seedlings (Grzybowski, 2019).

The mechanical beneficiation in the production of pulps for juices can be a fast and efficient alternative for obtaining the seeds. Currently, the seeds that are obtained from this process are discarded. However, they can be used in the production of seedlings, if they present a potential for the formation of normal and vigorous seedlings.

In this context, the present study had the objective to evaluate the effect of manual and mechanical processing on the physical and physiological quality of mangaba seeds.

Material and Methods

This research was conducted by using mature fruits (fruits with a yellowish reddish epicarp, reddish streaks, and with a soft texture) from Estância (located at 54 meters of altitude, with the following geographical coordinates: 11° 16' 7" S and 37° 26' 32" W), in the State of Sergipe, Brazil. They were provided by the Pomar Company. The fruits were submitted to manual and mechanical processing to remove the seeds. The manual processing was carried out with the aid of a polypropylene sieve, with a diameter of 16 cm, an 8 in thermoplastic mesh, and running water, lasting approximately for 2 hours. A total of 1,000 seeds were obtained. The mechanical processing was carried out by a pulping machine (Max Machine, Model: MDP 300), following the company's routine protocol. An amount of 2,470 kg of fruit was processed in 30 min and this generated a large number of seeds, which were then homogenized, and a sample of 2,000 seeds was obtained.

The experimental design was completely randomized, testing two treatments (manually processed and mechanically processed seeds), with eight repetitions. The experimental unit constituted 25 seeds, excepting the water concentration, duplicates of 10 g, and the length and the dry mass of the seedlings (15 seedlings). The water concentration of the seeds and the physical quality analyses were determined by the weight of 1,000 seeds, with an evaluation of the mechanical damage by the radiographic images. To evaluate the physiological quality, the following variables were evaluated, namely, the percentage of germination, the percentage of germination at the first germination count, the electrical conductivity, as well as the length and the dry mass of the seedlings.

The seeds were weighed by an analytical balance (Bell Model Analytical M214AI) to estimate the weight of 1,000 seeds when using seeds from the mechanical processing, as they were from the same provenance and harvested in the same period. The determination of water concentration were carried out at 105 ± 3 °C for 24 hours, according to the Rules for Seed Testing (Brazil, 2009).

For the X-ray tests, the seeds were fixed by double-sided transparent adhesive tape and they then adhered to transparent sheets. After this, they were exposed to the radiographic analyses. The radiation intensity (25 kV) and the exposure time (5 s) were determined by automatic calibration using a Faxitron® X-Ray Corp Model HP MX-20. The obtained images were categorized into classes according to the internal morphology of the seeds. Subsequently, the pixel density of the seeds was evaluated with ImageJ software and compared with the germination results (Abràmoff et al., 2004).

For the germination test, the seeds were distributed in rolls of Germitest® paper. They were moistened with 2.5 times of their dry weight with distilled water and they were kept in an incubator chamber (Marconi Model MA 403) at 25 °C, with a photoperiod of 12:12 hours (light/dark). Each seed that was used in the X-ray tests had its individual position identified in the germination test. At the 25th and 30th days after sowing, the first and the last count of the normal seedlings were both assessed (Brazil, 2009).

The normal seedlings that were obtained by the germination test were measured with a graded ruler to determine the seedling lengths. For the same seedlings, their dry masses were determined. Each replicate was packed in a paper bag, identified, and then placed in an oven with a forced air circulation at 80 °C for 24 hours (Nakagawa, 1999).

For the electrical conductivity tests, the seeds had been previously weighed to an accuracy of 1 mg, placed in plastic cups containing 75 mL of ultrapure water, and then kept at 25 °C for 24 hours in an incubator chamber. The electrical conductivity was read by a Digimed (CD-20) Conductivity Meter, with the results expressed in $\mu S\ cm^{-1}\ g^{-1}$.

The data was tested for normal distribution by the Shapiro-Wilk test and the homogeneity of the variances by the Bartlett test. The data was submitted to an analysis of variance by the F test, and the means were analyzed by the Student's t-test at $p \leq 0.05$. Subsequently, the Pearson (r) linear correlation coefficients were calculated for all combinations of the physiological and physical quality tests, and the significance of r was determined by the t-test ($p \leq 0.05$). The software used in the statistical analysis was R v. 4.0.2 (R Core Team, 2020).

