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Evaluation of Germination and Storage of Euterpe edulis Martius Seeds

Giovana Bomfim de Alcantara [a*], Angela Cristina Ikeda [a]

[a] Universidade Federal do Paraná, Brasil
[*] Autor correspondente (giobomfim@ufpr.br)

INFO

Keywords
Juçara palm
pericarp
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ABSTRACT

Euterpe edulis Martius is ecologically important in its native biome and threatened by illegal palm heart extraction. Despite the species' relevance, there is still a lack of information about the physiological behavior of its seeds under different germination and storage conditions. The aim of this work is to evaluate conditions of germination and storage of juçara palm seeds in different environments and substrates. Germination test was set in greenhouse and acclimatized room, combined with three substrates: Tropstrato Florestal®; sand and vermiculite, considering the absence and presence of pericarp. A storage test was set in two temperatures of 25°C and 4°C combined to six periods of storage: zero, 14, 28, 42, 56 and 70 days. The experimental design for both tests was completely randomized composed of four replications and 20 seeds each. They were analyzed: index of speed germination; average of seedling length; percentage of germination; relative frequency of germination and the average of germination time. The seeds' viability was verified by a tetrazolium test (0.05%). Germination test showed that better results are achieved by removing the pericarp from the seeds. At the acclimatized room the treatments with vermiculite showed superior results for the index of speed germination, average of seedling length and relative frequency of germination, whereas the best result for percentage of germination was verified with Tropstrato® substrate. In the greenhouse, the use of Tropstrato® was better for the index of speed germination and average of seedling length. The storage test showed that the treatments stored for fourteen days have the best averages considering the variables analyzed. The findings reinforce the role of the substrate and moisture maintenance in maximizing vigor and emergence. Our results contribute to strategies of conservation and production of E. edulis seedlings, supporting ecological restoration initiatives and the sustainable use of the species.

RESUMO

Palavras-chaves
palmito Juçara
pericarpo
substrato
tetrazólio

Avaliação da Germinação e Armazenamento de Sementes de Euterpe edulis Martius

Euterpe edulis Martius é ecologicamente importante em seu bioma nativo e ameaçado pela extração ilegal de palmito. Apesar da relevância da espécie, ainda há escassez de informações sobre o comportamento fisiológico de suas sementes em diferentes condições de germinação e armazenamento. Assim, o objetivo deste trabalho é avaliar essas condições em sementes de palmito juçara em diferentes ambientes e substratos. O teste de germinação foi realizado em casa de vegetação e sala climatizada e três substratos: Tropstrato Florestal®; areia e vermiculita, considerando a ausência e presença de pericarpo. O teste de armazenamento foi realizado em duas temperaturas, 25°C e 4°C e seis períodos de armazenamento: zero, 14, 28, 42, 56 e 70 dias. O delineamento experimental para ambos os testes foi inteiramente casualizado com quatro repetições e 20 sementes cada. Foram analisados: índice de velocidade de germinação; comprimento médio das plântulas; porcentagem de germinação; frequência relativa de germinação e a média do tempo de germinação. A viabilidade das sementes foi verificada pelo teste de tetrazólio (0,05%). O teste de germinação mostrou que melhores resultados são obtidos com a remoção do pericarpo das sementes. Na sala climatizada os tratamentos com vermiculita apresentaram resultados superiores para o índice de velocidade de germinação, comprimento médio de plântulas e frequência relativa de germinação, enquanto a porcentagem de germinação foi superior com substrato Tropstrato®. Em casa de vegetação o uso de Tropstrato® foi melhor para o índice de velocidade de germinação e comprimento médio de plântulas. O teste de armazenamento mostrou que o armazenamento por quatorze dias apresenta médias superiores considerando-se as variáveis analisadas. Os resultados reforçam o papel do substrato e da manutenção da umidade na maximização do vigor e da emergência das sementes. O trabalho traz resultados que contribuem para estratégias de conservação e produção de mudas de E. edulis, apoiando iniciativas de recuperação ecológica e uso sustentável da espécie.

