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Scientific Article

Biometry and storage of Triangle Palm seeds

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Abstract

Studies on biometry are considered important tools for understanding the germination process of seeds. Moreover, the ex-situ conservation of palm seeds can cause loss of viability over time. The present study aimed to evaluate the biometric characterization and the effect of storage on the seed germination of the Triangle Palm (*Dypsis decaryi*), a widely used species in Brazilian landscaping. Two independent studies were conducted: I) biometric characterization of diaspores and II) effect of storage in seed germination. For biometry, a sample of 100 diaspores was used and the length, width and thickness were measured. Biometric data were analyzed using descriptive statistics and correlation analysis. The seeds were packed in transparent plastic bags kept in a refrigerator (20 ± 2 ºC and 80% relative humidity) for storage evaluation. The experimental design was entirely randomized. The treatments consisted of six storage periods (0 - control, 30, 60, 90, 120 and 150 days); there were four repetitions and 25 seeds per plot. The germination percentage and germination speed index were evaluated. Diaspores showed little variation in their biometric characteristics, with a mean of 18.39 mm in length, 16.10 mm in width and 15.96 mm in thickness. There was significant and positive correlation between all biometric characteristics. It was observed that germination remained stable until 30 days of storage (90%), with a gradual percentage decrease in later periods. The seed storage allowed to maintain its viability and longevity with the ability to germinate, reaching a germination percentage of 63% after 150 days of storage.
Keywords: *Dypsis decaryi*, longevity, morphometry, ornamental plant.

Resumo

Biometria e armazenamento de sementes de palmeira-triangular

Estudos sobre a biometria são consideradas ferramentas importantes para a compreensão do processo germinativo das sementes. Além disso, a conservação *ex-situ* de sementes de palmeiras pode causar perda de sua viabilidade ao longo do tempo. Objetivou-se com o presente trabalho avaliar a caracterização biométrica e o efeito do armazenamento na germinação de sementes da palmeira triangular (*Dypsis decaryi*), espécie muito utilizada no paisagismo brasileiro. Foram conduzidos dois estudos independentes: I) caracterização biométrica dos diásporos e, II) efeito do armazenamento na germinação de sementes. Para a biometria, foi utilizada uma amostra com 100 diásporos sendo realizadas as medidas do comprimento, largura e espessura. Os dados biométricos foram analisados por meio de estatística descritiva e análise de correlação. As sementes foram acondicionadas em embalagem de saco plástico transparente dentro de refrigerador (20 ± 2 ºC e 80% de umidade relativa do ar) para avaliação do armazenamento. Adotou-se o delineamento experimental inteiramente casualizado. Os tratamentos consistiram em seis períodos de armazenamento (0 - controle, 30, 60, 90, 120 e 150 dias); foram quatro repetições e 25 sementes por parcela. Avaliou-se a porcentagem e o índice de velocidade de germinação. Os diásporos apresentam pequena variação nas suas características biométricas, com média de 18,39 mm de comprimento, 16,10 mm de largura e 15,96 mm de espessura. Houve correlação significativa e positiva entre todas as características biométricas. Observou-se que a germinação se manteve estável até 30 dias de armazenamento (90%), com queda gradual na porcentagem nos períodos posteriores. O armazenamento das sementes permitiu manter a sua viabilidade e longevidade com capacidade de germinar, obtendo percentagem de germinação de 63% após 150 dias de armazenamento.
Palavras-chave: *Dypsis decaryi*, longevidade, morfometria, planta ornamental.

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Introduction

Popularly known as triangle palm, *Dypsis decaryi* (Jum.) Beentje & J. Dransf. is an ornamental species native to the dry savannas of southern Madagascar, widely used in parks and gardens for its exuberance (Lorenzi et al., 2004; Bao et al., 2010). Its main ornamental attributes are the long blue-green or gray-green leaves, arranged in three different directions that resemble a triangle, characteristics that allow its cultivation to be very popular in several gardens and parks around the world (Garreta and Marfil, 2020).

As the majority of palm species, its propagation is predominantly done by seeds that can germinate a few days after sowing (Luz et al., 2008; Bao et al., 2010), however, they show sensitivity to desiccation in water contents below 20%, with critical content ranging between 10 and 15% (Batista et al., 2016). In this perspective, it is important to understand better the physiology of seeds to assist in their conservation, initial plant development, as well as to evaluate genetic variation in natural populations (Ferraz et al., 2021).