Results and Discussion

The mangaba seeds were oval and flattened, with a light brown color, and with a central hilum. The weight of 1,000 seeds corresponded to 254.25 g. This data was similar to that obtained by Gonçalves et al. (2013) for the mangaba seeds from Mato Grosso State, Brazil.

The differences in the seed size and the weight of the seeds depended upon the variations in the morphological and physiological characteristics of the mother plant and their interactions with the ecological variables. These variables promoted different responses in the germination, the seedling development, and the organization of the plants' community (Souza & Fagundes, 2014).

The weight had a direct relation with the quality and the maturity of the seeds, being influenced by the degree of humidity (Lima Júnior, 2010). When reaching maximum vigor, the seeds also presented their maximum weight, which then decreased as a function of respiration and reserve expenditure, to promote seedling germination and early development (Carvalho & Nakagawa, 2012).

The water concentration was 54.55% for manual processing and 53.61% for mechanical processing. In the literature, water concentrations have been reported for mangaba seeds ranging from 48 to 56% (Santos et al., 2010; Masetto et al., 2014).

The low water concentration that maintains the viability of the recalcitrant seeds varies greatly depending on the species. For *Theobroma cacao* L., the lowest safe water concentration corresponds to 23%; while for *Avicennia marina* (Forsk.) Vierh, it is 61.5% (Mumford & Brett, 1982; Farrant et al., 1996). For mangaba, it is not known what is the lowest water concentration that keeps the seeds viable, however, in a study on the physiological quality of the seeds due to the drying periods, the mangaba seeds with a water concentration of 38% (after 48 hours drying) decreased in germination (Santos et al., 2010).

A high water concentration is an indispensable elemental condition for maintaining the viability of recalcitrant seeds, such as mangaba. However, to perform an X-ray test, the water concentration is a variable to be considered, due to its influence on the quality of the radiographic images. The highest concentration contributes to the lowest visualization of the internal structures (Silva et al., 2014). Thus, to use the tests in recalcitrant seeds, a balance is required, in which the water concentration in the seeds allows for obtaining the images, without compromising the viability of the seeds.

Despite the high moisture, it was possible to visualize the internal structure of the mangaba seeds that presented damage in their different parts for the two processing methods (Figure 1).

These damages were present in 7% of the seeds that were processed manually and 9% of those that were processed mechanically, thus compromising the germination and the vigor. When relating by means of the radiographic images,

