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Physiological and molecular changes in seeds of *Hancornia speciosa* Gomes stored in conservative solutions

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ABSTRACT: For native species in Brazil, there is a great need for scientific information that allows efficient production and storage of seeds. The present work evaluated the physiological and molecular changes in *Hancornia speciosa* seeds stored in conservative solutions. The experiment was carried out in a completely randomized design, in a split-plot scheme, with eight replications. In the plots, the conservative solutions (A, B, C, and D) were allocated, and the subplots the storage periods (50, 100, 150, and 200 days). The following variables were analyzed: water content, X-ray, germination, shoot length, root, dry mass of seedlings, electrical conductivity, concentration and quality of ribonucleic acid, the concentration of peroxidase, and heat-resistant proteins. The water content remained above 50%, and 88% of the seeds are full and well-formed. There is a reduction in the germination, shoot length, root, and dry mass of seedlings; and an increase of electrical conductivity with the increase of the storage time. Heat-resistant proteins were not sufficient to protect seeds against macromolecular damage, and RNA and peroxidase concentrations decreased with the increase of the storage time. The seeds stored in solutions B and C are more vigorous, being such solutions indicated for the conservation of *H. speciosa*.

Index terms: *ex situ* conservation, heat-resistant proteins, *mangabeira*, recalcitrant, RNA.

RESUMO: Para espécies nativas do Brasil existe uma grande necessidade de informações científicas que permitam a produção e armazenamento eficiente de sementes. O presente trabalho avaliou as alterações fisiológicas e moleculares em sementes de *Hancornia speciosa* armazenadas em soluções conservativas. O experimento foi conduzido em delineamento inteiramente casualizado, em esquema de parcelas subdivididas, com oito repetições. Nas parcelas, soluções conservativas (A, B, C e D) e nas subparcelas, períodos de armazenamento (50, 100, 150 e 200 dias). Foram analisadas as variáveis: teor de água, raios-X, germinação, comprimento da parte aérea, raiz e massa seca de plântulas, condutividade elétrica, concentração e qualidade do ácido ribonucleico, concentração de peroxidase e proteínas resistentes ao calor. O teor de água manteve-se acima de 50% e 88% das sementes estão cheias e bem formadas. Com o aumento dos períodos de armazenamento, ocorre redução na germinação, comprimento da parte aérea, raiz e massa seca de plântulas e aumento da condutividade elétrica. Proteínas resistentes ao calor não foram suficientes para proteger as sementes contra danos macromoleculares e as concentrações de RNA e peroxidase diminuíram ao longo do armazenamento. As sementes armazenadas nas soluções B e C são mais vigorosas, sendo tais soluções indicadas para conservação de *H. speciosa*.

Termos para indexação: conservação *ex situ*, proteínas resistentes ao calor, mangabeira, recalcitrante, RNA.

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INTRODUCTION

Seed storage under conditions maintaining its post-harvest viability is one of the most convenient and commonly adopted methods for ex-situ conservation of plant germplasm. Generally, the necessary conditions to maintain the viability of these seeds during long-term storage are achieved by using water removal (drying) in equilibrium with low relative air humidity (about 15%) and temperatures below zero (commonly -18 °C), which is the approach adopted in most seed banks (FAO, 2013).

The seeds can be classified as orthodox, intermediate, or recalcitrant (Berjak and Pammenter, 2008). Orthodox seeds tolerate desiccation down to low moisture content (2-5%) and storage at low temperatures without damaging their structure. Seeds classified as intermediate tolerate drying to a humidity level of 7 to 10% and do not tolerate storage at low temperatures for a prolonged time. And recalcitrant seeds, sensitive to desiccation, lose their viability when reaching a water content of 12-31% and do not tolerate storage under low temperatures (Roberts, 1973; Hong and Ellis, 1996).

These classifications are important for the management of seed bank collections, developing strategies for the conservation of species, and the regeneration of forest areas, especially in tropical regions where alternative land use can endanger species conservation.

For the classification of seeds, tropical species present a wide variation of performance when storage (Walters, 2015). Those species require scientific information to allow technological development for efficient production and storage of propagules, specifically for recalcitrant seeds. Recalcitrant seeds cannot be stored employing the standard approach adopted in seed banks, and the development of technologies that allow storage for long periods is a challenge (Barbedo, 2018). Among the native species of Brazil with recalcitrant seeds, there is *Hancornia speciosa* Gomes (Apocynaceae), known as *mangabeira*.

H. speciosa is on the "List of Native Species of the Brazilian Flora with current or potential economic value" in the food category (Pereira et al., 2016), in other words, non-timber forest products. In addition to its potential as food, the species also stands out for its pharmacological potential (Torres-Rêgo et al., 2016; Neves et al., 2016; Bitencourt et al., 2019; Silva et al., 2019) and socioeconomic value, specifically for the traditional community called *Catadoras de Mangaba* (Sergipe, 2010).

Despite the social importance for the communities, the exploration of the species is threatened by forest fragmentation and irregular extraction without a sustainable management plan. In addition, there is an ecological implication for the natural regeneration of seedlings that are at risk without the presence of regenerants. These elements can promote habitat loss and cause the decline of the population and, consequently, fruit production for commercial use. Therefore, it is urgent and necessary to develop conservation strategies, currently occurring only *in situ*, in field collections, which are susceptible to anthropic pressure.

In this sense, technological innovations that allow the conservation and maintenance of the viability of seeds of *H. speciosa* can be an excellent strategy for species conservation. Also, to collaborate for seedling production planning in nurseries, which can enrich areas and maintain natural populations. To adopt this type of alternative, it is necessary to understand the effects on the viability, vigor and correlate the molecular alterations involved on seed quality. Therefore, in the present study, the physiological and molecular changes were evaluated in *H. speciosa* seeds stored in conservative solutions.

MATERIAL AND METHODS

The seeds were manually processed from ripe fruits (Nunes et al., 2021a), which correspond to yellowish to reddish skin, reddish streaks, and a soft texture. Obtained from a natural population in an area identified in previous studies as genetically diverse and used by the Catadoras de Mangaba community (Nunes et al., 2021b).

The fruits were dried at 25 °C for 24 h, the seeds homogenized, and a sampled was used to assess the initial quality by X-rays test, germination, water content, shoot length, root, and seedlings dry mass, electrical conductivity, concentration, and quality of ribonucleic acid (RNA), and concentration of peroxidase, and heat-resistant proteins. For storage, the seeds were packed in nylon mesh sachets containing 500 seeds. Seed storage was carried in four different conservative solutions (A, B, C, and D) based on processing described in patent BR 10 2021 009165 7 (Silva-Mann et al., 2021). The present patent refers to an osmoprotective solution for the storage of recalcitrant seeds. The solution has restrictive osmotic potential in water obtained by polietilenoglicol -0.8 MPa, an antimicrobial compound of natural origin, and can be kept in a domestic refrigerator. Solution A (without fungicide), B (with propolis extract - 100 mL per liter), C (with commercial fungicide Vitavax®-Thiram 200 SC), and D (with commercial fungicide Captan SC - 250 mg per 100 kg of seeds). The seeds were kept at 10 ± 2 °C in the dark for 50, 100, 150, and 200 days. Subsequently, the seeds were subjected to physiological and molecular quality assessments, as follows:

Water content (WC): oven method at 105 ± 3 °C for 24 h, in duplicate of 10 g (Brasil, 2009).