A major factor to be considered is the biometric characterization of fruits and seeds. The biometric characteristics such as size and weight are important for sorting seeds into classes, and those with better physiological quality, or with higher percentage of germination and vigor, usually have larger size or density and well-formed embryos and bigger quantities of reserves, behavior that highlights the size or density of the seed as a tool in seedling production, standardizing the emergence of seedlings and the seedlings size and vigor (Carvalho and Nakagawa, 2012). Biometry also assists in genetic variability assessment within and between populations and allows to establish if variability is related to edaphoclimatic factors (Zuffo et al., 2019), as well as assisting in identifying the optimal harvest point for the fruits (Araújo et al., 2022).

Another relevant aspect to be highlighted is the conservation of seeds, considering that as soon as their physiological maturity is reached, their vigor suffers an inevitable decrease with the deterioration process, which is an inevitable and irreversible process (Marcos Filho, 2015). During storage some factors can affect the longevity and physiological quality of seeds, such as seed water content, temperature and relative humidity and the type of packaging (Solberg et al., 2020).

Seed water content can lead to high respiration rate and energy reserve consumption (Ullmann et al., 2018). Temperature and relative humidity have a role in accelerating or reducing seed deterioration (Zhang et al., 2021). As well as the type of packaging during storage can cause changes in the water content of seeds (Capilheira et al., 2019), since permeable packaging allows the gas exchange between the seed and the environment, while hermetic packaging restricts this exchange (Ferreira and Bazzo, 2020). The conservation of genetic resources for future uses can be achieved through various methods; however, the best results are often obtained with traditional (in situ and ex situ conservation) and modern (in vitro conservation and cryopreservation) methods, or even through a combination of methods (Walters, 2015; Streczynski et al., 2019).

The ex-situ conservation of palm seeds can lead to viability loss over time (Soares et al., 2022) due to the challenging storage requirements of low temperature and relative humidity for these seeds (Meerow and Broschat, 2015). Therefore, it is necessary to conduct research that develops methods to determine characteristics that aid in the propagation process of different palm species, as well as evaluate seed viability, enabling proper classification regarding storage capacity and the possibility of ex-situ conservation. Also, the biometric information will help to classify the pattern of shape, size and mass of the seeds of this species.

Given the above, the present study aimed to evaluate the biometric characterization and the storage effect on the germination of *Dypsis decaryi* seeds.

Materials and Methods

Plant material

Mature fruits of *Dypsis decaryi* were harvested from three mother plants in the gardens of Universidade Estadual Paulista, Câmpus de Jaboticabal, São Paulo State, with the help of high-altitude pruning shears. The harvest was performed after verifying the beginning of fruit detachment from the branches, which showed a typical aspect of maturation evidenced by the whitish-yellow color of the epicarp (Lorenzi et al., 2004), in February, in the season of the year understood as summer in the collection region.

The fruits were packed in plastic boxes and transported to the Horticultural Seeds Laboratory (UNESP/FCAV), where the seeds were treated. After their preparation, two trials were performed, the first one aimed to evaluate the biometric characterization and the second one, the effect of storage on seed viability by the germination test.

Experiment 1: Biometric characterization

In the laboratory, the fruits were de-pulped (removal of the epicarp and mesocarp) by manual rubbing with a steel mesh sieve (6 mm) until the pulp was completely detached. Subsequently, the diaspores were soaked in sodium hypochlorite solution (2%) for 10 minutes, using a commercial solution (2.5% active chlorine) for asepsis, and then rinsed under running water to eliminate chlorine residues and dried at room temperature under shade for a period of 24-hours under a laboratory bench with diffused light coming from the windows.

Afterwards, a random sample of 100 diaspores was used for biometric characterization. The diaspores were measured for length, diameter and thickness using a digital caliper accurate to 0.01 mm (Western® PRO DC-6). The batch underwent a pre-selection process, in which seeds with significantly reduced size, deformed, or damaged were discarded, aiming at lot standardization (Neves et al., 2019). The number of samples was determined as indicated in the literature in research related to the biometry of fruits and seeds of different plant species (Santos-Moura et al., 2019; Bezerra et al., 2022; Souza et al., 2022).

