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Germination and emergence of *Astrocaryum aculeatum* G. Mey. seeds subjected to desiccation

Elizabeth Rodrigues Rebouças[1], Sidney Alberto do Nascimento Ferreira[1]*, Patrícia Nazário[2], Daniel Felipe de Oliveira Gentil[3]

[1]Instituto Nacional de Pesquisas da Amazônia, Manaus-AM, Brasil
[2]Instituto Água e Terra, Curitiba-PR, Brasil
[3]Universidade Federal do Amazonas, Manaus-AM, Brasil
*Corresponding author, e-mail: sanf@inpa.gov.br

Abstract

Astrocaryum aculeatum is a palm tree of food and economic importance in the Amazon, but little information is found for its propagation. The objective of this study was to evaluate the performance of *A. aculeatum* seeds as a function of different moisture contents. The seeds were desiccated in a vacuum oven for different periods (0, 12, 24, 48, 72, and 96 hours), which resulted in seeds and embryos with different moisture contents; the resulting seed moisture contents (18.6%, 16.3%, 14.7%, 13.1%, 11.3%, and 9.6%, respectively) were considered treatments. The seeds were divided into two lots: one immediately evaluated, and another evaluated after 30 days of storage, for each seed moisture content. Seed quality was evaluated through germination and seedling emergence in a completely randomized design with four replications, using a split-plot arrangement. *A. aculeatum* seeds and embryos presented different moisture contents, which decreased from 18.6% to 9.6% in seeds and from 39.1% to 20.9% in embryos after the 96-hour desiccation. Decreases in seed moisture content down to 14.4% (31.0% in embryos) do not affect seed germination and seedling emergence and can be considered the critical moisture content below which seed physiological quality is compromised.

Keywords: Arecaceae, moisture content, seed viability, tucumã

Introduction

The Amazon plant diversity includes many economically promising species that have potential to contribute to the food market and regional development. Tucumã or tucumã-do-amazonas (*Astrocaryum aculeatum* G. Mey. - Arecaceae) is particularly noteworthy among these species, as the pulp of its fruits can be consumed fresh and in sandwiches, tapiocas, and snacks or used in the production of ice creams and cremes, and its seeds present suitable properties for cosmetic and biofuel production (Macêdo et al., 2015).

Despite the regional economic importance of tucumã, there are few commercial crops, and the production is mostly extractive (Didonet & Ferraz, 2014). Thus, the lack of conservationist measures may significantly harm natural succession, causing the disappearance of the species in some areas and even compromising its genetic variability. However, the domestication of species is needed to meet the demand of these fruits or any other plant product of interest. In this process include the development of agricultural practices for seed management, which can contribute to the establishment of crops.

Understanding the physiological behavior during desiccation and storage is important for seed management due to varying tolerant to moisture loss among species, often needing special conservation conditions (Mayrinck et al., 2016). Consequently, research has focused on developing procedures to determine the best conditions for maintaining seed viability, being that decreasing metabolic rate by decreasing in seed moisture content is the main technique for seed conservation (Nery et al., 2014).

Identifying the critical moisture content threshold for seed viability is essential for planning and conducting desiccation and storage, focused on maintaining seed physiological quality (Martins et al., 2009). The storage of most seeds of palm trees is difficult when reducing

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moisture contents and storing in low temperatures conditions (Meerow & Broschat, 2015).

The effect of intrinsic (physiological, chemical, and physical) and environmental factors on the viability of *A. aculeatum* seeds remains poorly understood. In addition, seed germination and seedling development of this species are protracted (Gentil & Ferreira, 2005). However, the removal of the endocarp, combined with seed imbibition (Ferreira & Gentil, 2006) and the use of alternate temperatures (Ferreira et al., 2021) reduce the germination period. Ferreira et al. (2021) reported a high seed germination rate (73%) for diaspores (endocarp + seed) stored under ambient conditions for six months.

Therefore, information on the physiological dynamics of *A. aculeatum* seeds as a function of desiccation are essential for developing technologies for the collection, processing, storage, and germination of seeds of this species. In this context, the objective of this study was to evaluate the physiological quality of *A. aculeatum* seeds as a function of different moisture contents.