X-ray test: eight replicatons of 25 seeds were fixed by double-sided transparent adhesive tape and 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 classified according to the internal anatomy revealed by the radiography in full and well-formed or damaged seed (Brasil, 2009) and visualized the density with the ImageJ software.

Germination (G): the seeds of the X-rays test 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 at 25 °C with a 12 h photoperiod. The test lasted until the 35th day after sowing (Brasil, 2009).

Shoot (SL) and root length (RL) of seedlings: the hypocotyl and primary root were measured using eight replicatons of 15 normal seedlings randomly chosen at the 25th days after sowing (Nakagawa, 1999).

Seedling dry mass (SDM): the normal seedlings obtained were dried using oven with forced air circulation, at 80 °C for 24 h (Nakagawa, 1999).

Electrical conductivity (EC): 25 seeds were weighed and immersed in 75 mL of deionized water, at 25 °C for 24 h, using eight replicatons (Vieira and Krzyzanowski, 1999).

Extraction and quantification of RNA: the total RNA from embryonic axis (40 mg) were obtained by commercial kit Nucleospin® RNA II (Macherey-Nagel). The quantification of the extracted RNA was evaluated by means of spectrophotometry.

Peroxidase: 20 mg of cotyledons was obtained only from stored seeds. These were homogenized for 5 min and filtered through gauze. The filtrate was centrifuged at 6,800*x g*, 4 °C for 15 min, the supernatant was removed and used in the assay. To determine the peroxidase in the samples, 20 µg of protein + 25 mM citrate-phosphate buffer (pH = 5.4) + 1 mM guaiacol were used for each 1 mL of the reaction mixture. It was started with the addition of 10 µL of 30% hydrogen peroxide and the absorbance at 475 nm at 25 °C for 1 min with 10-sec intervals.

Extraction and quantification of heat-resistant proteins: the embryonic axis (150 mg) before storage and after storage were macerated using 1 mL of extraction buffer (50 mM Tris-HCl pH 7.5; 500 mM NaCl; 5 mM $MgCl_2$; 1 mM PMSF). The samples were shaken for 1 min and centrifuged at 4 °C, at 16,000 xg for 30 min, and the supernatant incubated in a water bath at 70 °C for 15 min. Protein quantification was performed by the Bradford method (1976) adding 5 µL of sample to 195 µL of Bradford's reagent. The samples reacted for 5 min at 25 °C. The readings were taken on the microplate spectrophotometer (Epoch ™) at 595 nm using bovine serum albumin (BSA) as reference.

Data statistical analysis was conducted in a completely randomized design, in a split-plot scheme, with eight replications. As plots the four conservative solutions (A, B, C, and D) and subplots the periods (50, 100, 150, and 200 days). Data were tested for homogeneity (Bartlett test) and normality (Shapiro-Wilk test) and subjected to analyses of variance using the F test. Tukey test compared the means of the treatments at 5% of probability. The data from the periods tested were subjected to regression analysis.

The principal component analysis (PCA) was also performed. Pearson's linear correlation coefficients (r) were calculated for all combinations between the physiological and molecular quality tests. The significance of the r values was determined by the t-test ($p \leq 0.05$).

RESULTS AND DISCUSSION

The X-ray test evaluated the analysis of the physical quality of the seeds before storage. The seeds were classified into full seeds (88%) (Figures 1 A, B, and C), that is, those that are well-formed and that did not present empty spaces, and seeds with damage (12%) (Figures 1 D, E, and F), those with changes in the formation of internal structures or empty spaces. This information is useful for decision-making when choosing the lot to be stored; considering the percentage of full seeds, the lot has potential for storage.

The initial seed water content was 55.6%. There were no changes in this content in all treatments ($p > 0.05$), and the values were kept above 50% for solution A (59.66%), B (53.32%), C (59.19%), and D (58.67%).

Recalcitrant seeds, such as *H. speciosa*, typically contain a high-water content at natural dispersion, and its maintenance is an obligatory condition for viability. The reduction in water content can trigger physical and macromolecular damage, such as changes in the activity of proteins and a decrease in cell membrane integrity, resulting in deterioration (Pammenter and Berjak, 2014; Umarani et al., 2015).

The minimum water content that maintains the viability of recalcitrant seeds varies depending on the species. *Talisia esculenta* (A. St. Hil) has recalcitrant seeds and the reduction in water content in these seeds from 40% to 24% on the 15th day of storage caused a reduction in the germination rate (Sena et al., 2016). For *H. speciosa* seeds, water content of 43% did not alter germination, while values below 38% lead to a gradual decrease in germination (Santos et al., 2010).

Independently of the applied solution, the seeds presented reduced germination (Figure 2). This situation was accentuated at 100 and 200 days with fungi presence such as *Aspergillus* and *Penicillium*, even in solutions C and D,

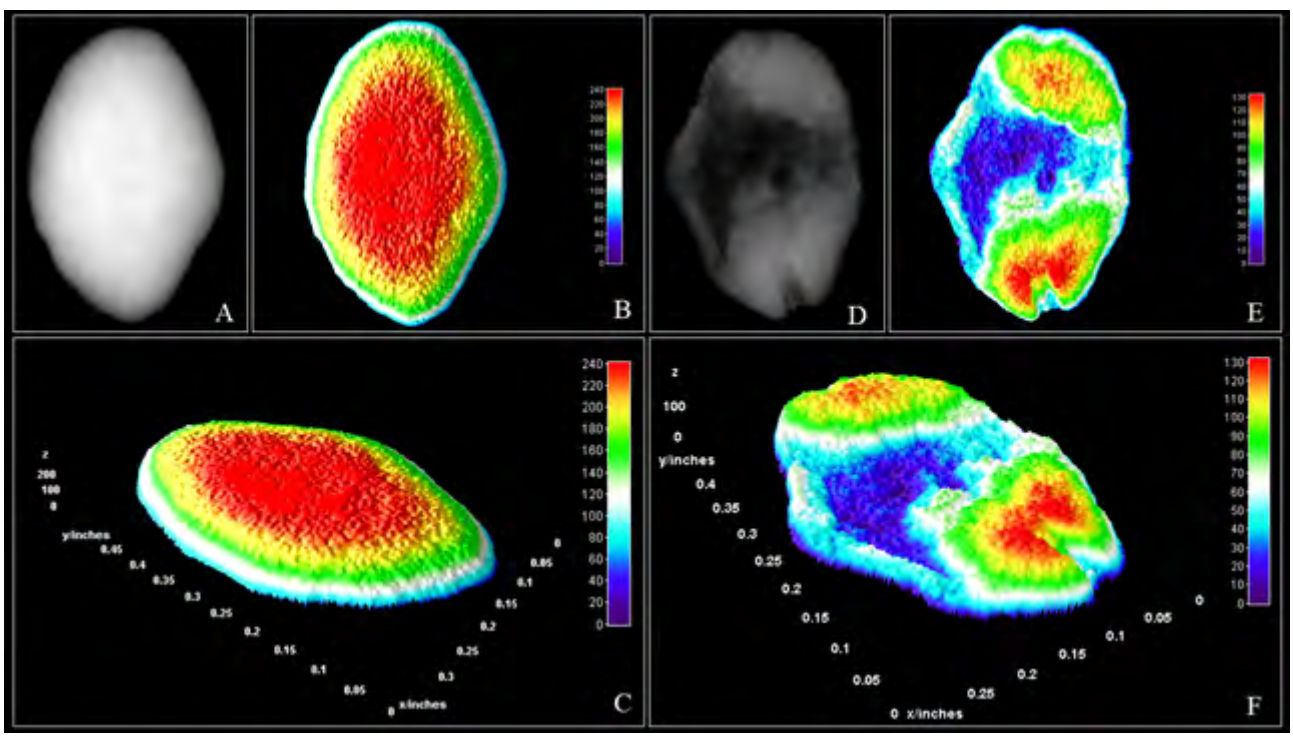

Figure 1. Radiographic images of *H. speciosa* seeds, combined with color representations (2D and 3D) of the density along the full (A, B, and C – relative density = 190.16) and damaged (D, E, and F- relative density = 67.53) seeds.