For each characteristic, data were submitted to descriptive statistical analysis, obtaining the lowest value, highest value, mean, variance, standard deviation, standard error of the mean, asymmetry, kurtosis and coefficient of variation. Values of reference adopted for the asymmetry coefficient were: S < 0, left skew distribution, and S > 0, right skew distribution. For the kurtosis coefficient were: K>3 ("leptokurtic"), more "tapered" distribution than normal, and K<3 ("platykurtic"), flatter distribution than normal, according to what was proposed by Pinheiro and Ferreira (2018).

Relative frequency was performed by frequency distribution and plotted in frequency histograms using Microsoft Excel® 365 software (2211 Build 16.0.15831.20098 version). Pearson's correlation coefficient (r) ($p < 0.05$) was also calculated among the variables.

Experiment 2: Effect of storage on seed viability

For storage, the seeds were sealed manually in transparent plastic bags without vacuum, to avoid changes in humidity, and placed in a refrigerator (20 ± 2 ºC and 80% relative humidity) during the 150 days storage period. Such conditions of temperature and relative humidity were chosen due to the recalcitrant behavior of the seeds of this species. In addition to this factor, as the packaging used did not allow the exchange of water vapor with the environment, thus not having influence of ambient humidity on seed storage.

Water content

The water content of the seeds was determined for each storage period by the drying oven method with forced air circulation, with two subsamples of 10 seeds maintained at 105 ± 3 °C for 24-h (Brasil, 2009), results were expressed in percentage of wet basis (% w.b.).

Experimental design

Experimental design was entirely randomized (DIC). Treatments consisted of six storage periods (0 - control, 30, 60, 90, 120 and 150 days); there were four repetitions of 25 seeds per plot. At time zero the seeds were submitted to the germination test immediately after their ripening and drying in the shade. After each storage period the seeds from each treatment were subjected to the germination test. So that they had the same conditions at the time of setting up the tests, a sample with 100 seeds was removed from the package, still inside the refrigerator, and then the package was manually sealed again.

Germination test conditions

Sowing was performed inside transparent "gerbox"-type plastic boxes (11 × 11 × 3 cm) with lids filled with medium-grained vermiculite as substrate, which was previously moistened with distilled water. This substrate was chosen because it is one of the most used in research on palm seed germination (Luz et al., 2008; Batista et al., 2016; Souza et al., 2022). The substrate was maintained at 100% of its water retention capacity with no need for water replacement during the evaluation. The boxes containing the seeds were placed on laboratory benches under room temperature and ambient lighting, in agreement with what was proposed by Luz et al. (2008), who reported that the seeds of *Dypsis decaryi* can be easily germinated under laboratory conditions.

Variables evaluated in the germination test

The germination evaluation was performed daily, and the seeds that emitted the germinative bud were considered germinated, until there was stabilization of germination for all treatments, being 20 days for both evaluation periods. The emission of the germinative bud was considered as a botanical criterion for evaluating germination because it is the most used in research with palm seeds. From the collected data were calculated: germination percentage (G%), (Brasil, 2009) and the Germination Speed Index (GSI) Maguire (1962).

Statistical analyzes

The data obtained were submitted to the residual normality test and homogeneity of variance, and the germination percentage was previously transformed to arc sine $(x/100)^{1/2}$ for statistical analysis. When normal, the data were submitted to analysis of variance (ANOVA) and, when significant, were analyzed by polynomial regression at 1% ($p < 0.01$) as a function of the storage period. All analyses were performed in the statistical software AgroEstat® 1.1.0.711 version (Barbosa and Maldonado Junior, 2015). Germination distribution over time graphs were also made.

Results and Discussion

Biometric characterization of seeds

Dypsis decaryi diaspores showed an average of 18.39 mm in length, 16.10 mm in diameter and 15.96 mm in thickness (Table 1). The results for length and diameter are close to the ones found by Bao et al. (2010), who reported mean values of 16.01 and 17.10 mm and by Batista et al. (2016), who reported mean values of 18.4 mm of width and 16.1 mm of diameter for the same species.

Table 1. Biometric characterization of *Dypsis decaryi* diaspores.