Material and Methods

The diaspores (endocarp + seed) used were from mature fruits harvested at the beginning of the seed dispersion from several racemes and plants in Manaus, Amazonas, Brazil, and were considered a mixture of half-sibling progenies. The research was developed 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.

To remove pulp residues adhered to the endocarp, the diaspores were initially immersed in water for three days, with daily exchange of water and then rubbed with sand and washed under running water (Ferreira & Gentil, 2006). They were then placed in polyethylene mesh bags and kept under ambient conditions (temperature of 28.6 ± 1.4 °C and relative humidity of 81.0 ± 12.9%) for 60 days, when all the seeds had detached from the endocarp. The seeds were then kept in double transparent polyethylene bags (0.75 mm thickness) for 15 days, until the extraction of seeds by breaking the endocarp (Ferreira & Gentil, 2006).

The seeds were then taken to a vacuum oven (TE-395; Tecnal) under pressure of -25 inHg and temperature of 30 °C for 0, 12, 24, 48, 72, and 96 hours. Moisture contents were determined after each desiccation period, using the oven-drying method at 105 ± 3 °C for 24 hours, as described in Brasil (2009), with four 4-seed (divided into four parts) replications and four replications of ten embryos each. The embryos were desiccated inside the seeds and excised moments before moisture content determination. Each seed moisture content was considered a treatment. The seeds were divided into two lots: one for immediate evaluation and another for evaluation after 30 days of storage, in glass pots with plastic lids, sealed with adhesive tape, wrapped in black polyethylene bags (absence of light), and kept at room temperature (27.6 ± 1.4 °C).

The seeds were soaked in water for nine days before sowing, as recommended by Ferreira & Gentil (2006). They were sown in perforated plastic boxes (60 × 40 × 20 cm), containing a 10 cm layer of partially decomposed wood sawdust substrate. The boxes were covered with a 100-μm thick, transparent anti-UV polyethylene film to create a mini-greenhouse and placed in a nursery with 50% shade (Ferreira et al., 2010). The substrate was moistened with water to field capacity before sowing and, afterwards, water was replenished to maintain the initial moisture.

Germination, defined as the formation of a germinative button, and emergence, defined as the emission of the first bifid leaf, were assessed every ten days until six months after sowing, as proposed by Gentil & Ferreira (2005). These data were used to calculate germination and emergence speed indexes, as well as mean germination and emergence times (Ranal & Santana, 2006). Seeds that did not germinate were evaluated through the cutting test (Brasil, 2009) and classified as dormant (no decay, with a firm, milky-white embryo) or dead (completely decayed or with only the embryo decayed).

A completely randomized experimental design with four 25-seed replications was used, in a split-plot arrangement; the main plots consisted of the “storage” factor (with and without storage) and subplots consisted of the “moisture content” factor (moisture levels of 18.6%, 16.3%, 14.7%, 13.1%, 11.3%, and 9.6%). In analysis of variance, the data expressed in percentages were transformed into arcsine $\sqrt{(x/100)+0.5}$. When the effect of the moisture content factor, or the interaction effect between factors was significant, polynomial regression analysis was performed up to the third degree. Results were presented and discussed using the original data, without transformation.

Results and Discussion

Increases in desiccation periods resulted in decreases in moisture contents, with different results for *A. aculeatum* seeds and embryos, fitting linear equations (**Figure 1**). After 96 hours of desiccation, moisture content decreased from 18.6% to 9.6% in seeds and from 39.1% to 20.9% in embryos. Embryos consistently exhibited higher moisture content than seeds throughout the desiccation process. The decrease in moisture content was lower for embryos (46.5%) than seeds (48.4%) when considering initial moisture contents and those obtained after 96 hours of desiccation. Prakash et al. (2019) evaluated *Elaeis guineensis* Jacq. (Arecaceae) seeds and excised embryos desiccated in silica gel for 96 hours and found higher decreases in moisture content compared to those found in the present study; with decreases from 23.4% to 6.1% (73.9%) for seeds, and from 37.9% to 18.1% (52.2%) for embryos. Additionally, Schlinwein et al. (2013) evaluated diaspores (endocarp + seed) of *Butia odorata* (Barb. Rodr.) Noblick (Arecaceae) desiccated in a forced- air oven at 30 °C for 0, 24, and 48 hours and found moisture contents of 17.0%, 8.6%, and 7.5%, respectively.

