Water immersion and one-year storage influence seed germination of Copernicia alba palm tree from a neotropical wetland

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Short communication

Water immersion and one-year storage influence seed germination of *Copernicia alba* palm tree from a neotropical wetland

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Abstract

Copernicia alba (Morong ex Morong & Britton) is a palm tree native to tropical and subtropical climates, commonly named "carandá", that often forms monodominant populations in the *Pantanal* wetland in Brazil. Currently, the knowledge of the seed germination behavior of *C. alba* is still limited, as well as seed viability in *ex-situ* conservation under laboratory conditions. Hence, this study investigated the effect of water treatments and a one-year storage period (at 19°C under a relative humidity of 45%) on its germination. We subjected fresh and stored seeds to immersion in water at room temperature (~25°C) for 24, 48, and 72 h; and hot water (~75°C) for 5 min and 10 min. The results showed that seeds do not have primary dormancy, with fresh seeds germinating 84% in the control. Additionally, fresh seeds had a significant increase in germination percentage (reaching 100%) and faster germination times after immersion in water for 48 and 72 h. On the other hand, hot water treatments decreased germination for both fresh and stored seeds. One year of storage reduced the germination capacity by almost 50%. We concluded that seeds of *C. alba* may be easily germinated under laboratory conditions for seedling production, and water immersion at room temperature can improve the germination of fresh seeds. However, long-term seed storage and hot-water treatments may jeopardize germination. Future studies should further investigate *ex-situ* conservation of seed of *C. alba* under extended periods, considering seed desiccation and alternative storage conditions, to improve seed conservation techniques.

Keywords: *carandá*; caranday palm; flooding; hot water; *Pantanal*; seed storage

Introduction

Tropical wetlands cover extensive areas of the Earth's surface and are one of the most diverse ecosystems influenced by seasonal flooding (Keddy et al. 2009). Frequently, palm species that dominate such flooding formations show fruit/seed dispersal and germination strategies related to the water pulse (Orozco-Segovia et al. 2003). According to the literature, palm seeds display morphophysiological dormancy due to underdeveloped embryos (Baskin and Baskin 2014) and the thick endocarp that hinders embryo growth (Carvalho et al. 2015; Oliveira et al. 2013; Pérez 2009). To overcome dormancy, water treatments have been used to optimize and accelerate the germination process of palm seeds by facilitating oxygen absorption and losing mechanical restrictions of the surrounding tissues (e.g., Bovi 1990; Martin et al. 1996; Ferreira and Gentil 2006; Fava and Albuquerque 2011; Rubio Neto et al. 2012; Pinto et al. 2012; Goudel et al. 2013). On the other hand, hot-water treatments (~75°C) have been tested on the germination of tropical palm seeds but may not promote a dormancy-break (Martin et al. 1996; Rodrigues-Junior et al. 2016).

Studies have shown that *ex-situ* conservation of palm seeds is limited and associated with seed viability loss (Broschat 1994; Orozco-Segovia et al. 2003). The knowledge of seed storage behavior and seed viability can contribute to the ex-situ conservation of the species in seed banks, allowing the conservation of the germplasm and supporting ecological restoration programs (León-Lobos et al. 2012). Several external factors influence the viability of tropical palm seeds during storage, such as temperature and time, and intrinsic factors like seed dormancy and desiccation sensitivity (recalcitrance) (Orozco-Segovia et al. 2003). Recalcitrant seeds do not tolerate water loss – typically below a threshold <12% – and thus rapidly fade in laboratory storage (Hong and Ellis 1996). In addition, the longevity of stored palm seeds varies among species, and the optimal conditions for *ex-situ* conservation may reduce metabolic activity under relatively low values of environmental humidity and temperatures (Pivetta et al. 2011). In laboratory conditions, temperatures ranging from 18 to 23°C have been recommended for most palm seeds (Broschat 1994) and tested for a few tropical species, such as the macaw palm (*Acrocomia aculeata*) from the *Cerrado* savannas (Ribeiro et al. 2012) and *Copernicia alba* from a Brazilian wetland (Masetto et al. 2012).