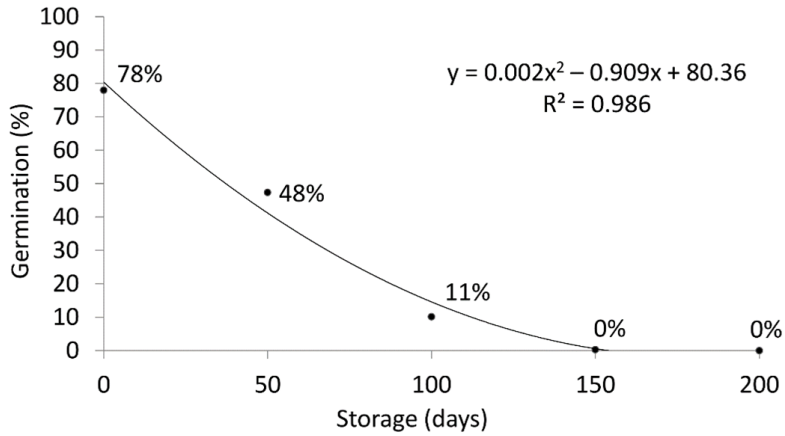

Figure 2. Average germination of *H. speciosa* seeds stored in conservative solutions during storage.

which contained commercial fungicides in their formulation. Both fungi were also reported in *mangaba* seeds in the Brazilian Cerrado. It was also previously reported *Chaetomium* spp., *Cladosporium* sp., *Fusarium* spp., *Paecilomyces* sp., *Pestalotiopsis* sp., *Phomopsis* sp., *Aureobasidium pullulans* (de Bary) Arnaud, and an unidentified yeast (Anjos et al., 2009).

Viability reduction in storage is an inevitable process for all classes of seeds. For recalcitrant seeds, reductions will vary mainly due to the intrinsic features of the species. This reduction can be drastic, as observed for *Aquilaria malaccensis* Lamk that presented germination corresponding to 92.0% (before storage), 39.6% after 5 days, 4.0% after 10 days, and 0% at 15 days of storage at the forest nursery (Tabin and Shrivastava, 2014).

The deterioration can be attenuated using osmoconditioning treatments, as seen in *Hevea brasilliensis* Muell. Arg. seeds treated with 30% PEG 6000 solution, which maintained germination (99%) after 16 days of storage (Charloq et al., 2016). It can also occur slowly, as seen in *Araucaria angustifolia* (Bertol.) Kuntze seeds germinate 64% after 180 days of storage in the refrigerator at 5 °C and 45% relative humidity (Garcia et al., 2014). And incubation the embryos of inga (*Inga vera* Willd. subsp. *affinis* (DC.) T.D. Pennington) in a PEG solution at -2.0 MPa increases their tolerance to desiccation (Bonjovani and Barbedo, 2014).

The decreasing germination under storage observed for *H. speciosa* seeds can also be influenced by the lipid content in its chemical composition (oil content 27.33 ± 0.37%, proteins 12.10 ± 1.60%, fibers 11.98 ± 0.46%, cellulose 17.07%, hemicellulose 22.57% and lignin 10.16%) (Santos et al., 2015), considering that it presents higher chemical instability and may cause faster deterioration such as hydrolytic reactions that result in hyperoxide (Harrington, 1972).

Seeds evaluated before storage showed the shoot length, root, and dry mass of seedlings corresponding to 5.92 cm, 10.26 cm, and 89.259 mg.seedlings^{-1}, respectively, values that decreased over storage (Figure 3).

At 50 days, the highest shoot seedlings were obtained for solution B and in relation to solution D, the A, C, and D solutions did not differ statistically. The root and the dry mass of seedlings did not differ ($p > 0.05$) among the treatments. At 100 days, more vigorous seedlings were observed in the seeds kept in solutions B and C (Table 1).

The values measured in this study for the shoot and root length of seedlings were similar to those obtained by Santos et al. (2010) and, for the seedling dry mass, agree to Soares et al. (2015). Vigor is an intrinsic factor for seeds and can be influenced by environmental conditions during its formation, mechanical damage, the action of microorganisms, insects, and storage conditions (Carvalho and Nakagawa, 2012). The vigor of seeds, evaluated through length shoot and root length, and dry mass of seedlings, is maintained in seeds stored in solutions B and C for 100 days (Table 1 and Figure 3). After this period, there is a reduction in vigor, which may be associated with high metabolic activity and respiratory rate. Those events can lead to considerable consumption of the reserve tissue and a decrease in seed energy, negatively influencing the continuity of the physiological processes.

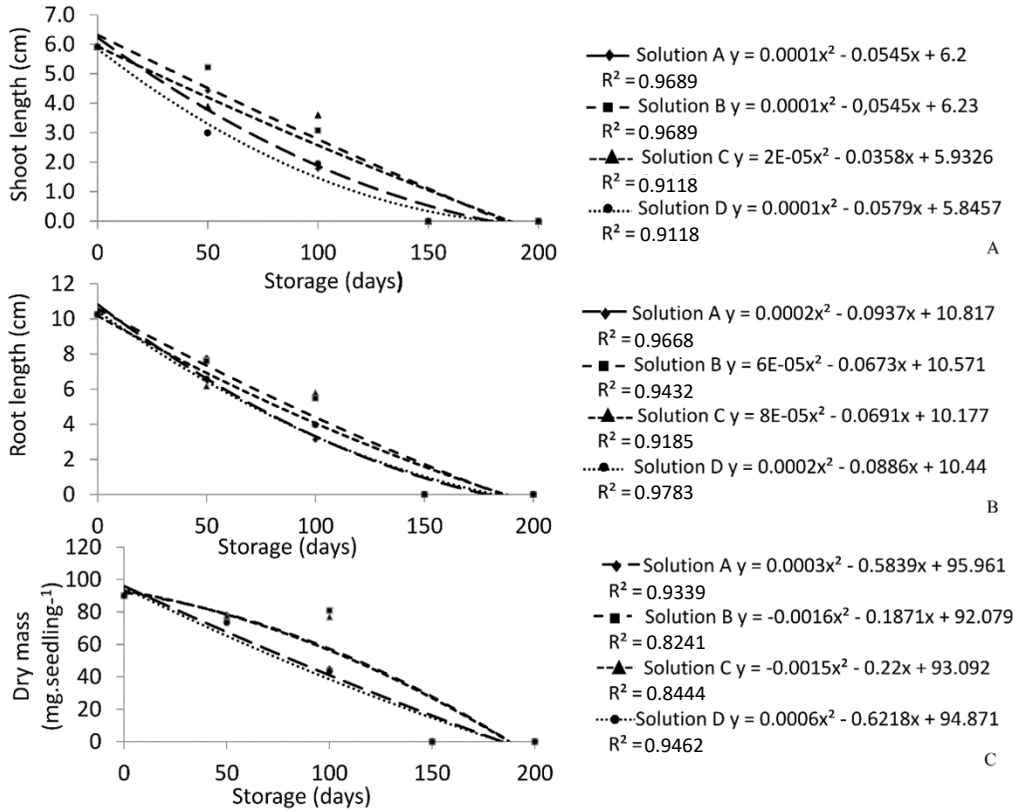

Figure 3. Shoot length (A), root length (B), and seedlings dry mass (SDM) of *H. speciosa* seeds stored in conservative solutions for different periods