Linear regression analysis revealed a strong correlation between seed and embryo moisture contents (R^2 = 0.9841), allowing for the estimation of *A. aculeatum* embryo moisture content from seed moisture content and vice versa (**Figure 2**). According to Jaganathan (2021), information on moisture content in specific tissues is important for a better understanding of the desiccation of diaspores from Arecaceae plants. For instance, Panza et al. (2004) found that tissues of embryos of *Euterpe edulis* Mart. can concentrate high water contents due to the presence of cells highly highly vacuolated, differently from reserve tissues (endosperm).

Germination showed a significant effect only for the "moisture content" factor of *A. aculeatum* seeds (**Figure 3**). Initially, it was 42.0% (with a moisture content of 18.6% of the seeds and 39.1% of the embryos), increasing with desiccation and reaching the maximum value of 53.1% germination (with a moisture content of 14.4% of the seeds and 31.1% of the embryos), then decreasing until reaching 37.8% germination (with a moisture content of 9.6% of the seeds and 20.9% of the embryos). According to Silva et al. (2012), the desiccation of *Attalea speciosa* Mart. (Arecaceae) seeds did not affect the viability of zygotic embryos; however, the embryo vigor decreased as the seed moisture content decreased. Seeds of other palm species, such as *Carpentaria acuminata* (H. Wendl. & Drude) Becc., and *Phoenix canariensis* Chabaud, tolerate desiccation up to 5% and 8%, respectively; however, *Dypsis decaryi* (Jum.) Beentje & J.Dransf. and

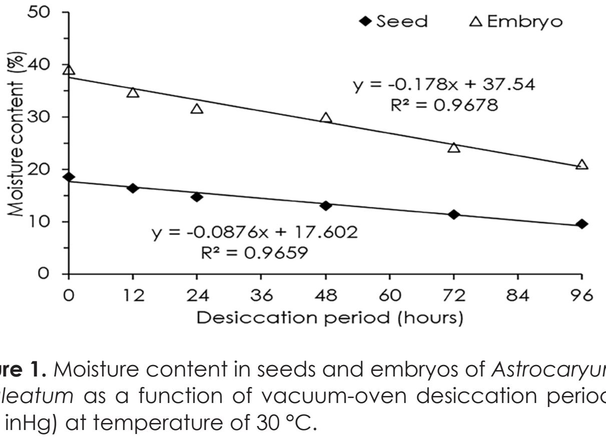

Figure 1. Moisture content in seeds and embryos of *Astrocaryum aculeatum* as a function of vacuum-oven desiccation periods (-25 inHg) at temperature of 30 °C.

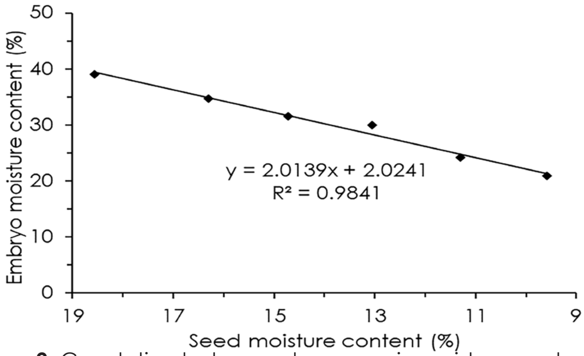

Figure 2. Correlation between decreases in moisture contents in seeds and embryos of *Astrocaryum aculeatum*.

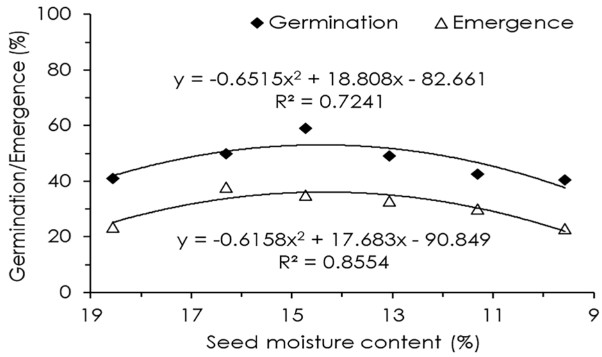

Figure 3. Seed germination and seedling emergence as a function of decreases in moisture content in *Astrocaryum aculeatum* seeds.