Copernicia alba (Morong ex Morong & Britton) is a palm tree (Arecaceae family) native to tropical and subtropical climates, commonly named "carandá" or caranday palm, which often forms monodominant populations in the Pantanal wetland in Brazil (Pott and Pott 1994). This species also occurs in the Chacos of Argentina, Paraguay, and Bolivia (Lorenzi et al. 2004). In the Brazilian *Pantanal* wetland, where it is mainly found, the areas can remain flooded for up to eight months per year (Pott and Pott 1994; Nunes-da-Cunha and Junk 2011, Fig. 1a). In the natural areas, *C. alba* exhibits early successional characteristics and may tolerate fires; its fruits are edible and provide food for wildlife such as macaws, parrots, and fishes (Pott and Pott 1994). The economic relevance of *C. alba* regards its wood durability, uses in rural constructions, corrals, fences, and as an ornamental plant (Lorenzi et al. 2004; Pivetta et al. 2011).

Considering the current limited knowledge in seed germination and storage behavior of *Copernicia alba*, and the fact that it colonizes the *Pantanal* areas, which are periodically subjected to flooding followed by dry periods, the present study aimed to evaluate the effect of water treatments and storage on seed germination. We investigated: (a) if water treatments (~ 25 and 75^{0}C) influence germination; and (b) germination behavior of seeds after one year of storage. The thick endocarp can hinder germination; thus, water immersion treatments are expected to enhance germination. However, we anticipated that seed viability might decrease after the relatively prolonged storage period.

Material and Methods

Seed collection and benefiting

We harvested fruits in July 2011 from 15 matrices from a natural population at the *Carandazal* Station, in a natural area located in the Miranda subregion of the *Pantanal* in Mato Grosso do Sul, Brazil (GPS coordinates 19º48'30.1''S e 57º10'13.5''W). Then we took them to the Seed Lab at *Universidade Federal do Mato Grosso do Sul*, Campo Grande, MS, where the experiments were carried out. We removed fruit coat and pulp by manual friction and then homogenized the seed lot. Fruits are elliptical and brown, endocarp fused to the seeds with 1-2 cm in length (Fig. 1b). Initial seed water content was determined by fresh-weighing two samples of 15 seeds each, and then reweighing the samples after oven-drying at 105 ± 3°C for 24 h, according to the Brazilian rules for seed testing (Brasil 2009). We repeated this procedure after one-year of storage to determine the seed water content of stored seeds.

Experimental factors and germination trials

To evaluate the influence of water immersion on germination, we subjected seeds to different treatments under laboratory conditions: (1) control, using untreated fresh-harvested seeds; (2) seeds immersed in water at room temperature (~25°C) for 24 h; (3) 48 h; and (4) 72 h. Besides, we tested two additional treatments using hot water immersion at 75°C for (5) 5 min; and (6) 10 min. Plastic trays (20 x 30 x 9 cm) were used to maintain the seeds immersed in water and kept under constant fluorescent light for the desired times, with daily water changing when necessary.

Each control and treatment consisted of four samples of 25 seeds (=100 individual seeds each). After water immersion treatments (at room temperature and hot water), we set seed germination test in a rolled paper towel (germitest paper) moistened with 2.5 times dry paperweight in distilled water (Brasil 2009). The rolls were kept inside plastic bags to avoid water loss and incubated in alternating temperatures of 20-30°C (Fava and Albuquerque 2011) under a 12 h photoperiod of fluorescent light (4 x 20 W). We daily recorded germination for up to 45 days, considering germinated seeds with radicle protrusion ≥2 mm.

