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Conservation of *Astrocaryum aculeatum* seeds as a function of storage conditions of diaspores

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Abstract

Information on the conservation of *Astrocaryum aculeatum* seeds can contribute to the sustainable management of this species, as commercial harvesting of its fruits is essentially extractivist. Thus, the objective of this study was to evaluate the germination and vigor of *A. aculeatum* seeds based on the storage conditions of diaspores (seeds with endocarp). A completely randomized experimental design was used, with a split-plot arrangement in time and four replications. Diaspores containing seeds with 21.1% moisture content were packaged and stored under the following conditions: a) permeable packaging in a natural environment (air temperature of 27.7 ± 1.5 °C and relative air humidity of 85.0 ± 11.9%); b) impermeable packaging in a natural environment; c) impermeable packaging in a cold chamber (18.0 ± 1.0 °C and relative air humidity of 68.1 ± 6.2%). Seed moisture content, germination, dormancy, and mortality, percentage of seedling emergence, emergence speed index, and mean emergence time were evaluated after different storage periods (0, 2, 4, 6, and 10 months). Storing diaspores of *A. aculeatum* in impermeable packaging in a natural environment effectively preserve seed physiological quality for ten months. Contrastingly, storing diaspores in permeable packaging in a natural environment and in impermeable packaging in a cold chamber reduces seed physiological quality after two to three months of storage.

Keywords: *Arecaceae*, tucumã, seed germination, seed longevity, seed vigor

Introduction

Astrocaryum aculeatum G. Mey., known in Brazil as tucumã, is an Amazonian palm found in Bolívia, Brazil, Venezuela, Trinidad, Guiana, and Suriname (Kahn, 2008). It is typically found in dryland (*terra firme*) areas, thriving particularly in deforested regions and secondary forests (Kahn & Granville, 1992).

The fruits of *A. aculeatum* are valued as food; their pulp is eaten fresh, either alone or as a filling in sandwiches and tapiocas, and is also used in creams and ice creams (Didonet & Ferraz, 2014; Macêdo et al., 2015). Additionally, their seeds have shown potential for producing biodiesel (Freitas et al., 2022) and cosmetics (Macêdo et al., 2015). Despite this economic potential, commercial plantations are practically nonexistent, with nearly all the production coming from extractivism (Macêdo et al., 2015). The lack of information on seed management has partly hindered the establishment of plantations with this species.

The germination of *A. aculeatum* seeds is slow, irregular, and often low, potentially taking 2 to 3 years to occur (Macêdo et al., 2015). This period can be shortened by drying the diaspores, extracting the seeds, and soaking then in water (Gentil & Ferreira, 2005; Ferreira & Gentil, 2006; Nazário & Ferreira, 2010). Germination is also favored by sowing in a mini greenhouse (Ferreira et al., 2010) and by positioning the germination pore laterally in the planting hole (Elias et al., 2006). Stratification of seeds under alternating air temperatures (26/40 °C) favors the overcoming of dormancy and, consequently, promotes germination (Ferreira et al., 2021). In the seed extraction process, drying the diaspores reduces seed moisture content to 14.5% to 24.0%, without affecting seed viability (Ferreira & Gentil, 2006; Nazário & Ferreira, 2010).

Seed deterioration is strongly affected by intrinsic and extrinsic factors, such as moisture content and

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initial seed quality, and air temperature in the storage environment (Popinigis, 2023). Therefore, reducing seed moisture content and environmental air temperature is essential for storage, as high seed moisture content and air temperatures accelerate metabolism, causing seed deterioration and loss of viability (Bewley et al., 2013).

Seed deterioration is also connected to the characteristics of the packaging used, particularly those promoting water vapor exchange between seeds and the storage environment (Marcos Filho, 2015). Packaging can be permeable, allowing these exchanges, or impermeable, preventing water vapor exchange; the choice of appropriate packaging depends on the species, seed moisture content, storage conditions, and storage period (Marcos Filho, 2015).