Ptychosperma elegans Blume seeds are sensitive to desiccation, even to moisture contents of 20% and 27%, respectively (Batista et al., 2016).

The emergence of *A. aculeatum* seedlings, in addition to presenting a significant effect for the "moisture content" factor, also showed significance for the "storage" factor, with no interaction effect. Non-stored seeds resulted in higher seedling emergence (34.0%) than stored seeds (26.8%) (**Table 1**), indicating that the hermetic packaging prevented gas exchange, compromising seed physiological quality and leading to increased mortality (Table 1). Ferreira et al. (2021) found 73% germination for *A. aculeatum* seeds after six months of storage of non-packaged diaspores under ambient conditions (temperature of 28.7 ± 1.4 °C and relative humidity of 76 ± 6.9%).

Regarding the "moisture content" factor of *A. aculeatum* seeds, seedling emergence values were significantly lower than germination, reaching a

Table 1. Seed germination, seedling emergence, and seed mortality as a function of storage of *Astrocaryum aculeatum* seed

Storage	Germination(%)	Emergence(%)	Mortality(%)
Without storage	50.3 a	34.0 a	26.3 a
With storage	43.7 a	26.8 b	44.8 b
C.V. (%)	21.5	31.1	21.2

Means followed by the same letter in the columns are not significantly different from each other by the Tukey's test at a 5% probability level.

maximum of 36.1%, whereas germination was 53.1%, at moisture contents of 14.4% (seeds) and 31.1% (embryos) (Figure 3). Therefore, the germination test, considering only the formation of germinative button, overestimate the physiological quality of *A. aculeatum* seeds, i.e., their capacity to form normal seedlings with potential to continue development and originating normal plants (Brasil, 2009). According to Panza et al. (2007), this is due to the seedling emergence greater sensitivity to desiccation compared to germination; they reported that the development of *E. edulis* seedlings is not connected to the emergence of the germinative button, since deaths can occur during the initial development of seedlings from desiccated seeds. A study evaluating *Oenocarpus bacaba* Mart. (Arecaceae) seeds, showed that sensitivity to desiccation is more expressive when taking into account more advanced stages of seedling development(Bastos et al., 2021).

The critical moisture content for *A. aculeatum* seeds, i.e., that below which seed viability starts to decrease (Probert & Longley, 1989), was 14.4% for germination and seedling emergence (Figure 3). By setting the equations for seed germination and seedling emergence equal to zero (y = 0) as a function of moisture content (Figure 3), the lethal moisture content of the seeds, defined as the point where viability is null (Hong & Ellis, 1992), was estimated to be 5.4% and 6.7%, respectively. Additionally, critical and lethal embryo moisture contents for germination and seedling emergence were higher: 31.0% and 12.9% for seed germination and 31.0% and 15.5% for seedling emergence, respectively. These results are noteworthy due to the greater sensitivity of emergence in relation to the degree of lethal moisture content, based on both the moisture content of the seeds and the embryos. This indicates that *A. aculeatum* seeds do not exhibit orthodox or recalcitrant behavior, as they tolerate some level of desiccation, indicating that they are intermediate type.

Ribeiro et al. (2012) classified *Acrocomia aculeata* (Jacq.) Lodd. ex R. Keith (Arecaceae) seeds as orthodox, based on their tolerance to desiccation (5%) and cooling (-20 °C), through evaluations of viability using *in vitro* culture of embryos. Additionally, Souza et al. (2016) found tolerance to desiccation up to moisture contents of 6% to 8% for *A. aculeata* seeds; however, they were sensitive to the temperature of 10 °C and were classified as intermediate. Félix et al. (2017) classified *Adonidia merrillii* (Becc.) Becc. (Arecaceae) seeds as recalcitrant, with reduced emergence and initial seedling growth when desiccated to moisture contents below 28%. According to Nazário & Ferreira (2012), *Oenocarpus bataua* Mart. (Arecaceae) seeds are recalcitrant and presented critical and lethal moisture contents of 35.0% and 24.3%, respectively.