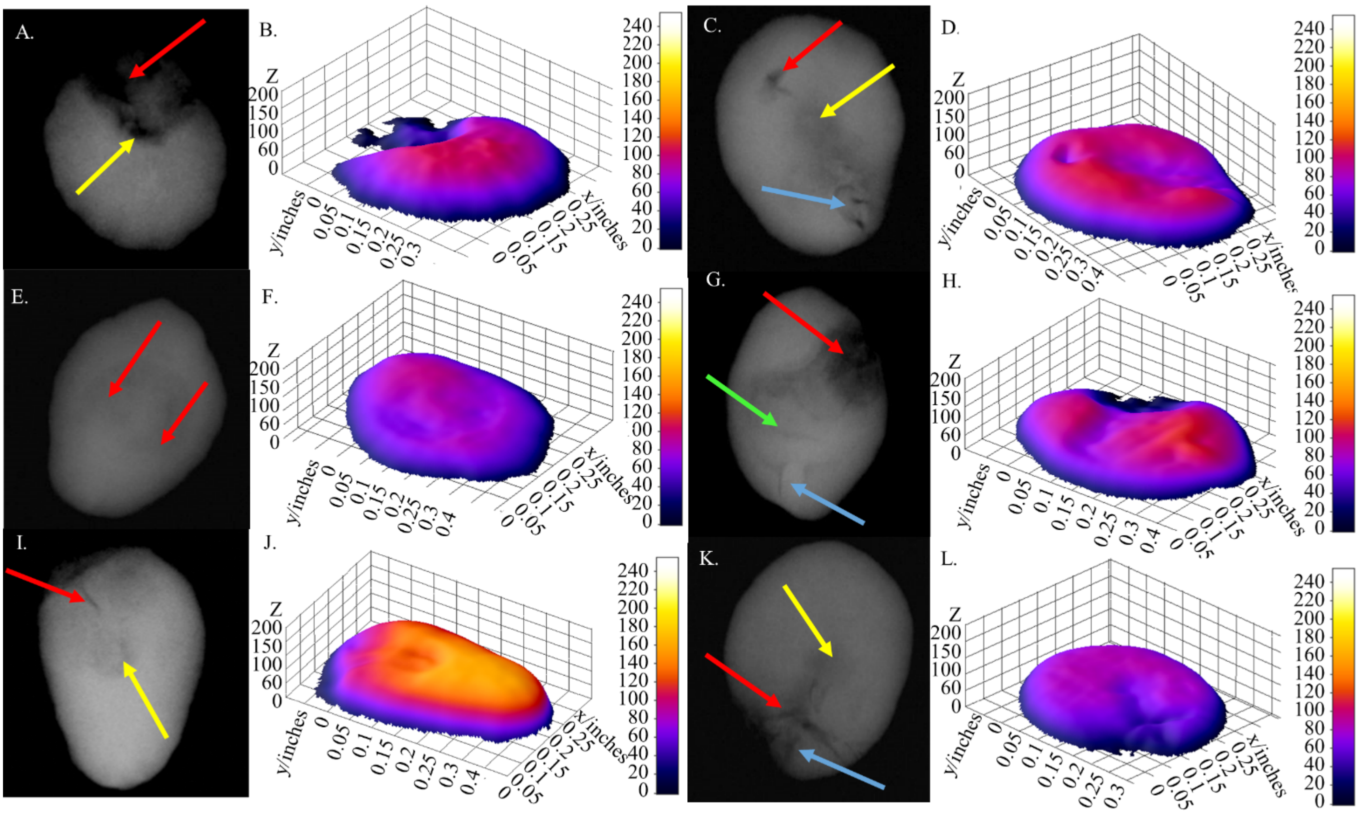

Figure 1. Radiographic images of the mangaba seeds (*Hancornia speciosa* Gomes), with damage to the seed hilum (yellow arrow), endosperm (red arrow), embryo (blue arrow), and cotyledons (green arrow) when obtained from manual processing (A, E, and I); and from mechanically (C, G, and K); as well as with their respective three-dimensional pixel density graphs (B, F, and J - manual; D, H, and L - mechanical)

with the data from the test of germination, it was noticed that for the two methods of processing, the non-germinated seeds and the deteriorated seeds were the seeds that presented damage. The filled seeds in the radiographic images presented normal seedlings.

It was verified that the observed pixel density for each seed might be associated with the formation of normal seedlings. This was affirmed when considering that for the regions of the seeds where no damages were observed in the internal structure, the density values were higher; consequently, the lower values were for the regions with damage (Figure 2).

The seed lot that was processed manually presented a higher pixel density, varying from 93 to 243 in, while for the mechanically processed seeds, the density ranged from 93 to 165 in. The highest values of densities that were verified for the seeds from the manual processing may be associated with the high water concentrations present in this lot. When considering that water influences the quality of the images obtained, this water may have filled the tissues and prevented the passage of the X-rays since they can cross more easily through the aerated parts, or through the softer tissues (lower density).

When relating the different classes of the germination test with the pixel density that was obtained by analyses of the radiographic images of the seeds, a tendency towards the formation of normal seedlings by the seeds with a higher pixel density was observed for both processing methods. However, additional studies are needed to validate this parameter (Figure 3).

The applications of the X-ray tests in those studies with other recalcitrant seeds of the *Eugenia dysenterica* (Mart.) DC.