Table 1. Shoot length (SL), root length (RL) and, dry mass (SDM) seedling of *H. speciosa* seeds stored in conservative solutions for different periods.

Initial quality	Storage (days)	Solutions -0.8 MPa			
		A	B	C	D
		SL (cm)			
5.92	50	4.43 ab	5.22 a	3.88 ab	2.92 b
	100	1.78 b	3.08 a	3.60 a	1.95 b
	150	0.00 a	0.00 a	0.00 a	0.00 a
	200	0.00 a	0.00 a	0.00 a	0.00 a
		RL (cm)			
10.26	50	7.77 a	7.60 a	6.17 a	6.58 a
	100	3.13 b	5.49 a	5.80 a	3.96 b
	150	0.00 a	0.00 a	0.00 a	0.00 a
	200	0.00 a	0.00 a	0.00 a	0.00 a
		SDM (mg.seedling^{-1})			
89.259	50	78.18 a	75.00 a	77.69 a	73.36 a
	100	45.00 b	80.75 a	77.00 a	43.25 b
	150	0.00 a	0.00 a	0.00 a	0.00 a
	200	0.00 a	0.00 a	0.00 a	0.00 a

Means followed by the same lowercase letters on the line do not differ by Tukey's test at 5% probability.

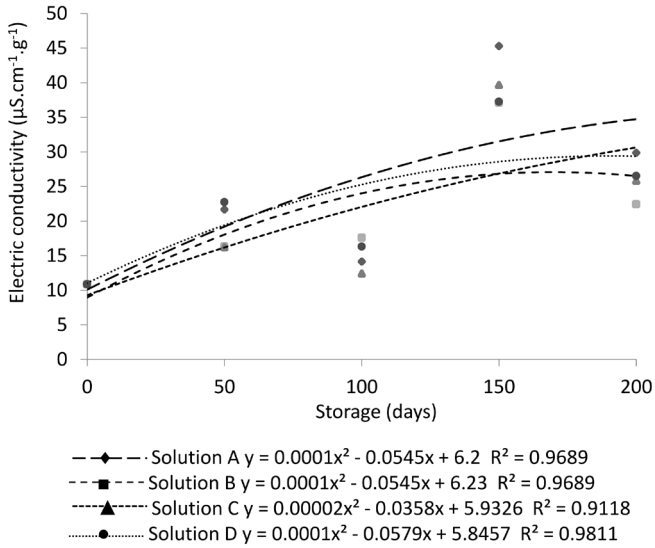

Figure 4. Electrical conductivity (EC) of *H. speciosa* seeds stored in conservative solutions for different periods.

With the extension of the storage time, there was an increase in the electrical conductivity for seeds stored in all solutions (Figure 4). The lowest values were verified at 50 days for solutions B and C.

The values of electrical conductivity obtained in the present study for 50 and 100 days of storage were close to those obtained in seeds of *H. speciosa* by Barros et al. (2006) that evaluated *mangaba* seeds obtained by different processing methods. There were higher electrical conductivity values at 150 and 200 days of storage, close to those cited by Barros et al. (2010), in seeds submitted to two drying methods.

The release of leachate by the seeds indicates seed deterioration due to the disruption of the cell membrane system culminating in the reduction of viability (Santos and Paula, 2005). These results obtained for *mangaba* seeds under storage in these solutions permit us to infer the deterioration of the material. Corroborating the results obtained for germination, shoot length, root length, and seedling dry mass decreased with the storage time.

RNA extraction was successful in all treatments. There are controversies in using the information on the concentration, quality, and integrity of RNA, which may vary depending on the method and other environmental conditions. In this study, the intention is to verify whether reductions in RNA concentrations may contribute to future gene expression studies. As RNA needs to present concentration, quality, and integrity, it hopes to contribute to future works of gene expression. The suggestion is to use treatments with a concentration higher than about 52%. It results in better planning and reduction in the economy of laboratory supplies.

The highest concentrations of RNA were found in the period of 50 days for all solutions, and during the other periods, the highest concentrations were observed in solution B (Figure 5A). The quality of the RNA is determined from its purity, which allows inferences about the composition of the extracted material such as salts, polysaccharides, phenols, and proteins, mainly in seeds (Fleming et al., 2017; Fleming et al., 2019). Despite undergoing several changes during storage, the seeds stored in solution B presented RNA purity values closer to those recommended in reports of the literature (Djami-Tchatchou and Straker, 2012; Dash, 2013).

RNA is important for germination to occur; however, it is a fragile molecule, easily damaged, which presents a simple chain, vulnerable to degradation by reactive oxygen species (ROS) (Bazin et al., 2011; Bai et al., 2017). ROS are produced in high quantities in recalcitrant seeds due to the lack of drying and metabolic activity reduction after dispersion. Thus, there is an imbalance of metabolism and high respiration rates, consequently generating ROS-related oxidative damage to macromolecules such as DNA and RNA, high production of free radicals as peroxides, and causing seed death.