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INTRODUCTION

Euterpe edulis Mart. is a native species from Atlantic Forest belonging to the Arecaceae family, also known as juçara palm, içara, jiçara, ripa and sweet-palm. The species occurs naturally in the middle stratum of the Ombrophilous Dense Forest and in the Seasonal Deciduous Forest (Bourscheid et al. 2011). Considering the studies of forest silviculture combined with nature conservation, the species is very important for preservation and regeneration of the Atlantic Forest, mainly as a food resource for birds and mammals. This interaction with the fauna collaborates with the dispersion of its seeds, supporting the maintenance of biodiversity from the secondary forests (Campos-Filho and Sartorelli 2015).

The palm tree also represents a source of income to the people who live close to the areas with remaining vegetation as part of the extraction of non-wood forest products (Elias et al. 2016). In the 1930s, an intense exploration of the species began and the most advantageous way of obtaining food from the juçara palm was by knocking down the tree and removing its meristem, sold as palm heart (Milanesi et al. 2013). However, after cutting and removing the apical meristem, the juçara palm has no ability to regrow and ends up dying. Such a scenario reinforces the urgency of developing strategies to ensure its dissemination. Thus, since 2008 the species was included in the official list of species of Brazilian flora threatened with extinction, the National Red List (Martinelli and Moraes 2013).

The seeds' germination occurs if the embryo receives enough water, the lack or excess can affect germination according to the desiccation tolerance of each species (Kerbauy 2019). The seed of juçara palm is recalcitrant, therefore, below a certain degree of humidity, it dehydrates and physiological damages occur, affecting the germination rate. In addition, the seed does not tolerate low temperatures and prolonged storage, which result in loss of seeds' viability. These characteristis affect ecological restoration once they unable the stocking of seeds in nurseries for long periods. So, it is important to look for alternatives to increase the germination rate of juçara seeds by overcoming seed dormancy (Ribeiro et al. 2015). Also, the mucilage adhered to the seeds becomes a substrate to microorganisms' development which interfere in the radicle emission, therefore, removing the pericarp and its mucilage may increase the chances of germination and the vigor of the seedlings (Bezerra et al. 2015). So, considering juçara palm as an endangered species, it is extremely important to know the behavior of its seeds during the storage.

In the process of seed germination, factors of the substrate such as structure, aeration and capacity of water retention can vary from one to another, interfering in the process of germination and seedling development. A substrate to be considered ideal should have characteristics such as good capacity of water retention, distribution of particles that allow good aeration and low cost to the producer (Torres et al. 2013).

The aim of the present work is to evaluate the germination behaviour and development of seeds of *E. edulis* considering that presence of pericarp, substrates and temperature may interfere in the sucess of seedling formation. We tested absence and presence of pericarp, three types of substrates and two environmental conditions. Also, to analyze the storage of seeds submitting them to two environments with different temperature during periods from zero to 70 days.

MATERIAL AND METHODS

The fruits of *E. edulis* Mart. were ramdomly collected from a matrix located in the city of Curitiba, Paraná, Brazil, coordinates 25°30'20.3"S 49°16'27.9"W according to fruit avaiability and harvesting. The experiments were set in the nursery and at the laboratory of Forest Biotechnology (BiotecFlor), Department of Forest Sciences, Federal University of Paraná.

According to BRASIL (2009) there are regulations for seed germintation, depending on the species analyzed. Considering this document we adapted the rules to test some conditions for the species. The germination test was carried out combining three factors: 1. absence and presence of pulp and pericarp (Figure 1A); 2. two environments: greenhouse (Figure 1B) and acclimatized room (Figure 1C); 3. three substrates: Tropstrato Florestal®; medium grained sand and fine expanded vermiculite. The acclimatized room is located at the BiotecFlor, with temperature of 25°C ± 2°C, photoperiod of 16/8 hours and manual irrigation being performed three times a week. The greenhouse is located at the BiotecFlor nursery with automated nebulization, relative humidity around 90% and temperature of 25°C ± 2°C. The treatments were designed in a completely randomized scheme with four replications of 20 seeds each BRASIL (2009). The seeds' pericarp was removed manually using a serrated anatomical clip (Figure 1D) and the seeds were placed in 35x20 cm aluminum pots containing the different substrates.