Statistical Parameters	Length	Diameter	Thickness
	----------mm----------		
Lowest value	17.00	14.70	14.20
Highest value	19.50	17.20	17.10
Mean	18.39	16.10	15.96
Variance	0.24	0.26	0.31
Standard deviation	0.49	0.51	0.55
SEM	0.04	0.05	0.05
Asymmetry	-0.45	-0.31	-0.64
Kurtosis	-0.10	0.18	1.02
CV (%)	2.66	3.18	3.50

SEM: Standard error of the mean; CV (%): Coefficient of variation as percentage.

The variations found in the biometric characteristics of *Dypsis decaryi* diaspores are justified by the fact that they occur between individuals of this species and may be the result of genetic variability promoted by the action of environmental factors, since each species presents diversity due to its ecological plasticity, evidenced by the presence of these individuals in different regions (Santos-Moura et al., 2019; Souza et al., 2022). Furthermore, morphological patterns defined during seed maturation may vary, since the mother plant will generate seeds of different sizes depending on the resources available for their development (Bezerra et al., 2022), as observed for *Mauritia flexuosa* palm (Serpa et al., 2022).

The thickness showed the highest value (0.55) for standard deviation (Table 1), indicating greater sample variance among the characteristics evaluated. The coefficient of variation (CV) and standard error of the mean (SEM) showed low values for all biometric characteristics. Similar CV results for length and diameter of diaspores for the same species were found by Bao et al. (2010) in which the values were 2.87 and 7.01%, respectively. It should be noted that both studies used a sample composed of 100 diaspores, which may be an indicative that the sample number was taken from the population accurately and has low variability.

As for asymmetry (Table 1), there was a left skew distribution for all characteristics; however, the characteristic thickness showed the most negative coefficient (-0.64), indicating a high frequency of diaspores in the sample with higher thickness values. For kurtosis (Table 1), the data showed a platykurtic distribution ($K < 3$), with diameter and thickness showing positive values. It is also possible to observe that the values obtained are close to zero, setting an approximately normal distribution for the analyzed characteristics. For the *Geonoma máxima* subsp. *Chelidonura* palm, Pinheiro and Ferreira (2018) observed a right-skewed distribution for diameter and length of diaspores, and also a flatter distribution than normal ($K < 3$) only for the length of diaspores, indicating a smaller range of data distribution.

The frequency distribution for all biometric characteristics of the diaspores was grouped into eight classes (Figure 1). The highest frequencies for length are in the classes 18.6-18.9 mm (30%), 18.28-18.6 mm (22%) and 17.96-18.28 mm (17%) (Figure 1A). The diameter showed higher frequencies in classes 15.98-16.3 mm (30%), 16.3-16.62 (23%), and 15.66-15.98 mm (Figure 1B). For thickness, frequencies were grouped mostly within the classes 15.68-16.05 mm (35%), 16.05-16.42 mm (23%), and 15.31-15.68 mm (12%) (Figure 1C).