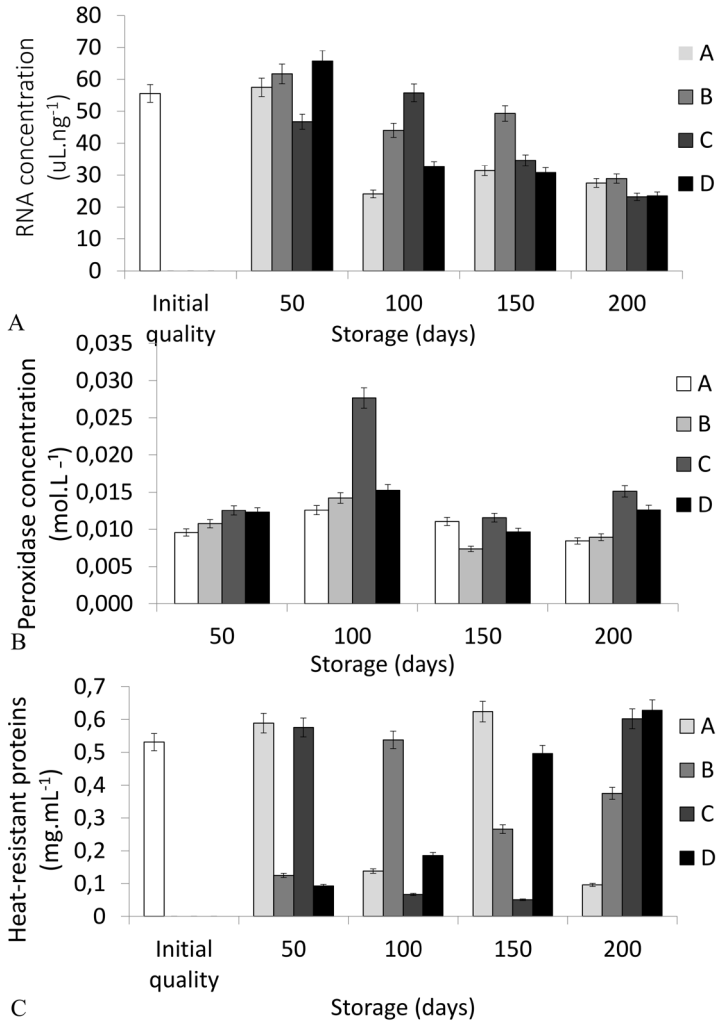

Figure 5. Concentration of ribonucleic acid (A), peroxidase (B), and heat-resistant proteins (C) extracted from *H. speciosa* seeds stored in conservative solutions (A, B, C, and D) for different periods.

The cellular damage caused by these free radicals is usually reduced or prevented by a protective mechanism involving enzymes that eliminate peroxidase. Its activities can be quantified over these effects, looking to correlate its contents to the physiological seed performance of this species (Viana et al., 2020).

The concentration of the peroxidase enzyme (Figure 5B) ranged from 0.0074 mol.L^{-1} (Solution B at 150 days) to 0.0277 mol.L^{-1} (Solution C at 100 days). Peroxidase belongs to oxidoreductase enzymes and iron-containing proteins, which can degrade hydrogen peroxide in various cellular processes (Mohammadnia et al., 2019). Its main role is to reflect the plant's metabolic response to stress, being, therefore, one of the most susceptible enzymes to environmental stress (Castillo, 1986). A rapid decrease in enzymic protection as peroxidase was correlated to desiccation sensitivity in *Garcinia gardneriana* (Viana et al., 2020).

The lower concentrations of peroxidase were observed for seeds stored in the periods of 150 and 200 days. They may be associated with the intense metabolic activity of seeds and consequently actions as scavengers on free radicals.

For the analysis of heat-resistant proteins, during the first 50 days of storage, the seeds in solutions A and C presented proteins heat-resistant concentrations to initial seed quality. At 100 days, seeds from solution B have values close to the initial quality (Figure 5C).

Heat-resistant proteins are generally expressed under stress conditions and act as chaperones, stabilizing the structure of other proteins (Siddique et al., 2008). The expression of these proteins can be induced by oxidative stress,

cold stress, heavy metals, ozone, UV, and radiation (Kalemba and Pukacka, 2007). These protective proteins can assist in the repair mechanisms in recalcitrant seeds and their expression patterns are attributes that are difficult to understand when studied in recalcitrant species (Kalemba and Pukacka, 2012).

The variations in these proteins in *H. speciosa* seeds may occur due to oxidative stress rates, one of the factors responsible for inducing the expression of these proteins. No direct relationship has been identified between variations in protein concentrations and seed viability and vigor.

From the Principal Component Analysis (PCA) correlating the four conservative solutions and the storage periods, the first two components (PC1 and PC2) represented 62.64% of data variability (Figure 6). Thus, through various linear combinations, it was possible to reduce from eight dimensions to just two, explaining a significant percentage of the observations for *H. speciosa* seeds.

The correlation analysis was performed to verify possible correlations between the physiological and molecular variables (RNA, heat resistant proteins, and peroxidase) (Figure 7). The germination is positively correlated ($p < 0.05$) with the shoot length ($r = 0.83$), root ($r = 0.81$), and seedlings dry mass ($r = 0.82$) variables. There are also significant and positive correlations for shoot length and root ($r = 0.34$) and seedling dry mass ($r = 0.41$) with peroxidase concentration, and these results are consistent considering that peroxidase is also affected by the vigor of the seeds, showing higher concentrations in vigorous seeds (Mohammadnia et al., 2019).

Negative correlations were found for electrical conductivity and practically all other variables, with $r = -0.5$ for germination and peroxidase concentration, $r = -0.77$, $r = -0.77$, and $r = -0.75$ for shoot length, root, and seedlings dry mass, respectively. The one with the highest electrical conductivity is also obtained with the highest degree of deterioration and, therefore, the least is as chances of forming normal seedlings.

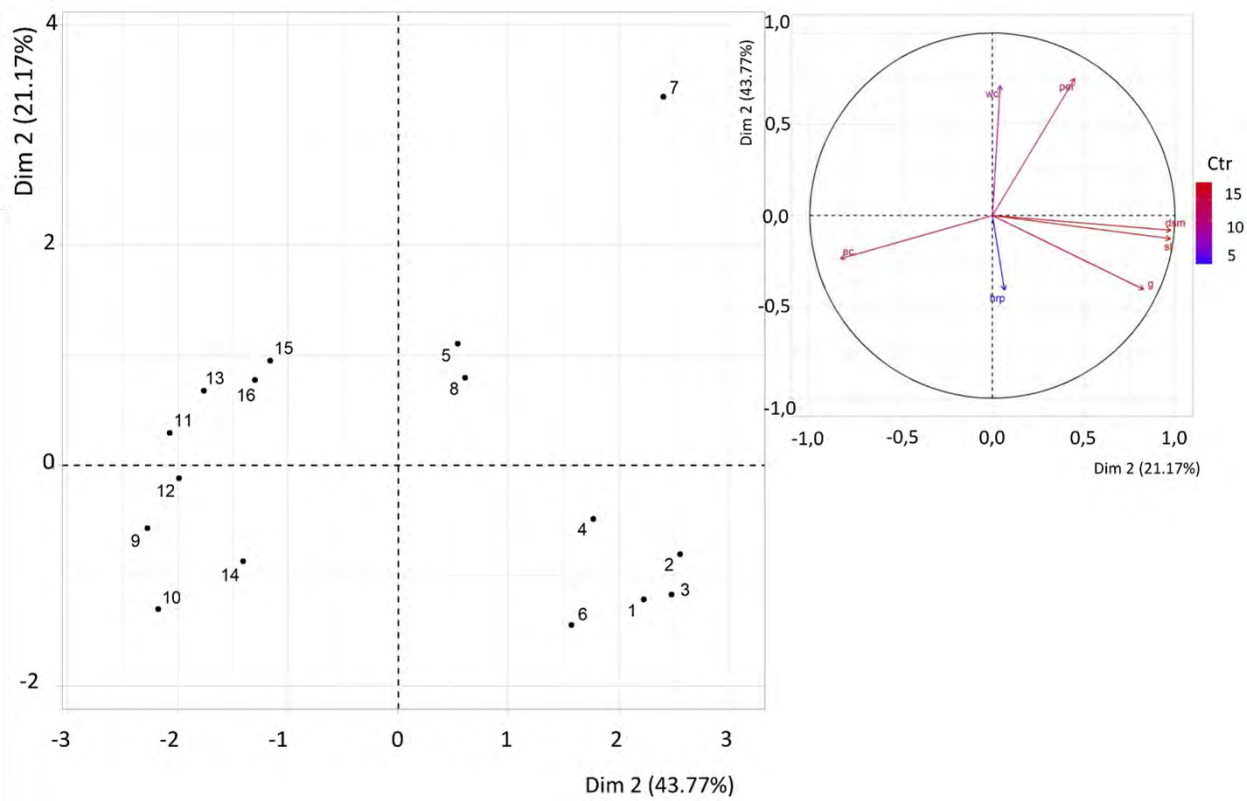

Figure 6. Biplot obtained by the linear combination of variables related to the physiological and molecular characteristics of *H. speciosa* seeds stored in conservative solutions for different periods.