In this context, considering the need of information on longevity of palm seeds, the objective of this study was to evaluate the germination and vigor of *A. aculeatum* seeds as a function of storage conditions of diaspores (seeds with endocarp).

Material and methods

The study was conducted at the Laboratory of Seeds and the Germination Nursery of the Biodiversity Coordination (COBIO) of the National Institute of Amazonian Research (Instituto Nacional de Pesquisas da Amazônia - INPA), in Manaus, Amazonas, Brazil. The diaspores used were from ripe fruits obtained in Manaus, representing a mixture of half-sibling progenies from various racemes and plants.

The diaspores were cleaned to remove pulp residues adhered to the endocarp; they were immersed in water for three days with daily water changes and then subjected to friction with sand and washed under running water (Ferreira & Gentil 2006). Subsequently, they were placed in polyethylene mesh bags and kept under ambient conditions (air temperature of 26.5 ± 1.5 °C and relative air humidity of 88.4 ± 11.6%) until the seeds naturally detached internally from the endocarp (60 days).

Diaspores were stored under the following conditions: a) permeable packaging (polyethylene mesh bags) in a natural environment (air temperature of 27.7 ± 1.5 °C and relative air humidity of 85.0 ± 11.9%); b) impermeable packaging (individually sealed double 0.75 mm transparent polyethylene bags) in a natural environment; c) impermeable packaging (individually sealed double 0.75 mm transparent polyethylene bags) in a cold chamber (18.0 ± 1.0 °C and relative air humidity of 68.1 ± 6.2%). The endocarps were broken to extract seeds after 0, 2, 4, 6, and 10 months for moisture content determination and seed imbibition for nine days (Ferreira & Gentil, 2006), followed by sowing.

Seed moisture content was determined using the oven method at 105 ± 3 °C for 24 hours (Brasil, 2009), with four 5-seed replications (Ferreira & Gentil 2006) using seeds cut in half.

The seeds were sown in drained plastic boxes (60 × 40 × 20 cm) containing partially decomposed sawdust as a substrate filling half of each box (10 cm height), using four 25-seed replications per treatment. The boxes were then covered with anti-ultraviolet transparent plastic film to create a mini greenhouse, and placed in the nursery under 50% shade (Ferreira et al., 2010). Seeds were sown by positioning the germination pore laterally in the planting hole, forming a 90° angle relative to the imaginary perpendicular line at to the substrate levell (Elias et al., 2006). Irrigation was performed as needed.

Percentage of seedling emergence was assessed every ten days for six months post-sowing, considering seedlings emerged when the first cataphyll was above the substrate level. After determining the emergence percentage, the emergence speed index and mean emergence time were calculated (Ranal & Santana, 2006). At the end of the experiment, the number of seeds that germinated but did not emerge as seedlings above the substrate level was added to the number of emerged seedlings; this total was used for germination calculation. Additionally, non-germinated seeds were classified as dormant (not rot, with firm and milky-white embryo) or dead (fully rotten or with a rotten embryo only), using the cut test method (Brasil, 2009).

The experiment was conducted in a completely randomized design, with a split-plot arrangement in time and four replications. The main factor was storage conditions (with three levels), and the secondary factor was storage period (with five levels). Data expressed as percentages were transformed using arcsine $\sqrt{(x+0.5)/100}$ for analysis of variance. The means of storage condition levels were then compared by the Tukey's test at a 5% significance level. When the effect of the storage period factor or the effect of the interaction between factors was significant, the data were subjected to polynomial regression, considering significance up to the third degree. All statistical analyses were performed using Assistat 7.7 beta software (Silva & Azevedo, 2016).

Results and discussion

The initial seed moisture content (21.1%) was significantly affected by the interaction between the factors (storage condition and storage period). The moisture contents of seeds from diaspores stored in impermeable packaging remained nearly constant over the ten-month storage period, regardless of storage conditions (**Figure 1**A). However, the moisture content of seeds from diaspores stored in permeable packaging in a natural environment decreased; these seeds reach 11.9% moisture content after ten months of storage. Changes in moisture content in seeds stored in permeable packaging are expected due to oscillations in environmental air temperature and relative humidity (Colville, 2017).