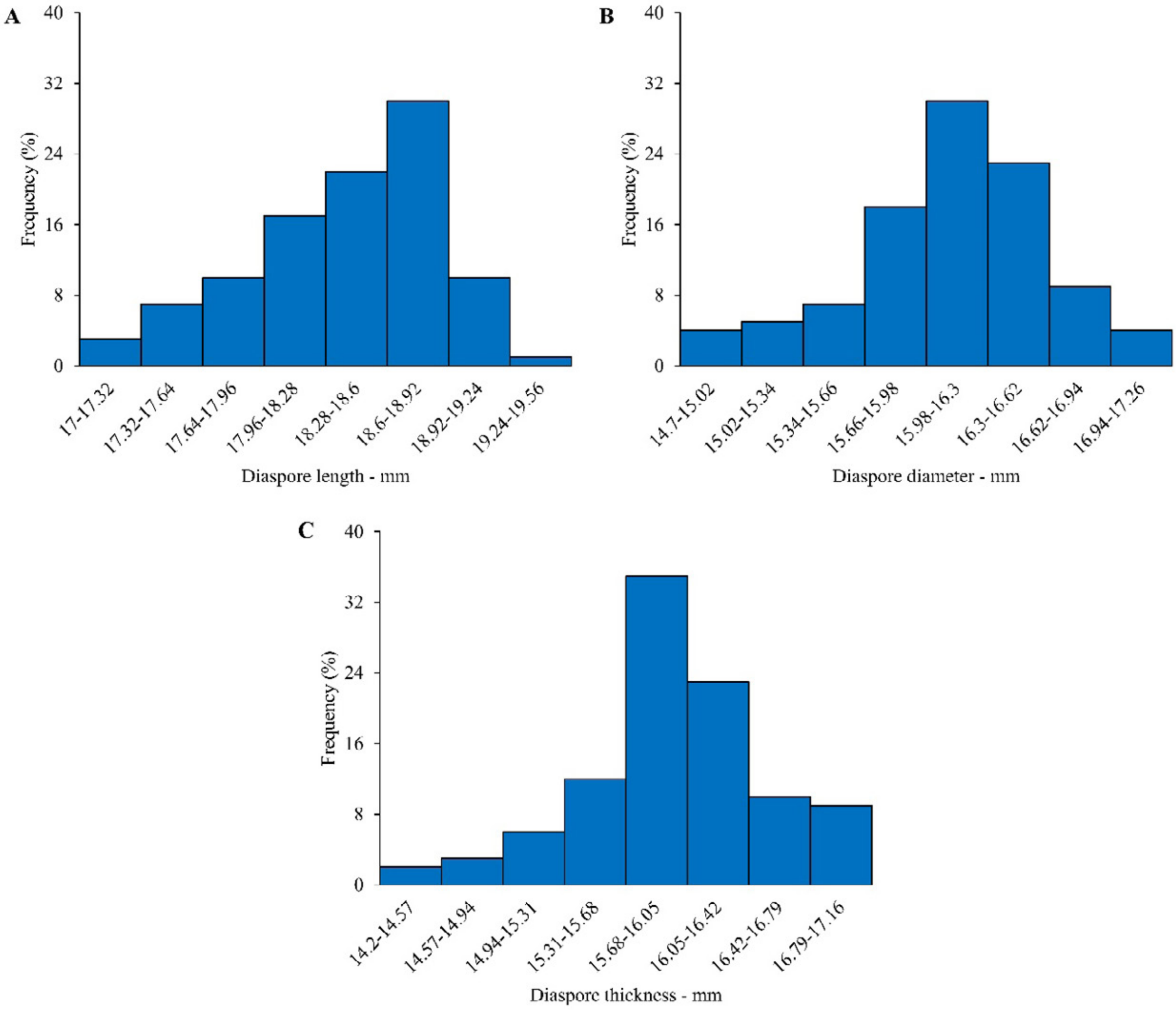


Figure 1. Frequency distribution for biometric variables of *Dypsis decaryi* diaspores.

By establishing fruit and seed classification based on biometric variables such as size, it is possible to standardize seedling emergence, and thus produce seedlings with similar sizes and improve some of its characteristics, since those with better physiological quality, or with a higher percentage of germination and vigor, usually have larger size or density, well-formed embryos and larger amounts of reserves (Carvalho and Nakawaga, 2012).

According to the correlation matrix (Figure 2), there was a significant and positive correlation between the biometric characteristics which involve length, diameter and thickness of *Dypsis decaryi* diaspores, where the highest correlation was observed between length and diameter (0.52). Similar results were obtained by Pinheiro and Ferreira (2018) who observed significant and positive correlation between the biometric characteristics of diaspores of *Geonoma máxima* subsp. *Chelidonura* palm.