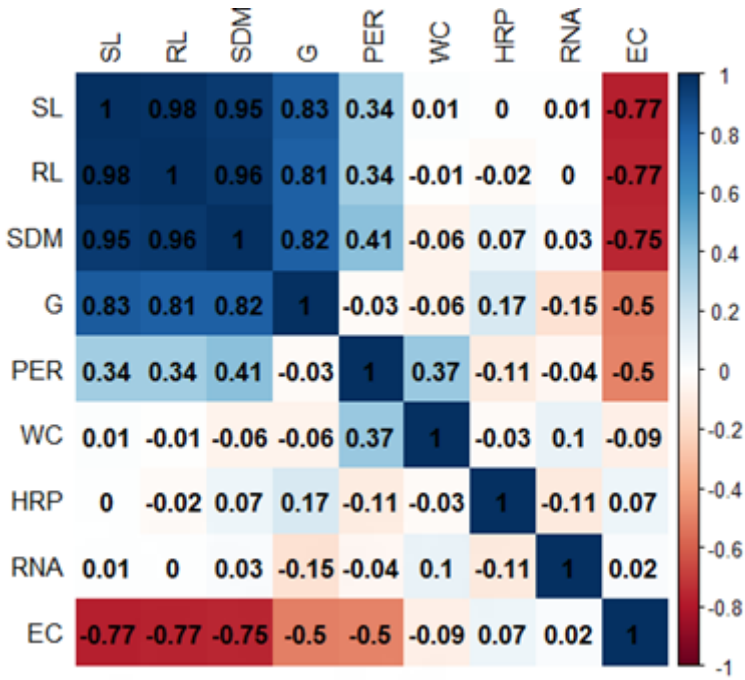

Figure 7. Pearson's correlation between the variables physical and physiological (water content – WC; germination – G; shoot length – SL; root length - RL; seedlings dry mass – SDM; eletrical condutivity - EC) and, molecular analysis (ribonucleic acid - RNA; Heat-resistant proteins – HRP; peroxidase - PER) of *H. speciosa* seeds stored in conservative solutions for different periods.

Species producing short-lived seeds require non-conventional storage methods. Information on seed storage behavior is fundamental for species management, especially in tropical areas, where the number of recalcitrant species is high (Tweddle et al., 2003; Wyse and Dickie, 2016). Thus, seed banks and other conservation strategies must be improved to avoid species loss. Technologies to improve the storage of recalcitrant seeds are continuously discussed (Mayrinck et al., 2019). This is the first study in the literature gathering information on *mangaba* seeds under storage and evaluating seed quality from molecular and physiological aspects.

CONCLUSIONS

H. speciosa seeds can be preserved for up to 50 days in the developed conservative solutions.

Solutions B and C are recommended to store *H. speciosa* seeds as they promote the maintenance of vigor.

Heat-resistant proteins expressed in the stored seeds were not sufficient to protect the *H. speciosa* seeds against macromolecular damage. The RNA and peroxidase concentrations decreased with the increase of the storage period.

In the case of recalcitrant seeds, success in storage even for a short period represents an advance. It contributes not only to conservation, but also to the planning and use of seeds in nurseries.

This work can be used as a basis for the development of alternative conservation strategies for recalcitrant seeds in *ex situ* banks.

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REFERENCES

ANJOS, J.R.N.; CHARCHAR, M.J.D.; LEITE, R.G.; SILVA, M.S. Survey and pathogenicity of fungi associated with mangaba (*Hancornia speciosa* Gomes) seeds in the Cerrado region of Central Brazil. *Revista Brasileira de Fruticultura*, v.31, n.3, p.911-915, 2009. https://www.scielo.br/pdf/rbf/v31n3/a40v31n3.pdf

BAI, B.; PEVIANI, A.; VAN DER HORST, S.; GAMM, M.; SNEL, B.; BENTSINK, L.; HANSON, J. Extensive translational regulation during seed germination revealed by polysomal profiling. *New Phytologist*, v.214, p.233-244, 2017. https://nph.onlinelibrary.wiley.com/doi/full/10.1111/nph.14355

BARBEDO, C.J. A new approach towards the so-called recalcitrant seeds. *Journal of Seed Science*, v.40, n.3, p.221-236, 2018. https://doi.org/10.1590/2317-1545v40n3207201

BARROS, D.I.; BRUNO, R.L.A.; NUNES, H.V.; CABRAL, G.C.; PEREIRA, W.E.; MENDONÇA, R.M.N. Métodos de extração de sementes de mangaba visando à qualidade fisiológica. *Revista Brasileira de Fruticultura*, v.28, n.1, p.25-27, 2006. http://www.scielo.br/scielo.php?pid=S0100-29452006000100010&script=sci_abstract&tlng=pt

BARROS, D.I.; BRUNO, R.L.A.; NUNES, H.V.; MENDONÇA, R.M.N., PEREIRA, W.E. Comportamento fisiológico de sementes de mangaba submetidas à dessecação. *Revista ACTA Tecnológica*, v.5, n.1, p.31-43, 2010. https://periodicos.ifma.edu.br/actatecnologica/article/view/19

BAZIN, J.; LANGLADE, N.; VINCOURT, P.; ARRIBAT, S.; BALZERGUE, S.; EL-MAAROUF-BOUTEAU, H.; BAILLY, C. Targeted mRNA oxidation regulates sunflower seed dormancy alleviation during dry after-ripening. *The Plant Cell*, v.23, p.2196-2208, 2011. http://www.plantcell.org/content/23/6/2196.short

BERJAK, P.; PAMMENTER, N.W. From *Avicennia* to *Zizania*: seed recalcitrance in perspective. *Annals of Botany*, v.101, n.2, p.213-228, 2008. https://academic.oup.com/aob/article/101/2/213/186304

BITENCOURT, M.A.O.; TORRES-RÊGO, M.; LIMA, M.C.J.S.; FURTADO, A.A.; AZEVEDO, E.P.; EGITO, E.S.T.; SILVA-JUNIOR, A.A; ZUCOLOTTO, S.M.; FERNANDES-PEDROSA, M.F. Protective effect of aqueous extract, fractions and phenolic compounds of *Hancornia speciosa* fruits on the inflammatory damage in the lungs of mice induced by *Tityus serrulatus* envenomation. *Toxicon*, v.164, p.1-9, 2019. https://www.sciencedirect.com/science/article/pii/S0041010119300960

BONJOVANI, M.R.; BARBEDO, C.J. Induction of tolerance to desiccation and to subzero temperatures in embryos of recalcitrant seeds of inga. *Journal of Seed Science*, v.36, n.4, p.419-426, 2014. https://doi.org/10.1590/2317-1545v36n41027

BRADFORD, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, v.72, n.1-2, p.248-254, 1976. https://pubmed.ncbi.nlm.nih.gov/942051/

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. *Regras para Análise de Sementes*. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília: MAPA/ACS, 2009. 399p. https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/arquivos-publicacoes-insumos/2946_regras_analise__sementes.pdf

CARVALHO, N.M.; NAKAGAWA, J. *Sementes: Ciência, Tecnologia e Produção*. Jaboticabal: FUNEP, 2012. 590p.