Germination was also significantly affected by the interaction between the evaluated factors. Seeds from diaspores stored in impermeable packaging in a natural environment showed constant germination (68.4%) throughout the evaluated period (Figure 1B). However, germination was lower in the other conditions, decreasing progressively with longer storage period, mainly for seeds from diaspores stored in permeable packaging under natural environment.

Percentage of seedling emergence showed similar trends to the other variables, as it was significantly affected by the interaction between the factors. After two months of storage, stored seeds exhibited a higher percentage of seedling emergence than non-stored seeds, regardless of the diaspore storage conditions (**Figure 2**A). This may be attributed to reduction of dormancy during a 2-month storage period. This result was not reflected in the germination data; considering that the initial mean germination was 67% (Figure 1B) and seedling emergence was 42% (Figure 2A), this indicates that dormancy may had a strong effect on the initial development of seedlings, preventing 37% of germinated seeds from reaching the seedling stage (first cataphyll).

Seeds from diaspores stored in impermeable packaging in a natural environment stood out by presenting high percentage of seedling emergence over the ten-month storage period (Figure 2A). However, diaspores stored in impermeable packaging in a cold chamber presented decreases in percentage of seedling emergence after from two months of storage onwards, despite maintaining nearly constant seed moisture content (Figure 1A). The percentage of seedling emergence from seeds stored in permeable packaging in a natural environment decreased from 2.7 months of storage onwards.

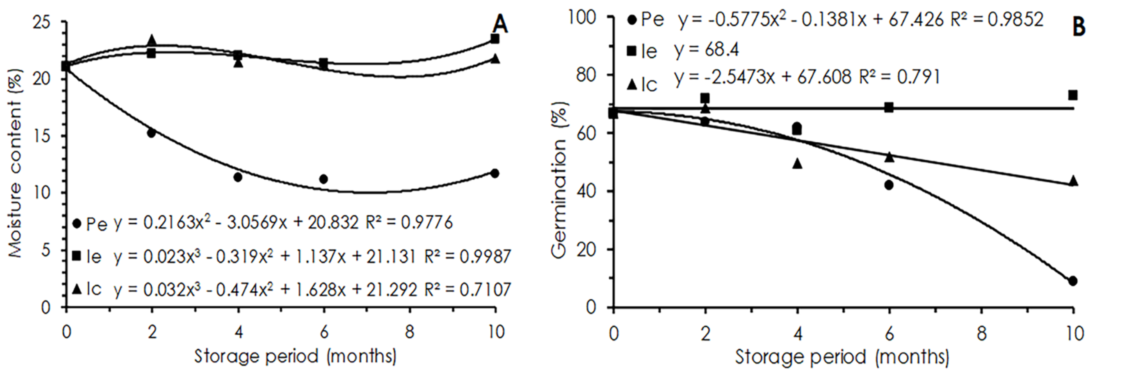

Figure 1. Seed moisture content (A) and germination (B) of *Astrocaryum aculeatum* seeds as a function of storage conditions and storage periods of diaspores (seeds with endocarp). Storage conditions: Pe: permeable packaging in a natural environment (27.7 ± 1.5 °C); Ie: impermeable packaging in a natural environment (27.7 ± 1.5 °C); and Ic: impermeable packaging in a cold chamber (18.0 ± 1.0 °C).