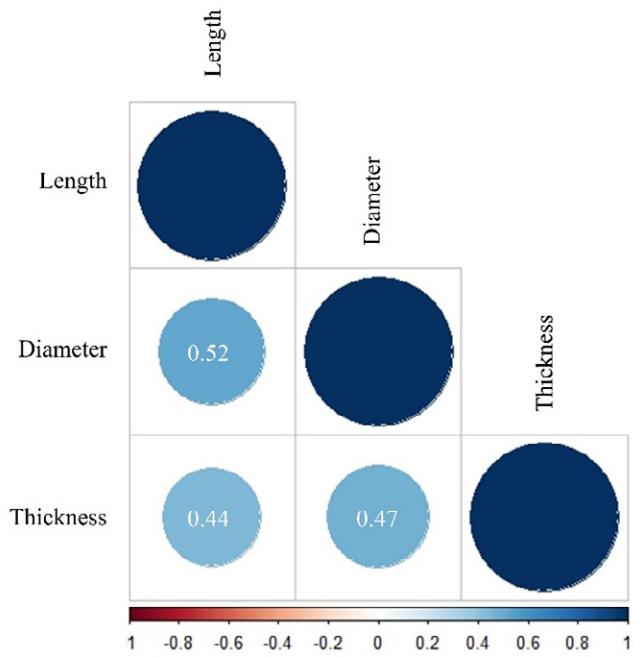
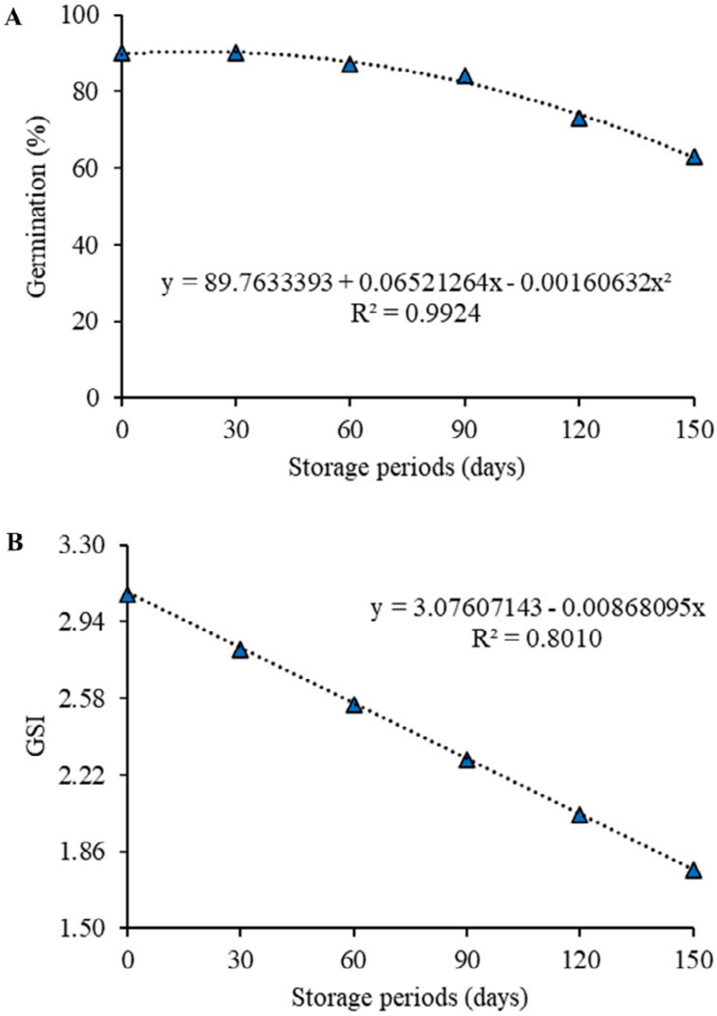


Figure 2. Pearson's correlation (r) between the biometric variables of *Dypsis decaryi* diaspores. Significant at 5% probability.

Effect of storage on seed germination

The initial water content of freshly harvested seeds (storage = 0) was 44.83%, which ranged by a few percentage points in the later periods, being 44.80, 44.59, 43.45, 43.25, and 43.15% for 30, 60, 90, 120, and 150 days of storage, respectively. The water content of the seeds, for each evaluation period, had few changes during storage, which suggests that under the conditions used, the seeds were basically in equilibrium with the environment, in addition to demonstrating the efficiency of the packaging in maintaining moisture the same. Still, the little variation observed in the water content of the seeds during storage may be due to their being hygroscopic, being able to receive and lose moisture depending on their physical state and chemical composition.

The percentage and the germination speed index (GSI) of the seeds decreased through the storage period (Figure 3). In freshly harvested seeds (storage = 0), 90% of germination was observed, remaining stable until 30 days of storage (Figure 3A). In the subsequent periods a decrease in germination was observed with percentages of 87, 84, 73, and 63% for 60, 90, 120, and 150 days, respectively. These results indicate that *Dypsis decaryi* seeds stored in refrigerator (20 ± 2 ºC and 80% relative humidity) maintain their capacity to germinate, reaching a percentage of 63% after five months (150 days) of storage. Therefore, it can be understood that the seeds were stored in an environment that possibly allowed to decrease metabolism and, consequently, deterioration, maintaining their viability during storage, since the germination percentage was only 30% lower when compared to freshly harvested seeds (storage = 0) to those stored for 150 days (Figure 3A).