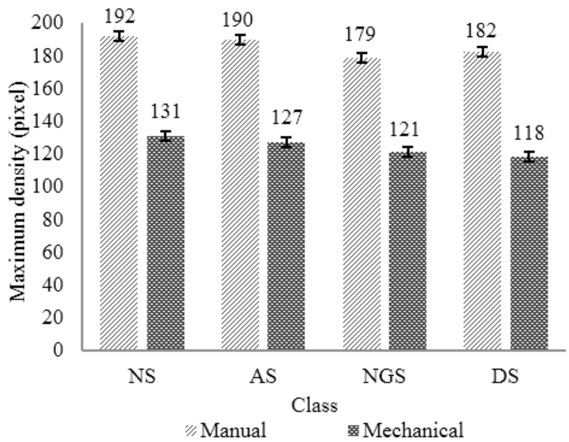

Figure 3. Mean pixel density for the normal seedlings (NS), the abnormal seedlings (AS), the non-germinated seeds (NGS), and the deteriorated seeds (DS) of mangaba (*Hancornia speciosa* Gomes) that were obtained from the manual and mechanical processing

species also allowed for definitions of the direct relationships of the free spaces inside the seeds, their water concentrations, as well as for the emergence of the seedlings during the germination, and the physical detections of their conditions in the radiographic images (Silva et al., 2017).

The observed pixel density for each seed favored and inferred the formation of normal seedlings. The efficiency of the X-ray test in a study of the physical structures and the internal morphology of the seeds of a forest species was reported for identifying the seeds of *Terminalia argentea* Mart. LC that had embryonic abnormalities (Gomes et al., 2014). The various changes that were observed in their radiographic

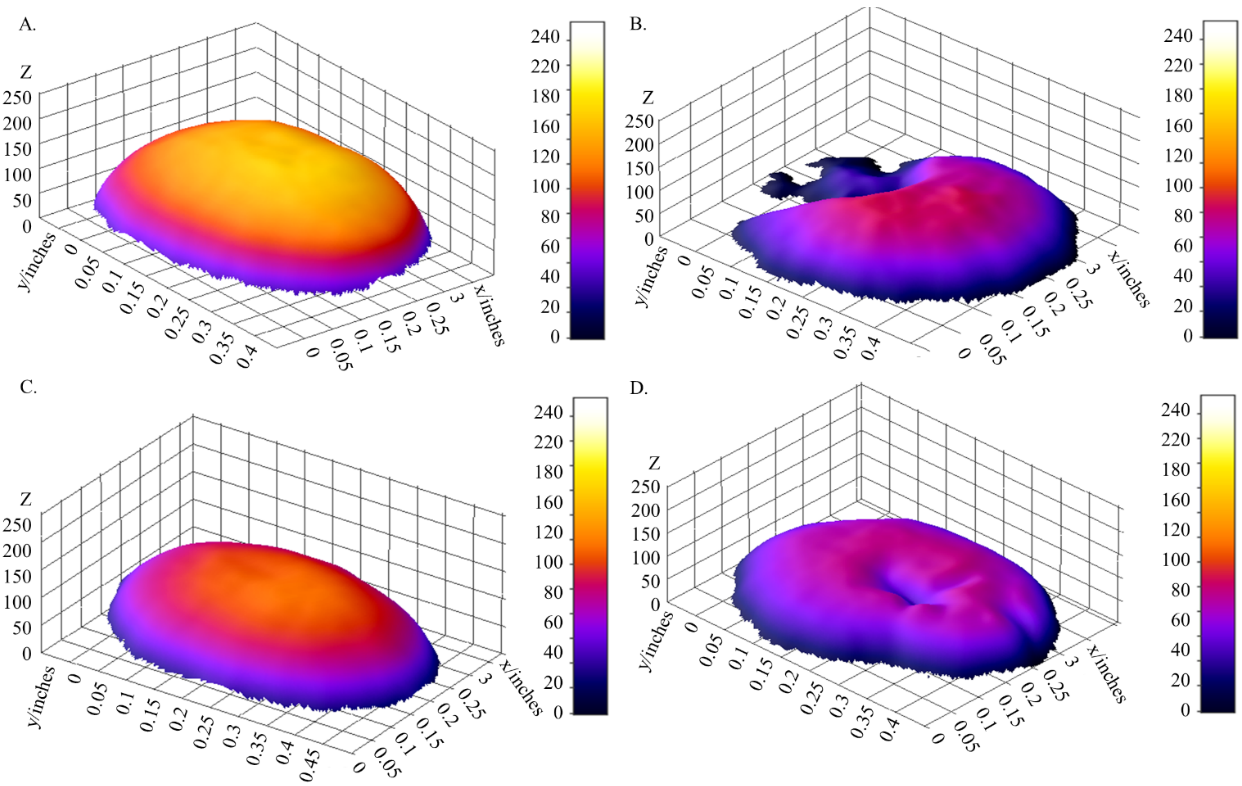

Figure 2. The three-dimensional pixel density graphs plot for the seeds that were obtained from manual processing (full and well-formed seeds – A; and with endosperm and cotyledons damage - B); together with the mechanically processed seeds (filled and well-formed seeds– C; with damage to the seed hilum and the embryo - D)