In the storage experiment, fresh seeds were wrapped in kraft paper bags and kept them stored in a cold chamber at 19°C (relative humidity 45 ± 5%) for one year. After this period, two samples of 15 seeds each were used to determine the seed water content (Brasil 2009) (see seed collection and benefiting subsection). Then we subjected stored seeds to immersion in water treatments (at room temperature and hot water) and used a sample of stored seeds as control (untreated seeds). Seeds (treated and control) were set to germinate in a rolled paper towel as described above. By the end of the germination trials, we determined the germination percentage and mean germination time based on the number of daily germinated seeds (Labouriau 1983).

Data analysis

Germination variables (germination percentage and mean germination time) were evaluated using generalized linear models with the *lme4* package (Bates et al. 2015) in R statistical software (R Core Team 2020). The preliminary analysis detected a significant difference between fresh and stored seeds (P-value <0.001), then we proceeded with the analysis separately for each storage period. Hence, models compared variable values as a function of water immersion treatments (water immersion in room temperature for 24, 48, and 72 h and hot water for 5 and 10 min) compared to their respective controls.

Results and Discussion

Initial seed water content was ~10% for fresh-harvested seeds and decreased to 7% after the one-year period of storage. *Copernicia alba* seed shows no dormancy type. Fresh-harvested seeds germinated 84% (Fig. 2a), indicating a high germination percentage, with no primary dormancy type, contrary to previous studies (Fava and Albuquerque 2011). Genetic factors may affect seed germination by influencing seed viability and quality in the same specie from different areas (Soler-Guilhen et al. 2020). Lack of dormancy in *C. alba* seeds can warrant recruitment in the *Pantanal* wetlands where seeds are subjected to flooding conditions, demonstrated by a rapid flush of seedling emergence in the post-flood environment (Bao et al. 2018). Even though the majority of palm seeds may display morphophysiological dormancy related to the undeveloped embryo and mechanical restrictions imposed by the thick endocarp (Baskin and Baskin 2014; Pérez 2009), seeds of *C. alba* can be easily germinated under

Water treatments influenced germination. The fresh-harvested seeds had an increase in germination compared to control after water immersion for 48 and 72 h, in which nearly all seeds germinated (98-99%, Fig. 2a). Stored seeds germinated 32% after water immersion for 24 h and differed significantly from the 48, 72 h (~50%), and the stored control (Fig. 2b). Water treatments (48 and 72 h) reduced the mean germination time values of fresh seeds (around 20 days) compared to control (25 days, Fig. 2c), providing faster germination. In contrast, there was no change in the mean germination time of stored seeds (Fig. 2d). A short period of immersion in water may improve the germination of *C. alba* fresh seeds for seedling production. As well, water immersion treatments promoted germination of *Copernicia prunifera* (*carnaúba*), a closely related species of economic relevance in the Brazilian Northeast region (Reis et al. 2011), displaying flood-tolerant seedlings (Arruda and Calbo 2004). Both species occur in flooded environments. The enhancement of germination ability after immersion in water can improve the competitive performance following flooding events in their natural habitats. The tolerance of *C. alba* seeds to prolonged water immersion should be investigated to understand if the heightened germination is sustained. High and fast germination after flooding can be important to maintain monodominance since most buried propagules are recruited at the end of the seasonal flooding period in the Pantanal wetland (Bao et al. 2018).

In contrast, hot water treatments decreased the germination percentage of fresh seeds to 62 and 50% for 5 and 10 minutes of exposure, respectively (Fig. 2a, b). Such treatments also decreased the germination percentage of stored seeds to 21% for 5-min and 16% for 10-min of exposure (Fig. 2b). Additionally, the 5-min hot water treatment delayed the mean germination time of fresh seeds for 30 days (Fig. 2c), with no effect on this parameter after storage (Fig. 2d). Likewise, hot water treatments jeopardized the germination of *Euterpe oleracea* seeds from the Amazon rainforest (Bovi and Cardoso 1976). Thermal shock also did not promote the seed dormancy of *Acrocomia aculeata* (macaw palm) but rather caused high seed deterioration rates (Rodrigues-Junior et al., 2016).