CASTILLO, F. Extracellular peroxidases as markers of stress? In: GREPPIN, H.; PENEL, C.; GASPAR, T. (Eds). *Molecular and physiological aspects of plant peroxidases*. Genève: Université de Genève, *Centre de Botanique*, 1986. p.419-426. https://pdfs.semanticscholar.org/4611/c9e8b83bb6dc95a979faecb398df9539c650.pdf

CHARLOQ, Z.L.; SIREGAR, T.H.; DAMANIK, S.B.; YAZID, A.; HUSNI, M. Physiology changes of shelled rubber (*Hevea brasilliensis* Muell. Arg.) seed after 16 days storage with PEG 6000 30% coating to induce secondary dormancy. *Journal of Agronomy*, v.15, p.11-18, 2016. https://scialert.net/fulltextmobile/?doi=ja.2016.11.18

DASH, P.K. High quality RNA isolation from polyphenol-polysaccharide and protein-rich tissues of lentil (*Lens culinaris*). *Biotech*, v.3, n.2, p.109-114, 2013. https://link.springer.com/article/10.1007/s13205-012-0075-3

DJAMI-TCHATCHOU, A.T.; STRAKER, C.J. The isolation of high quality RNA from the fruit of avocado (*Persea americana* Mill.). *South African Journal of Botany*, v.78, p.44-46, 2012. https://www.sciencedirect.com/science/article/pii/S0254629911000585

FAO. Genebank Standards for Plant Genetic Resources for Food and Agriculture. Rome: FAO, 2013. http://www.fao.org/3/a-i3704e.pdf Access in: 23 nov. 2018.

FLEMING, M.B.; HILL, L.M.; WALTERS, C. The kinetics of ageing in dry-stored seeds: a comparison of viability loss and RNA degradation in unique legacy seed collections. *Annals of Botany*, v.123, n.7, p.1133-1146, 2019. https://academic.oup.com/aob/article/123/7/1133/5253623

FLEMING, M.B.; RICHARDS, C.M.; WALTERS, C. Decline in RNA integrity of dry-stored soybean seeds correlates with loss of germination potential. *Jounal of Experimental Botany,* v.68, n.9, p.2219-2230, 2017. https://academic.oup.com/jxb/article/68/9/2219/3603459

GARCIA, C.; COELHO, C.M.M.; MARASCHIN, M.; OLIVEIRA, L.M. Conservação da viabilidade e vigor de sementes de *Araucaria angustifolia* (Bert.) O. Kuntze durante o armazenamento. *Ciência Florestal*, v.24, n.4, p.857-867, 2014. https://periodicos.ufsm.br/index.php/cienciaflorestal/article/viewFile/16586/pdf_1

HARRINGTON, J.F. *Seed storage longevity*. In: KOZLOWSKI, T.T (Ed.). Seed Biology. New York: Academic Press, 1972. p.145-245.

HONG, T.D.; ELLIS, R.H. A protocol to determine seed storage behaviour. VIPGRI Technical Bulletin n. 1, (ENGELS J.M.M., TOLL J., eds). Rome: International Plant Genetic Resources Institute. 1996. https://cropgenebank.sgrp.cgiar.org/images/file/learning_space/technicalbulletin1.pdf

KALEMBA, E.M.; PUKACKA, S. Association of protective proteins with dehydration and desiccation of orthodox and recalcitrant category seeds of three *Acer* genus species. *Journal of Plant Growth Regulation*, v.31, n.3, p.351-362, 2012. https://link.springer.com/article/10.1007/s00344-011-9246-4

KALEMBA, E.M.; PUKACKA, S. Possible roles of LEA proteins and sHSPs in seed protection, a short review. *Biological Letters*, v.44, p.1-8, 2007. http://citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.607.2377

MAYRINCK, R.C.; VILELA, L.C.; PEREIRA, T.M.; RODRIGUES-JUNIOR, A.G.; DAVIDE, A.C.; VAZ, T. A. Seed desiccation tolerance/sensitivity of tree species from Brazilian biodiversity hotspots: considerations for conservation. *Trees*, v.33, n.3, p.777-785, 2019. https://link.springer.com/article/10.1007/s00468-019-01815-8

MOHAMMADNIA, F.; TORABIAN, S.Y.; REZAIE, A.; HASHEMI, S.A. The effects of seed coat removal on the activity of peroxidase enzyme in *Acer velutinium*. *Acta Ecologica Sinica*, v.39, n.3, p.190-193, 2019. https://www.sciencedirect.com/science/article/abs/pii/S1872203217301919?via%3Dihub

NAKAGAWA, J. Testes de vigor baseados no desempenho das plântulas. In: KRZYZANOWSKI, F.C.; VIEIRA, R.D.; FRANCA-NETO, J.B. (Eds.). *Vigor de sementes: conceitos e testes*. Londrina: Abrates, 1999. p.2.1-2.24.

NEVES, J.S.; FRANCHIN, M.; ROSALEN, P.L.; OMAR, N.F.; SANTOS, M.A.; PASCHOAL, J.A.R.; NOVAES, P.D. Evaluation of the osteogenic potential of *Hancornia speciosa* latex in rat calvaria and its phytochemical profile. *Journal of Ethnopharmacology*, v.183, p.151-158, 2016. https://www.sciencedirect.com/science/article/pii/S037887411630085X?casa_token=Ug6jSbS3STQAAAAA:7f9kRzNftNGsUq4qN7n2_iZcXAap2bfguKrUxdd6jpmuUuKOwp_DUYop0OEk7wb-dOFuUAV7Zd8

NUNES, V.V.; SILVA-MANN, R.; SOUZA, J.L.; CALAZANS, C.C. Geno-phenotypic diversity in a natural population of *Hancornia speciosa* Gomes: implications for conservation and improvement. *Genetic Resources and Crop Evolution*, v.68, n.7, p.2869-2882, 2021b. https://doi.org/10.1007/s10722-021-01160-1

NUNES, V.V.; SILVA-MANN, R.; VASCONCELOS, M.C.; RODRIGUES, A.M.B.; SOUZA, J.L. Physical and physiological quality of mangaba seeds obtained by different processing methods. *Revista Brasileira de Engenharia Agrícola e Ambiental*, v.2, n.6, p.429-435, 2021a. https://doi.org/10.1590/1807-1929/agriambi.v25n6p429-435

PAMMENTER, N.W.; BERJAK, P. Physiology of desiccation-sensitive (recalcitrant) seeds and the implications for cryopreservation. *International Journal of Plant Sciences*, v.175, n.1, p.21-28, 2014. https://www.journals.uchicago.edu/doi/abs/10.1086/673302

PEREIRA, A.V.; PEREIRA, E.B.C.; SILVA JÚNIOR, J.F.; SILVA, D.B. *Hancornia speciosa* (mangabeira). In: VIEIRA, R.F; CAMILO, J.; CORADIN, L. (Eds.) Espécies Nativas da Flora Brasileira de Valor Econômico Atual ou Potencial. Brasília, DF. Ministério do Meio Ambiente, 2016, 12p.