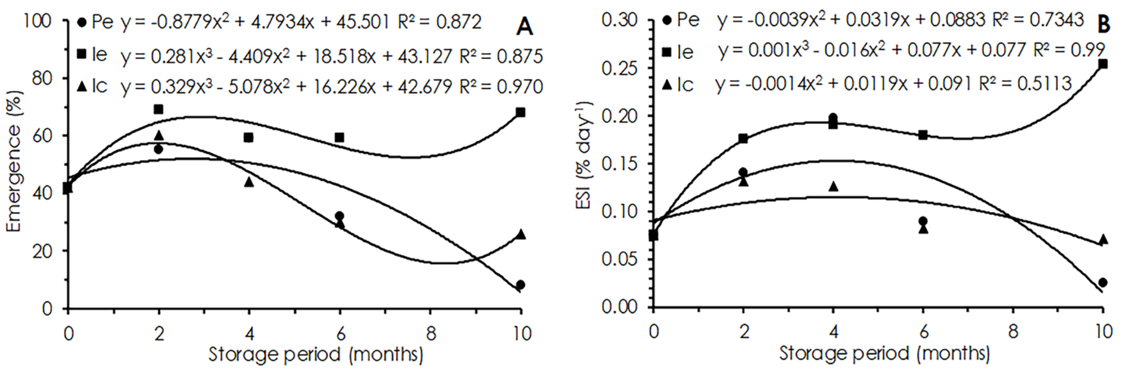

Figure 2. Emergence percentage (A) and emergence speed index (ESI) (B) of *Astrocaryum aculeatum* seedlings as a function of storage conditions and storage periods of diaspores (seeds with endocarp). Storage conditions: Pe: permeable packaging in a natural environment (27.7 ± 1.5 °C); Ie: impermeable packaging in a natural environment (27.7 ± 1.5 °C); and Ic: impermeable packaging in a cold chamber (18.0 ± 1.0 °C).

Most tropical palm seeds present significant viability loss when stored at air temperatures below 15 °C, as found for *Elaeis guineensis* Jacq. (Kumar et al., 2015). The lower temperature limit tolerated by *A. aculeatum* is probably above this mean, as the percentage of seedling emergence from seeds stored at 18 °C decreased. Thus, the hypothesis that a seed moisture content of 21.1% is too high for storing seeds at an ambient temperature of 18 °C cannot be discarded.

The emergence speed index showed similar trends to seedling emergence, mainly for seeds stored in impermeable packaging in a natural environment (Figure 2B).

The mean emergence time was significantly affected by the factors (storage condition and storage period), but not by their interaction. Seedlings from seeds stored in impermeable packaging in a natural environment showed a shorter mean emergence time (100.3 days), although not significantly different from those from seeds stored in permeable packaging (103.2 days), but both were significantly shorter than that found for those from seeds storage in impermeable packaging in a cold chamber (116.0 days) (**Figure 3**A).

The mean emergence time progressively decreased as the storage period increased (Figure 3B). The initial mean emergence time was 148.5 days, decreasing to 90.1 days after ten months of storage. This indicates that dormancy was gradually reduced as the storage period extended. This is consistent with reports by Bewley et al. (2013), who stated that seed dormancy decreases over storage time. Similar trends were reported for *Copernicia alba* Morong seeds, which showed an initial mean germination time of 58.3 days, decreasing to 21.3 days after one month of storage (Masetto et al., 2012).

The low percentage of seedling emergence and emergence speed index and the high mean emergence time (Figures 2A, 2B, and 3B, respectively) found for *A. aculeatum* seeds at the beginning of storage may be connected to embryonic underdevelopment, common in palms, which can be associated with physiological mechanisms inhibiting germination (Baskin & Baskin, 2014).

Dormancy was significantly affected by the interaction between the factors. The use of impermeable packaging in a cold chamber resulted in a higher percentage of seed dormancy, although a decrease in dormancy was found over the ten-month storage period for all conditions studied (**Figure 4**A).

There is little information available on the possible causes of dormancy in *A. aculeatum* seeds. However, the results of this study reinforce its occurrence, mainly considering the long emergence time, which averaged 148.5 days at the beginning of storage (Figure 3B). According to Baskin & Baskin (2014), most palm seeds exhibit morphophysiological dormancy involving aspects related to embryonic underdevelopment and the presence of germination inhibitors; seeds that do not exhibit morphophysiological dormancy are morphologically dormant.