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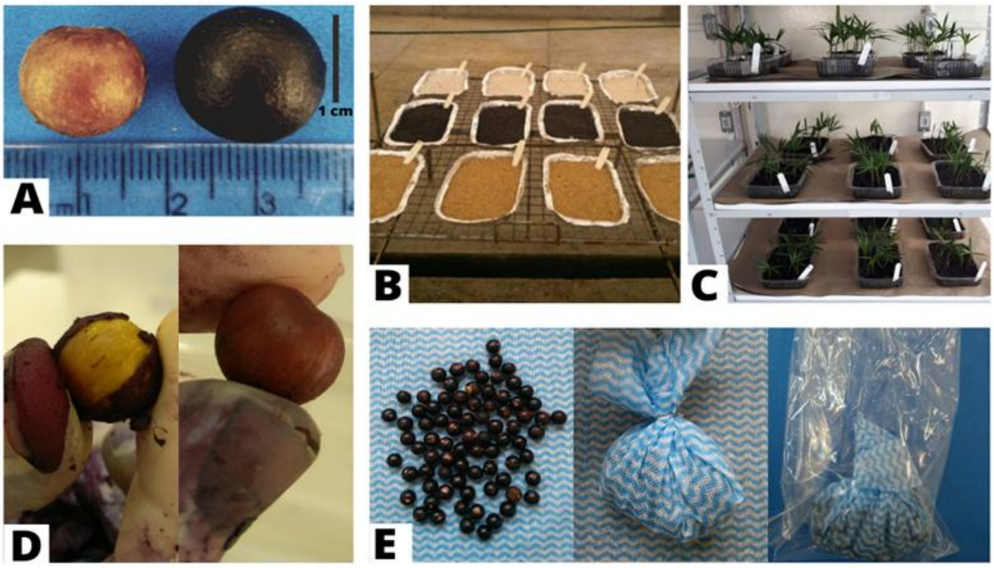

Figure 01 – Seeds of *Euterpe edulis* Martius. **A.** Absence and presence of pericarp and pulp in the fruit; **B.** Greenhouse, substrates to test seeds' germination; **C.** Acclimatized room, substrates to test seeds' germination; **D.** Pulp and pericarp removed by mechanical scarification; **E.** Wet chamber with absorbent tissue and plastic bags used for the storage test.

The evaluations were performed during seven months and the variables analyzed were: average of seedling length (cm); percentage of seed germination; relative frequency of germination and index of speed germination (IVG) using the formula proposed by Maguire (1962). The seed was considered germinated when the integument was ruptured and the epicotyl was emitted over the substrate surface. The height of the seedlings was measured weekly with a millimeter ruler considering since the base of the stem until the tip of the leaf.

After the results of the germination test, a seed storage test was carried out in two places with temperatures of 25°C and 4°C considering room temperature and refrigeration. The temperatures were combined with storage days: zero, 14, 28, 42, 56 and 70 days. Fruits with pericarp were wrapped in absorbent tissue, placed in plastic bags to test a possible wet chamber (Figure 1E) and disposed at the acclimatized room with a temperature of 25°C ± 2°C and inside the refrigerator at 4°C ± 2°C, maintaining humidity by manual irrigation three times a week. Same as germintation test 960 seeds were used in total.

Considering each storage period, the seeds were placed in plastic pots of 25x15x7 cm containing Tropstrato® substrate for germination. The pericarp was previously removed using a serrated anatomical clip. After seven months, the storage test was evaluated, and the variables analyzed were: index of speed germination; average of germination time; average of seedling length (cm) and percentage of germination. The experiment was designed in a completely randomized scheme with four replications and 20 seeds per plot.

The germination test was installed in a triple factorial: 2 environments x 3 substrates x 2 (absence or presence of pericarp). The storage test was installed in double factorial: 2 environments x 6 storage periods. The homogeneity of the data was verified by Bartlett test, followed by the analysis of variance and means' comparison by Tukey with confidence level of 95% using the Sisvar® software (Ferreira 2011).

For germination and storage experiments, the tetrazolium test was performed using 50 seeds of each treatment sectioned longitudinally in order to remove the embryo, immersed in 0.05% triphenyl tetrazolium chloride solution (Oliveira et al. 2014). The embryos were placed in an incubator at 40°C during three hours and those with reddish color were considered as viable embryos.