Significant at 1% probability.

Figure 3. Germination (%) (A) and germination speed index - GSI (B) of *Dypsis decaryi* seeds as a function of storage period.

The GSI showed a quadratic behavior (Figure 3B) similar to the germination percentage, however, there was greater variance between the values found. For freshly harvested seeds (storage = 0) the observed index was 3.07, which decreased to 2.81 soon after 30 days of storage. The decrease in germination speed intensified even more in the later storage periods, reaching the lowest index (1.77) at 150 days, and this may be related to the acceleration of the seed deterioration process (Ratajczak et al., 2019), considering that soon after physiological maturity is reached their vigor suffers a drop with the deterioration process, this being an inevitable and irreversible process (Marcos Filho, 2015).

Chemical degradation of seed compounds occurs during storage (Bewley et al., 2013; Silva et al, 2022), and the decrease in germination speed of seeds, verified in the increase in the GSI (Figure 3B), is the first sign of a performance drop and is usually caused by the rupture of the membrane system, due to the attack of its cellular constituents by free radicals, which generate physiological and biochemical damage in seeds that do not display any mechanisms of repair or maintenance over time, commonly observed in recalcitrant seeds (Marcos Filho, 2015).

For *Archontophoenix alexandrae* palm seeds, the storage in polyethylene bag with vermiculite in a proportion of 10:1 (seeds:vermiculite, v:v), with saturation of 25% water proved to be suitable to maintain their viability and vigor under refrigerated conditions for eight months (Lone et al., 2020). However, *Copernicia alba* seeds stored for one year at 19 °C under 45% relative humidity showed a ≤ 50% reduction in germination rate (Soares et al., 2022).

In general, according to the literature, it is possible to verify that storage under appropriate conditions can reduce the speed of seed deterioration (Silva et al., 2022). In addition, certain factors such as the type of packaging and the initial quality of the seeds can affect the maintenance of the physiological quality of the seeds (Carvalho and Nakagawa, 2012), as observed by Beltrame et al. (2022), in which *Phoenix roebelenii* palm seeds showed a germination percentage of 88% even after desiccation and hermetic storage for 90 days at -20 °C.

When analyzing the distribution and the peak of seed germination over time, it can be observed a variation according to the storage periods (Figure 4). The beginning of germination occurred between the 7th and 9th days after the experiment was initiated. Freshly harvested seeds (storage = 0) and stored for 30 days exhibited the same germination behavior (Figures 4A and 4B). In later periods germination was irregular, being distributed over time as the number of days of storage increased. The beginning of the germination observed in this study are consistent with those reported by Luz et al. (2008) who verified germination on the 4th day after the establishment of the experiment and by Bao et al. (2010) that occurred on the 10th day.