Seed mortality was significantly affected by the interaction between the studied factors. The mortality of seeds from diaspores stored in impermeable packaging in a cold chamber increased over the storage period. This increase was even more pronounced in seeds stored in permeable packaging in a natural environment (Figure 4B), as these seeds presented visible changes in color, cracks in the endosperm, and embryo shrinkage from six months of storage onwards (**Figure 5**). Seeds from diaspores stored in impermeable packaging in a natural environment maintained a constant mortality rate (approximately 21.6%) during the study period. This

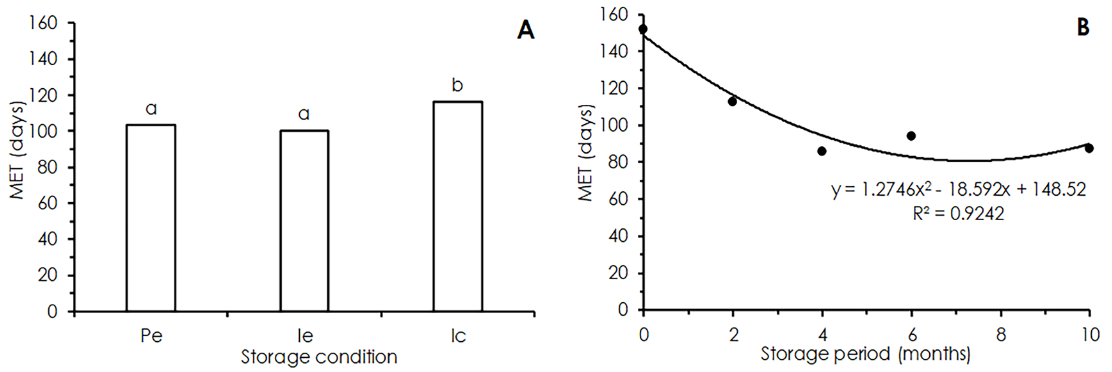

Figure 3. Mean emergence time (MET) of *Astrocaryum aculeatum* seedlings as a function of storage conditions (A) and storage periods (B) of diaspores (seed with endocarp). Storage conditions: Pe = permeable packaging in a natural environment (27.7 ± 1.5 ˚C); Ie = impermeable packaging in a natural environment (27.7 ± 1.5 ˚C); Ic = impermeable packaging in a cold chamber (18.0 ± 1.0 ˚C). Bars with the same letter are not significantly different from each other by the Tukey's test at a 5% significance level.

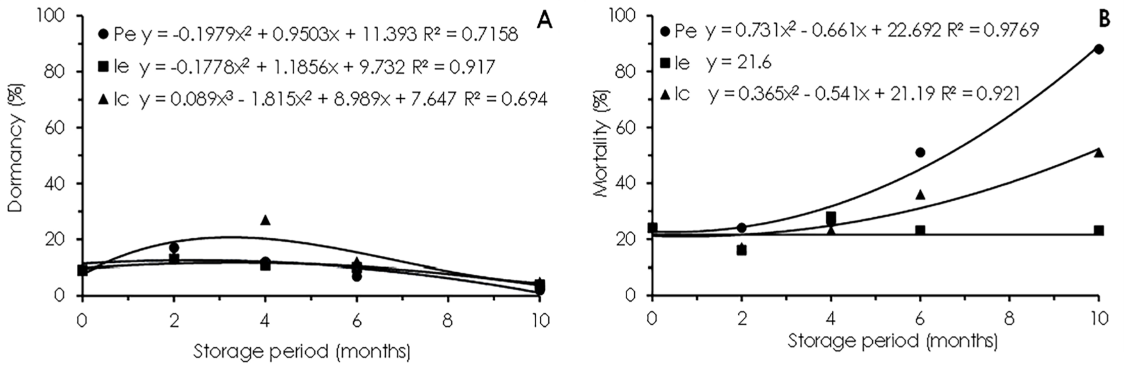

Figure 4. Dormancy (A) and mortality (B) of *Astrocaryum aculeatum* seeds as a function of storage conditions and storage periods of diaspores (seed with endocarp). Storage conditions: Pe: permeable packaging in a natural environment (27.7 ± 1.5 °C); Ie: impermeable packaging in a natural environment (27.7 ± 1.5 °C); and Ic: impermeable packaging in a cold chamber (18.0 ± 1.0 °C).