RESULTS AND DISCUSSION

Considering the total of 960 seeds evaluated, the presence of pericarp adhered to the seed contributed 1.25% to the germination percentage and the removal of the pericarp 27.08%. Thus, the seeds with the pericarp were not statistically evaluated due to the low percentage of germination.

Considering that the absence of pericarp showed better results for seed germination it is important to highlight that the oleaginous pulp kept around the fibrous endocarp covering the seed contributed to reduce the index of speed germination. As a rich substrate for microorganisms' development, it can

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also affect the final percentage of seedling emergence (Beckmann-Cavalcante et al. 2012). Mechanical removal of parts of the fruit is already recommended for other genera belonging to the Arecaceae family in order to accelerate and standardize the germination process (Meerow 2004). Some authors observed a positive effect of this technique on seeds of certain palm trees, such as *Syagrus schizophylla* (Pivetta et al., 2005).

Combined with the advantages of increasing the percentage of germination and reducing possible contamination, the method of removing the pericarp and other parts adhered to the seed have positive impacts on rural communities. In the region of Guaraqueçaba (PR) the pulp represents a versatile food with high nutritional power. Consumed by the inhabitants, it stimulates local commerce and enables projects using the pulp from seeds as the main raw material for the preparation of other products (Esteves et al. 2016).

Germination test showed that the best responses substrate-dependent varied according to the environmental condition (greenhouse or acclimatized room) and to the variable analyzed. The index of speed germination exhibited the best results with Tropstrato® for the greenhouse, and vermiculite was superior for the acclimatized room also considering the relative frequency of germination, the average of seedling length and the percentage of germination (Table 1).

Table 01 – Index of speed germination (%), relative frequency of germination, average of seedling length (cm) and percentage of seed germination resulted from interaction between greenhouse and acclimatized room, and substrates (Tropstrato Florestal®; fine expanded vermiculite and medium grained sand) of seeds from *Euterpe edulis* Martius. GH = greenhouse. AR = acclimatized room.

Substrate	Index of speed germination		Relative frequency of germination		Average of seedling length		Percentage of seed germination	
	GH	AR	GH	AR	GH	AR	GH	AR
Vermiculite	0.37^{ab}B	0.96^{a}A	0.69^{a}A	0.84^{a}A	8.32^{a}A	9.89^{a}A	8.32^{a}A	9.89^{a}A
Tropststrato®	0.67^{a}A	0.11^{b}B	0.89^{a}A	0.38^{b}B	11.64^{a}A	3.03^{c}B	11.64^{a}A	3.03^{c}B
Sand	0.22^{b}A	0.19^{b}A	0.73^{a}A	0.40^{b}B	6.21^{a}A	6.21^{b}A	6.21^{a}A	6.21^{b}A
CV (%)	46.39		25.47		21.41		23.28	

Means followed by the same vertical letter for substrate and the same horizontal capital letter for environments do not differ statistically by Tukey test (5%).

The substrate is another important factor to the germination success. Using three different substrates we observed different responses, and once Tropstrato® was better for the greenhouse experiment, vermiculite was better for the acclimatized room. Seeds in the greenhouse were under automatized irrigation and the relative humidity of the air remained around 90%, unlike the acclimatized room, where the seeds were manually irrigated three times a week, so vermiculite was good to keep the moisture, but humidity was better controlled in the greenhouse.

Vermiculite also has an important feature that makes it attractive to agricultural activities, which is its capacity of water retention and it usually represents an adequate agent to improve the physical qualities of the soil. The advantages are that vermiculite is easy to obtain, it is economically viable, it has a homogeneous chemical and granulometric composition and low density (Martins et al. 2012).

Once water availability influences as one of the main factors for seed germination, a stimulant effect is generated by the pre-imbibition treatment influencing the final germination percentage. As observed by Honorio et al. (2017) it is possible that the capacity of water retention of each substrate influenced the results, since the vermiculite in the drier condition of the acclimatized room has a greater capacity of water absorption and provided higher index of speed germination, relative frequency of germination and average of seedling length. This was not confirmed in the greenhouse, a more humid environment in which Tropstrato® was superior, these results confirm the importance of water availability for plant development and seed germination.