ROBERTS, E.H. Predicting the storage life of seeds. *Seed Science and Technology*, v.1, n.3, p.499-514, 1973.

SANTOS, P.C.G.; ALVES, E.U.; GUEDES, R.S.; SILVA, K.B.; CARDOSO, E.D.A.; LIMA, C.R. Quality of *Hancornia speciosa* Gomes seeds in function of drying periods. *Semina: Ciências Agrárias*, v.31, n.2, p.343-352, 2010. https://www.cabdirect.org/cabdirect/abstract/20103225628

SANTOS, R.M.; SANTOS, A.O.; SUSSUCHI, E.M.; NASCIMENTO, J.S.; LIMA, Á.S.; FREITAS, L.S. Pyrolisis of mangaba seed: Production and characterization of bio-oil. *Bioresource Technology*, v.196, p.43-48, 2015. https://www.sciencedirect.com/science/article/pii/S0960852415010226

SANTOS, S.R.G.; PAULA, R.C. Teste de condutividade elétrica para avaliação da qualidade fisiológica de sementes de *Sebastiania commersoniana* (Bail.) Smith e Downs (branquilho) - Euphorbiaceae. *Revista Brasileira de Sementes*, v.27, n.2, p.136-145, 2005. http://www.scielo.br/scielo.php?pid=S0101-31222005000200020&script=sci_arttext&tlng=pt

SENA, L.H.M.; MATOS, V.P.; MEDEIROS, J.É.D.; SANTOS, H.H.D.; ROCHA, A.P.; FERREIRA, R.L.C. Storage of pitombeira seeds [*Talisia esculenta* (A. St. hil) Radlk - Sapindaceae] in different environments and packagings. *Revista Árvore*, v.40, n.3, p.435-445, 2016. http://www.scielo.br/scielo.php?pid=S0100-67622016000300435&script=sci_arttext

SERGIPE. Lei nº 7.082 de 16 de dezembro de 2010. Diário Oficial do estado de Sergipe, Poder Executivo, Aracaju, SE, 16 dez. 2010. https://al.se.leg.br/Legislacao/Ordinaria/2010/O70822010.pdf. Accessed: 23, Nov. 2018.

SIDDIQUE, M.; GERNHARD, S.; VON KOSKULL-DÖRING, P.; VIERLING, E.; SCHARF, K.D. The plant sHSP superfamily: five new members in *Arabidopsis thaliana* with unexpected properties. *Cell Stress Chaperones*, v.13, n.2, p.183-197, 2008. https://link.springer.com/article/10.1007/s12192-008-0032-6

SILVA, M.R.M.A.; PACHECO, C.M.F.; MADEIRA, M.F.M.; SARAIVA, A.M.; FREITAS, E.A.; VALVERDE, T.M.; GOMES, J.H.S.; PÁDUA, R.M.; KITTEN, G.T.; ALVES, S.Y.F.; BRAGA, F.C.; SILVA, T.A. Effect of the extract and constituents from *Hancornia speciosa* fruits in osteoclasts. *Planta Medica International*, v.6, n.01, p.e7-e14, 2019. https://www.thieme-connect.com/products/ejournals/html/10.1055/a-0829-0454

SILVA-MANN, R.; NUNES, V.V.; RABBANI, A.R.C.; FREITAS, B.A.L.; CANDIDO, I.J.; SOUZA, J.L. Solução e processo para conservação de sementes recalcitrantes. Universidade Federal de Sergipe. BR 10 2021 009165 7. 11 mai. 2021

SOARES, A.N.R.; MELO, M.F.V.; VITORIA, M.F.; SILVA, A.V.C. Physiological quality of mangaba seeds submitted to drying. *African Journal of Agricultural Research*, v.10, n.52, p.4808-4813, 2015. https://pdfs.semanticscholar.org/c469/7bed054d89b69f33f71cd8f02e5f5d7af806.pdf

TABIN, T.; SHRIVASTAVA, K. Factors affecting seed germination and establishment of critically endangered *Aquilaria malaccensis* (*Thymelaeaceae*). *Asian Journal Plant Science Research*, v.4, n.6, p.41-6, 2014. https://www.imedpub.com/articles/factors-affecting-seed-germination-and-establishment-of-critically-endangeredaquilaria-malaccensis-thymelaeaceae.pdf

TORRES-RÊGO, M.; FURTADO, A.A.; BITENCOURT, M.A.O.; LIMA, M.C.J.S.; ANDRADE, R.C.L.C.; AZEVEDO, E.P.; SORES, T.C.; TOMAZ, J.C.; LOPES, N.P.; SILVA-JUNIOR, A.A.; ZUCOLOTTO, S.M.; FERNANDES-PEDROSA, M.F. Anti-inflammatory activity of aqueous extract and bioactive compounds identified from the fruits of *Hancornia speciosa* Gomes (Apocynaceae). *BMC Complementary and Alternative Medicine*, v.16, n.1, p.275, 2016. https://bmccomplementmedtherapies.biomedcentral.com/articles/10.1186/s12906-016-1259-x

TWEDDLE, J.C.; DICKIE, J.B.; BASKIN, C.C.; BASKIN, J.M. Ecological aspects of seed desiccation sensitivity. *Journal of Ecology*, v.91, n.2, p.294-304, 2003. https://doi.org/10.1046/j.1365-2745.2003.00760.x

UMARANI, R.; AADHAVAN, E.K.; FAISAL, M.M. Understanding poor storage potential of recalcitrant seeds. *Current Science*, v.108, n.11, p.2023-2034, 2015. https://www.jstor.org/stable/24905571?seq=1

VIANA, W.G.; LANDO, A.P.; SILVA, R.A.D.; COSTA, C.D.D.; VIEIRA, P.H.M.; STEINER, N. Physiological performance of *Garcinia gardneriana* (Planch. & Triana) Zappi: a species with recalcitrant and dormant seeds. *Journal of Seed Science*, v.42, e202042001, 2020. https://www.scielo.br/scielo.php?pid=S2317-15372020000100101&script=sci_arttext

VIEIRA, R.D.; KRZYZANOWSKI, F.C. *Teste de condutividade elétrica*. In: KRZYZANOWSKI, F.C.; VIEIRA, R.D.; FRANÇA-NETO, J.B. (Eds.). Vigor de sementes: conceitos e testes. Londrina: Abrates, 1999. p.4.1-4.26.

WALTERS, C. Orthodoxy, recalcitrance and in-between: describing variation in seed storage characteristics using threshold responses to water loss. *Planta*, v.242, n.2, p.397-406, 2015. https://link.springer.com/article/10.1007/s00425-015-2312-6

WYSE, S.V.; DICKIE, J.B. Predicting the global incidence of seed desiccation sensitivity. *Journal of Ecology*, v.105, n.4, p.1082-1093, 2016. https://doi.org/10.1111/1365-2745.12725C