The germination (GSI) and emergence (ESI) speed indexes were significantly affected by the interaction between factors "storage" and "moisture content" of *A. aculeatum* seeds. Seeds immediately evaluated (without storage) presented, in general, higher GSI and ESI (**Figures 4A and 4B**). Furthermore, the daily emergence (ESI) was lower than daily germination (GSI), regardless of the storage.

Regarding the mean germination times (MGT) and mean emergence times (MET), there was a significant interaction between the factors "storage" and "moisture content" of *A. aculeatum* seeds. The MGT and MET were lower for non-stored seeds (**Figures 5A and 5B**, respectively). Schlindwein et al., (2013) found no significant effect of storage (90 days) on emergence percentage, emergence speed index and mean time for emergence for diaspores of *B. odorata* with different moisture contents (17.0%, 8.6%, and 7.5%); the differences found for these variables were only related to the different moisture contents, with the lowest contents resulting in more favorable results.

For the variable dormant seeds there was significant interaction effect between the factors "storage" and "moisture content" of *A. aculeatum* seeds. Overall, dormancy was higher in seeds with higher moisture contents, progressively decreasing as the moisture content decreased (**Figure 6**). Additionally, dormancy was higher in non-stored seeds. These results partly explain the lower germination and emergence percentages obtained at higher moisture contents (Figure 3). Decreases in moisture content decreased dormancy and increased emergence percentage and speed index in diaspores of *B. odorata* (Schlindwein

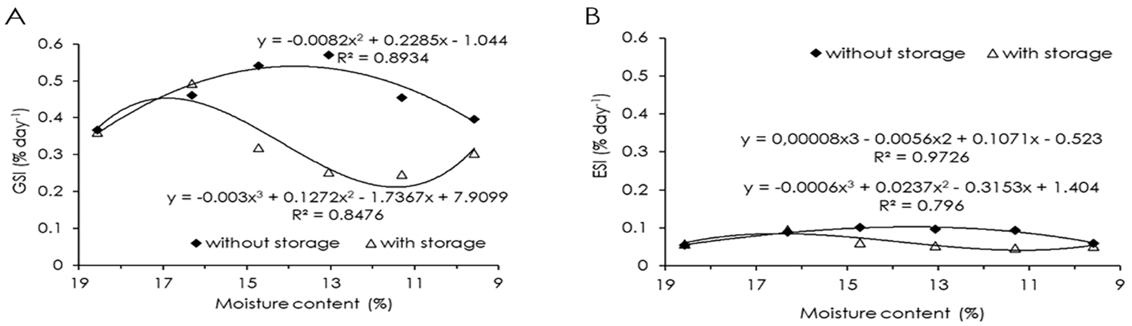

Figure 4. Germination speed index (GSI) (A) and emergence speed index (ESI) (B) as a function of decreasing moisture content and storage of *Astrocaryum aculeatum* seeds.

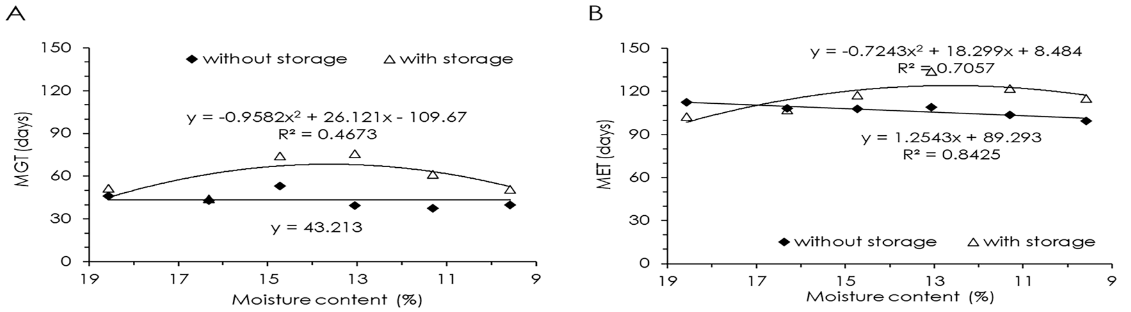

Figure 5. Mean germination time (MGT) (A) and mean emergence time (MET) (B) as a function of decreasing moisture content and storage of *Astrocaryum aculeatum* seeds.