Sand used as substrate, was the less efficient and it is associated to its porosity. Sand has macroporosity, high permeability and a low number of intra-aggregates, which correspond to the soil micropores, responsible for slower emptying and water conduction through the soil. Thus, the sand does not present satisfactory characteristics of water retention to maintain the necessary moisture for seed germination. Similar results were observed with the germination of the Australian royal palm (*Archontophoenix alexandrae* H. Wendl. & Drude), where sand showed lower germination results compared to

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germination in fine vermiculite moistened with water (Martins et al. 2011).

Considering the index of speed and the percentage of seed germination, no statistical difference was found among the storage periods at 25ºC neither at 4ºC. Considering both temperatures the averages of germination time were between 76 and 100%. The average of seedling length showed the lower results at 28 days (25ºC) and at 56 days (4ºC), and higher responses were observed at 70 days of storage at 4°C and 25°C (Table 2).

Table 02 – Data of index of speed germination (%), average of germination time, average of seedling length (cm) and percentage of seed germination resulted from interaction between days (14, 28, 42, 56 and 70 days) and temperatures (25 and 4ºC) of storage of seeds from Euterpe edulis Martius.

Storage	Index of speed germination		Average of germination time		Average of seedling length		Percentage of seed germination	
	25°C	4°C	25°C	4°C	25°C	4°C	25°C	4°C
No storage	0.15^{ab}	-	82.83^{a}	-	12.70^{a}	-	71.25^{ab}	-
14 days	0.16^{a}A	0.14^{a}A	84.38^{a}A	95.24^{a}A	11.21^{ab}A	11.95^{ab}A	77.50^{a}A	67.50^{a}A
28 days	0.14^{abc}A	0.10^{ab}A	81.75^{a}B	100.50^{a}A	8.61^{b}B	11.81^{ab}A	58.75^{abc}A	55.00^{a}A
42 days	0.10^{bc}A	0.10^{ab}A	89.95^{a}A	92.10^{a}A	12.70^{a}A	10.36^{b}B	48.75^{bc}A	62.50^{a}A
56 days	0.06^{c}A	0.09^{ab}A	75.92^{a}A	82.29^{a}A	11.76^{a}A	9.72^{b}A	41.25^{c}A	56.25^{a}A
70 days	0.06^{c}A	0.07^{b}A	81.69^{a}B	97.37^{a}A	14.24^{a}A	14.40^{a}A	46.25^{c}A	51.25^{a}A
CV (%)	25.88		11.24		12.08		19.56	

Means followed by the same vertical letter for days of storage and the same horizontal capital letter for temperatures do not differ statistically by Tukey test (5%).

About the percentage of germination, at 25°C the best result was verified with 14 days of storage, whereas at 4°C there was no statistical difference among the storage periods. Also, there was no difference between the two temperatures tested for the days of storage. The evaluation of storage time in two environments showed that the index of speed germination at temperature of 25°C and 4°C presented superior results after 14 days of storage (Table 2).

Regarding the experiment comparing two temperatures there was no difference in germination between acclimatized room and inside the refrigerator, but we observed differences according to the days of storage. The increase in the storage period reduced the index of speed germination, possibly because the storage causes a decrease in moisture, and since it is a recalcitrant seed, the loss of viability occurs when there is a drop in moisture (Cursi and Cicero 2014).

In recalcitrant seeds, subcellular water is associated with macromolecular surfaces ensuring, in part, the stability of membranes and macromolecules. Structural loss of water in recalcitrant seeds causes changes in metabolic systems and cell membranes, resulting in the process of seed deterioration. In the present study the critical water threshold seems to have been exceeded after 14 days. This deterioration initially leads to a reduction in several performance and vigor attributes, and results in the loss of capacity of germination, which was also verified in studies involving the drying of *Euterpe* spp. (Nascimento et al. 2010).

It is important to emphasize that seed deterioration occurs from a threshold of moisture loss. Cursi and Cicero (2014) tested the drying of *E. edulis* seeds and found that the germination percentage is affected by moisture and water content lower than 25.6% causes seeds' death. The authors also observed that in a drying process of seeds, the volume occupied by the embryo is reduced, what can be associated to the physiological performance of seeds to germinate.