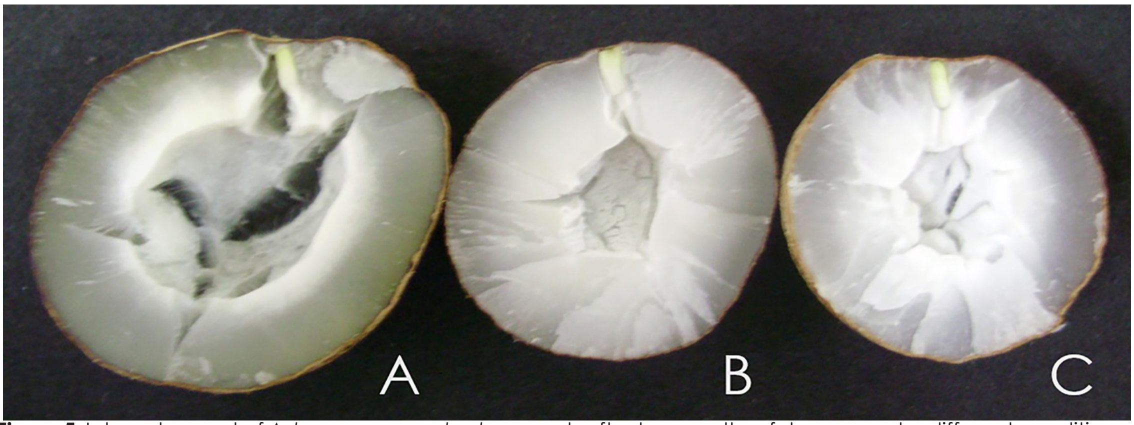

Figure 5. Internal aspect of *Astrocaryum aculeatum* seeds after ten months of storage under different conditions: A = permeable packaging in a natural environment (27.7 ± 1.5 ˚C); B = impermeable packaging in a natural environment (27.7 ± 1.5 ˚C); C = impermeable packaging in a cold chamber (18.0 ± 1.0 ˚C).

high mortality rate correlates with the low germination found for seeds stored in permeable packaging in a natural environment and impermeable packaging in a cold chamber. The loss of seed viability during storage has been associated with the accumulation of cellular damage caused by oxygen reactive species, affecting proteins, lipids, cell membrane integrity, and nucleic acids (Colville, 2017). Several antioxidants are essential for the process of removing oxygen reactive species and the protection against oxidative damages (Colville, 2017).

The presence of the fungus *Aspergillus* sp. was generally found during the experimental period, which may be connected to the mortality of *A. aculeatum* seeds. This microorganism belongs to a group termed storage fungi, which develop more rapidly when the relative air humidity and seed moisture content exceed 80% and 14%, respectively (Marcos Filho, 2015). Thus, further studies on practices for controlling fungi during storage of *A. aculeatum* seeds and diaspores are necessary.

A. aculeatum diaspores can present an average water losses of 39% during the seed extraction process, reducing moisture content from 23% to 14%, while seeds themselves have a moisture content of 20%, which does not compromise seed viability (Ferreira & Gentil, 2006). The germination percentage (Figure 1B) and mainly the emergence (Figure 2A) found in the present study were low when seed moisture content reached approximately 11% (Figures 1A). Thus, seeds of this species exhibit intermediate behavior, according to the classification proposed by Hong & Ellis (1996). Seeds of other palm species from different environments and regions have also been classified as intermediate, such as *Elaeis guineensis* Jacq. (Kumar et al., 2015), *Butia capitata* (Mart.) Becc. (Dias et al., 2015), *Acrocomia aculeata* (Jacq.) Lodd. ex R.Keith (Souza et al., 2016), and *Pritchardia pacifica* Seem. & H.Wendl. (Felix et al., 2017).

Conclusions

The storage of diaspores (seeds with endocarp) of *Astrocaryum aculeatum* with seeds at 21.1% moisture content in impermeable packaging in a natural environment (27.7 ± 1.5 °C) effectively preserves seed physiological quality for ten months of storage.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.