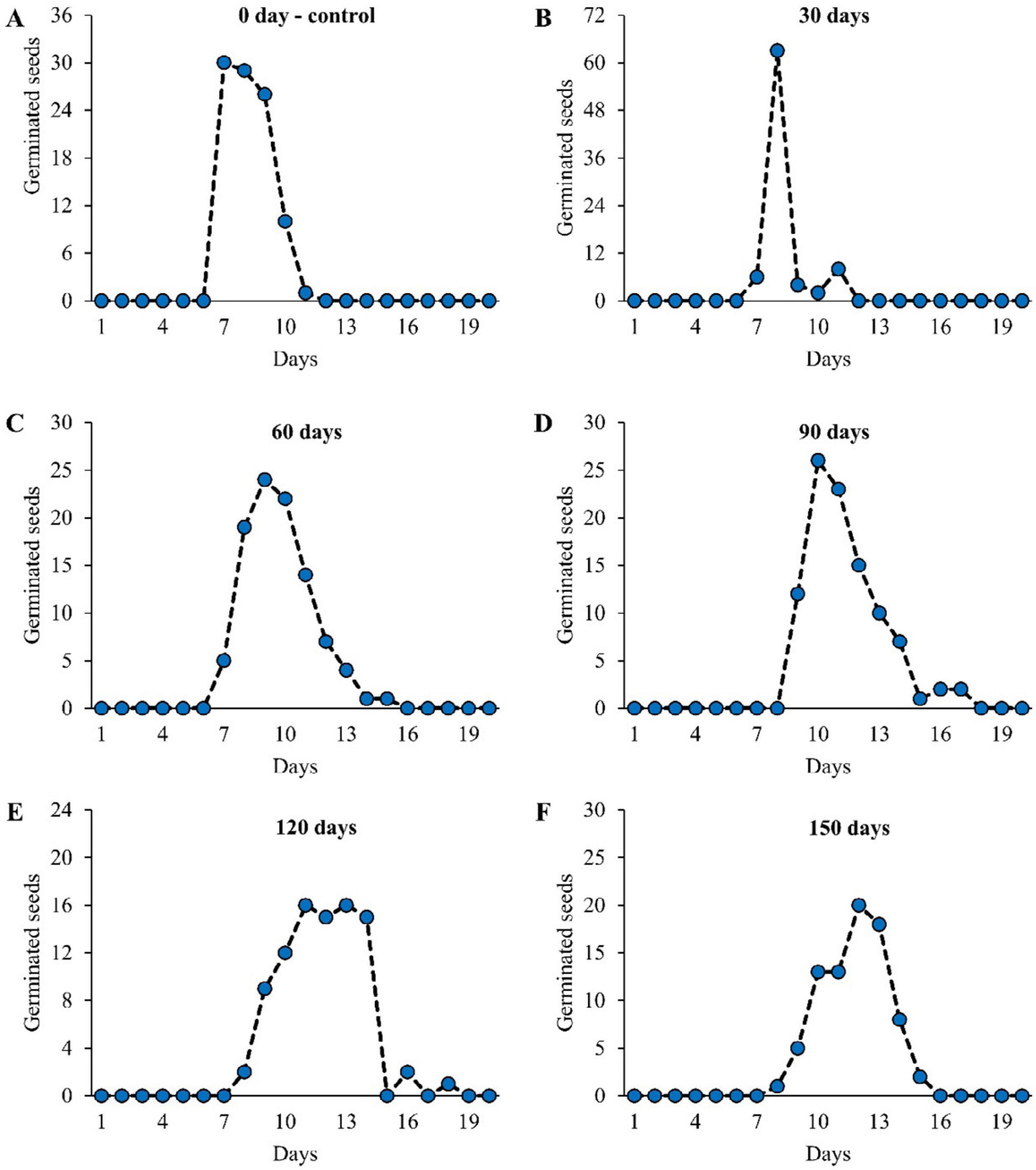


Figure 4. Distribution of seed germination of *Dypsis decaryi*, from a sample of 100 seeds, subjected to different storage periods.

Although the germination process occurred relatively quickly, with stabilization occurring between the 15th and 18th day (Figure 4), germination was uneven, reinforcing the comments of Meerow and Broschat (2015) who report that palm seed germination tends to be slow and uneven, occasioned by several factors such as genotype, maturation stage, and seed treatment, which make the seedling establishment stage long and difficult, especially on a large scale.

Conclusions

Diaspores show little variation in their biometric characteristics, averaging 18.39 mm in length, 16.10 mm in width, and 15.96 mm in thickness.

The percentage and germination speed index of the seeds decrease throughout storage; however, their germination capacity remains above 60% after 150 days of storage in a refrigerator (20 ± 2 ºC and 80% relative humidity).

It is suggested that more studies be carried out so that it can be known to what extent the seeds can be stored without losing their total germination capacity, thus maintaining their viability.

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Author Contribution

AMBS: Conceptualization, data curation, formal analysis, investigation, methodology, software, visualization and writing-original draft. **GRV**: Conceptualization, data curation, formal analysis, investigation, methodology, software, visualization, writing-review and editing and translation. **TSC**: Conceptualization, investigation, methodology, visualization and writing-original draft. **MPP**: Conceptualization, data curation, investigation, methodology, visualization and writing-original draft. **ACB**: Conceptualization, data curation, investigation, methodology, visualization and writing-original draft. **KFLP**: Funding acquisition, methodology, project administration, resources, supervision, validation and writing-review and editing.

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