images allowed for inferences about the physiological quality of the seeds of *Tabebuia heptaphylla* (Vell.) Toledo (Amaral et al., 2011).

When correlating the variables of the radiographic images and the evaluation of germination, the first germination count, the length of the seedlings, and the dry mass presented no significant correlations (Figure 4).

There were significant correlations ($p \leq 0.05$) for the germination, the first germination count, the seedlings length, the dry mass (r = 0.7), the electrical conductivity, and the average pixel density (r = 0.69). There were also significant negative correlations for the variables of dry mass, electrical conductivity (-0.58), no damage and damaged seeds (r = -1), germination, first count, and average pixel density (-0.51).

It is noteworthy that the percentage of full and well-formed seeds that were identified in the X-ray tests, and the formation of normal seedlings in the germination test, were similar, so the X-ray test is promising for verifying the qualities of different lots of mangaba seeds. Regardless of the processing methods, the germination percentages were above 80% (Table 1).

In order to obtain high percentages of germination from the recalcitrant seeds, a short period between the processing and the sowing must be considered. This would be associated with the necessity for the complete removal of the pulp, which contains phenolic compounds that inhibit germination (Soares et al., 2006). In this sense, mechanical processing is an option to be considered since it promotes a level of germination that is not different from the manual processing method but with the advantage of being faster.

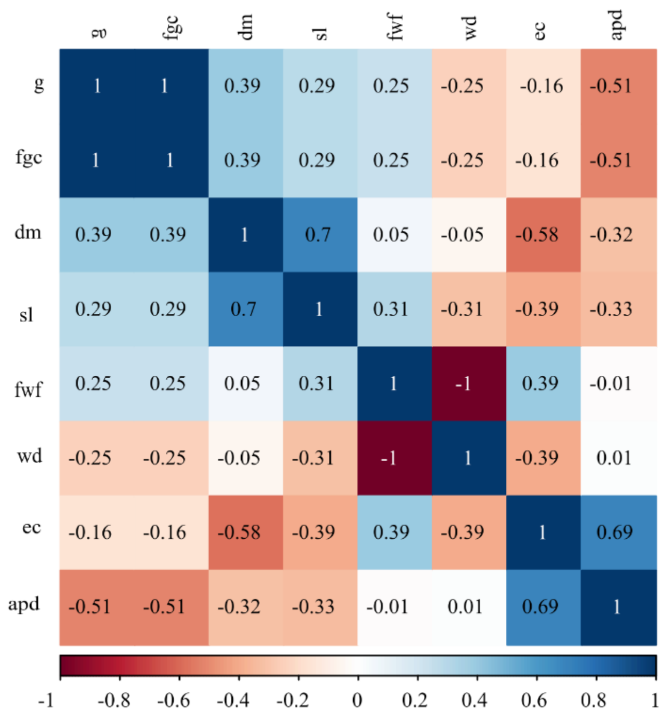

Figure 4. Pearson correlation between the variables of the analyses in the radiographs (full and well-formed - fwf, with damage – wd, and average pixel density - apd) and the evaluation of the germination (g), first germination count (fgc), seedlings length (sl), dry mass (dm), and electrical conductivity (ec) for the seeds of mangaba (*Hancornia speciosa* Gomes)

Table 1. Mean values obtained from the manual and the mechanical processing for the germination (G), first germination count (FGC), seedlings length (SL), dry mass (DM), and electrical conductivity (EC) of the mangaba seeds