Since loss of moisture in seed is related to the storage time, the longer this time, the greater the chances of seed deterioration. The main consequence of this deterioration is the total loss of germination, preceded by the presence of abnormal seedlings and reduction in speed of germination and seedling emergence (Matthews 1985). On the other hand, the average of germination time may

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also be influenced by the environment besides the storage period. In the present work we observed that even with long storage periods, up to 70 days, the seeds did not lose viability, reaching about 40% of germination.

Considering the average of germination time, the means are close to each other and may be a result from the removal of the pericarp, which contributed to greater homogeneity in germination (Bezerra et al. 2015). The slowness observed in the averages of germination time are associated with the presence of microorganisms in the stored seeds. During the storage period, we observed an increase of fungi in the seeds inside the humid chambers. This fact usually occurs during the storage of *Euterpe* spp. due to the water content of seeds and the deterioration process in the absence of fungicide treatment (Nascimento et al. 2010).

Although no statistical differences were evidenced for average of seedling lengths considering the temperatures, the analysis of this characteristic is important once it is related to seed vigor and since vigorous seeds have well-developed seedlings. Also, seedlings with a fully developed root system can express the vigor of the seeds that originated them.

Percentage of germination showed no difference between temperatures, but best results were found at 25ºC with 14 days of storage (77.50% of seed germination and 0.16 as index of speed germination) and once again, the condition of moisture maintenance is one of the main factors for the storage of seeds. However, with 14 days of storage the highest averages of germination were observed, and may have occurred because the seeds were submitted to a pre-imbibition process that guaranteed part of the seed moisture. The humidity of the humid chambers was maintained and influenced the seed germination rate.

A decreasing in the percentage of germination was observed according to the increasing of days in the storage periods and there was a reduction of 31.25% in seed viability. According to the tetrazolium test, at 4°C ± 2°C there was the same tendency of seed viability until 28 days of storage (Figure 2).

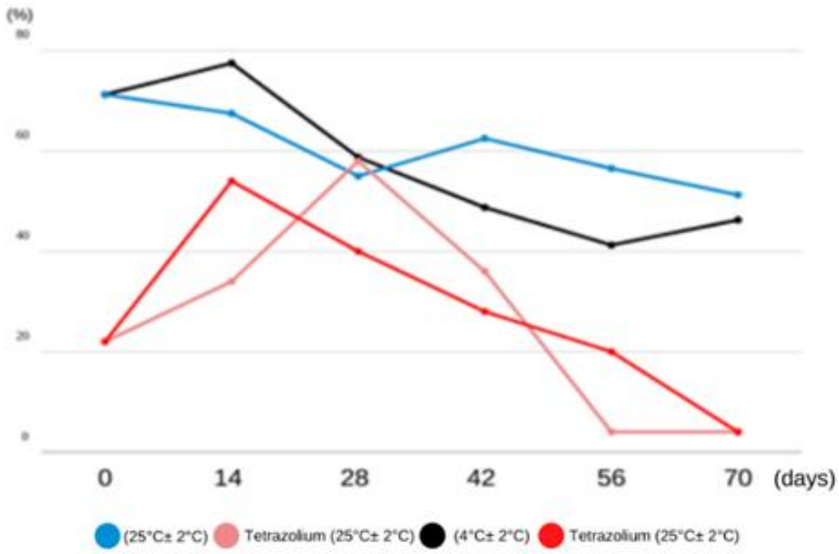

Figure 02 – Percentage of germination of Euterpe edulis Martius seeds according to storage periods considering two environments with temperatures of 25 and 4 ºC and tetrazolium test considering periods of storage.

The tetrazolium test showed no relation to the germination percentage in stored seeds. It occurred due to the conditions of tetrazolium test and, therefore, adjustments are recommended for future tests, such as increasing the concentration of tetrazolium, the time of exposure and reducing the temperature of immersion (Oliveira et al. 2014). In this way, adjustments to the tetrazolium method are essential for recalcitrant species such as *E. edulis*.

Conclusions

The germination of *Euterpe edulis* seeds was favored by pericarp removal, high relative humidity, and the use of substrates with good water retention, such as vermiculite and Tropstrato Florestal®. Storage for 14 days at 25 °C or 4 °C resulted in the best germination rates. These results support seedling production and species conservation.

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