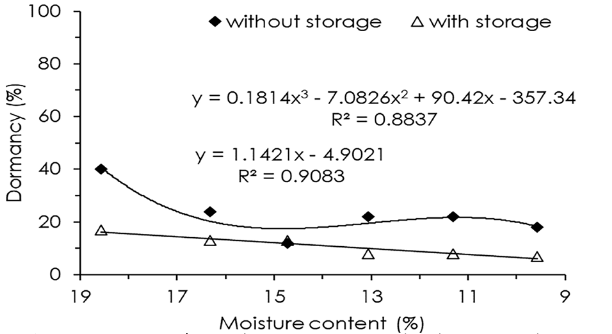

Figure 6. Dormancy in *Astrocaryum aculeatum* seeds as a function of decreasing seed moisture content and seed storage.

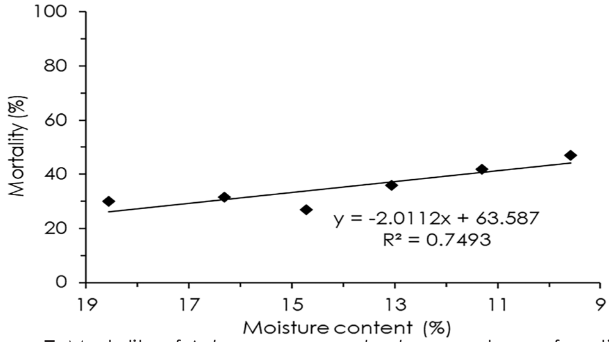

Figure 7. Mortality of *Astrocaryum aculeatum* seeds as a function of decreasing moisture content.

et al., 2013). According to Schlindwein et al. (2013), decreased dormancy in *B. odorata* seeds is due to periods of desiccation, rehydration, and exposure to high temperatures.

The variable dead seeds showed a significant effect for the factors "storage" and "moisture content" separately, with no interaction effect. Mortality increased as the moisture content of *A. aculeatum* seeds decreased (**Figure 7**). Moreover, it was higher in stored seeds (44.8%), compared to those immediately evaluated after desiccation (26.3%) (**Table 1**). According to Walters (2015), during the desiccation, the cytoplasm solidifies and recently formed spatial relations between molecules determine the period the seeds will be viable. Desiccated seed survival depends on high-performance cell protection mechanisms. Reducing the metabolic activity up to a quiescent state is an important mechanism to keep the cells alive under limited water conditions. The most common strategy to reduce cell metabolism is to limit molecular mobility, accumulating non-reducing soluble sugars and transforming the cytoplasm into a vitreous state (Sano et al., 2016).

Considering the peculiarities of stored palm seed behavior (Pérez et al., 2012; Meerow & Broschat, 2015), additional studies evaluating the desiccation and storage of *A. aculeatum* seeds under different temperatures and packaging conditions are needed for a better understanding of their physiological dynamics. Furthermore, assessments of seedling emergence and seed and embryo moisture contents should be included for a more accurate evaluation of the effects of desiccation on *A. aculeatum* seeds.

Conclusions

Seeds and embryos of *Astrocaryum aculeatum* exhibited different moisture contents after a 96-hour desiccation period; seed moisture content decreased from 18.6% to 9.6% and embryo moisture content decreased from 39.1% to 20.9%.

Decreases in seed moisture content down to 14.4% (31.0% in embryos) do not negatively impact seed germination and seedling emergence and can be considered the critical moisture content below which seed physiological quality is compromised.

The lethal moisture contents for seed germination and seedling emergence were estimated to be 5.4% and 6.7% in seeds and 12.9% and 15.5% in embryos, respectively.

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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.