Method	G (%)	FGC (%)	SL (cm)	DM (g per seedling)	EC ($\mu S\ cm^{-1}\ g^{-1}$)
Manual	81	81	19	0.62	12.4
Mechanical	86	86	20.1	0.67	7.5
p-value	0.3234^{ns}	0.3234^{ns}	0.1227^{ns}	0.01672*	0.000005*

*, ns - Significant at $p \leq 0.05\%$ (column) and not significant by the Student's t-test, respectively

There were no differences between the mangaba seed processing methods at the first germination count. For both methods, the germination percentage was above 80% for the 25th day of germination. In the literature, variations have been reported for mangaba seeds when they were evaluated on the 35th day, for which 67% of normal seedlings were identified (Santos et al., 2010). These differences may be associated, among other factors, with vigor. The seeds that were obtained from the use of both methods promoted a higher percentage of normal seedlings at the first count.

Rapid germination is an advantage for those seedlings that are to be established under field conditions (Parsons, 2012). Most species with recalcitrant seeds have thin and water permeable teguments with embryos that are completely developed at the moment of dispersion (Franchi et al., 2011).

Some recalcitrant species germinate soon after dispersion by forming a pool of seedlings in the understory of a forest. This rapid transition from maturation to germination is the main strategy for these kinds of seeds (Obroucheva et al., 2016). In addition, for this current study, the data that was obtained for the germinations, and the germination at the first count (25th day), was similar.

This may have indicated that there be a requirement for reducing the period that is currently established for the first germination count, together with the total time for the evaluations. When considering these perspectives, the species is a native and many of the tests may not adequately represent the seeds from different provenances, or from differing genotypes. Thus, additional studies should be carried out with different lots, in order to identify the best period for the evaluations of germination.

The vigor that was indirectly determined by the length of the seedlings did not differ for the two processing methods that were used. This finding has implied that the detections of high vigor for the seeds in this study when considering the values that were observed for seedling lengths, were higher than those that have been reported in the literature for mangaba seeds that have been previously studied, such as 13.46 cm (4.96 cm aerial part, and 8.5 cm root) (Santos et al., 2010) and 16.21 cm (Dresch et al., 2016).

The dry mass of the seedlings was different between the methods of processing, with higher masses being verified in those seedlings that were obtained from the fruits that were processed mechanically. The values that were analyzed here were higher than those that have been reported in other literature reports (Barros et al., 2006; Pinto et al., 2014).

It is important to emphasize that biotic factors, together with the quality and the concentration of the seed reserves, as well as the functional morphology of the cotyledons, exerted an influence on the establishment, development, and survival of the seedlings. The high mass of the seedlings permits one to infer that there was a vigorous and a high potential for field survival of these seeds (Melo et al., 2004).

The seeds presented differences in electrical conductivity of 12.4 μS cm^{-1} g^{-1} for the manual processing and 7.5 μS cm^{-1} g^{-1} for the mechanical processing. These values were lower when compared with data from the literature for mangaba seeds that ranged from 33.4 to 50.3 μS cm^{-1} g^{-1} (Barros et al., 2010).

The high electrical conductivity was verified for the seeds that benefited from the manual method and this may be related to the duration of the beneficiation process (2 hours), in which the seeds were exposed to running water and friction in the sieve mesh. Electrical conductivity was associated with the electrolytes that were leached by the seeds, which were released in high or low quantities, according to the level of damage of the internal structures. Another factor that can influence this work are the water concentrations. However, for the two lots that were studied, this influence did not occur, as the variations in the water concentrations were within a favorable limit, < 2%. The manual processing of the seeds presented more changes in the membrane systems, possibly resulting in lighter seedlings.

Therefore, it is possible to suggest that the manual processing method promoted changes in the seed membrane systems. However, these changes were probably small, and they did not compromise the viabilities and the vigor of the seeds. The use of manual or mechanical processing equipment for removing the pulp, allows for the production of seeds, with the potential to produce normal and vigorous seedlings. This information is relevant since it contributes to the use of these seeds from the fruit pulp industries, which are normally considered as being ready for disposal.

Conclusions

1. The manual processing and the mechanical processing of fruits in a pulp mill did not affect the physiological potential of the studied mangaba seeds.

2. The X-ray test is promising for verifying the qualities of different lots of mangaba seeds.

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