In general, the one-year storage period decreased germination percentage to ≤50%, with no change in germination time (Fig. 2b, d). *Ex-situ* conservation of seed plants is a worldwide concern (Walters et al. 2013), and a similar germination patterns after storage can be expected in other tropical palm species (Orozco-Segovia et al. 2003; Souza et al. 2016). Therefore, *ex-situ* conservation strategies remain a matter of inquiry for most tropical palm trees. Seeds of *C. alba* should be considered orthodox, given the seeds water content ranges from 10 to 7% depending on the storage period. Hong and Ellis (1996) define as recalcitrant seeds those sensitive to desiccation ≤12%; thus, seeds of *C. alba* can be classified as desiccation-tolerant (orthodox), due to their relatively low initial seed water content. The natural habitats where this species occurs can experience prolonged events of drought in pluriannual dry years of the *Pantanal* (Thielen et al. 2020), such as those that occurred in 2020; therefore, the desiccation tolerance may play a critical role to maintain this species in the ecosystem under a set of dry years.

Longevity patterns remain yet to be clarified in tropical palm seeds (Broschat 1994; Pivetta et al. 2011). Seed storage behavior of *C. alba* might be similar to the *Acrocomia aculeata*, whose seeds are desiccation-tolerant and can be stored over one year under low temperatures (10°C, see Ribeiro et al. 2012). Nevertheless, the role of temperature remains unstudied on the storage of seed of *C. alba*, including cryopreservation, and *in vitro* culture propagation methods.

Conclusions

Our study highlights some important considerations for studies involving water immersion as a method to accelerate seed germination and the *ex-situ* storage for the conservation of palm seeds from a tropical wetland. The results reveal those seeds of *Copernicia alba* do not present dormancy. Water immersion for 48 e 72h improved germination rate and time; conversely, hot treatments reduced the germination capacity, increasing the germination time. The seed storage for one year at 19°C under a relative humidity of 45% decreased the germination rates by almost 50%. Future studies should further investigate *ex-situ* conservation of seed of *C. alba* under extended periods considering seed desiccation and alternative storage conditions, to improve seed conservation techniques.

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Author contributions

Vanessa C. Soares: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Formal analysis, Writing - original draft, Writing - review & editing, and Visualization. L. Felipe Daibes: Formal analysis, Data Curation, and Writing - Review & Editing, and Visualization. Geraldo A. Damasceno-Junior: Conceptualization, Resources, Writing - Review & Editing, and Funding acquisition. Liana B. De Lima: Supervision, Conceptualization, Methodology, Writing - original draft, Validation, Formal analysis, Investigation, Resources, Data

Curation, Writing - Review & Editing, Visualization, and Funding acquisition. All authors read and approved the final manuscript.

Code Availability

Not applicable.

Declarations Conflicts of Interest/Competing Interests

The authors have no conflicts of interest.

Ethics Approval

Not applicable.

Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Availability of data and material

Not applicable.

Fig.1 (a) Individuals of *Copernicia alba* from a natural population (locally named *carandazal*) occurring in a natural area of Pantanal, Mato Grosso do Sul, Brazil. (b) Seeds of *C. alba* after cleaning under laboratory conditions. Photos by the first author

Fig. 2 Germination percentage (%) and mean germination time (MGT, days) of fresh-harvested (a) and one-year stored seeds (b) of *Copernicia alba*, from a natural population, under water immersion (W) treatments for 24, 48, and 72 h (at room temperature), and hot water (Hot, 75°C) for 5 and 10 minutes of exposure compared to their respective controls. Asterisks indicate significant differences (blue refers to a significant increase, red to a significant decrease, always compared to controls). * = P <0.05; ** = P <0.01; *** = P <0.001; n.s = non-significant; Cont = control

Figures

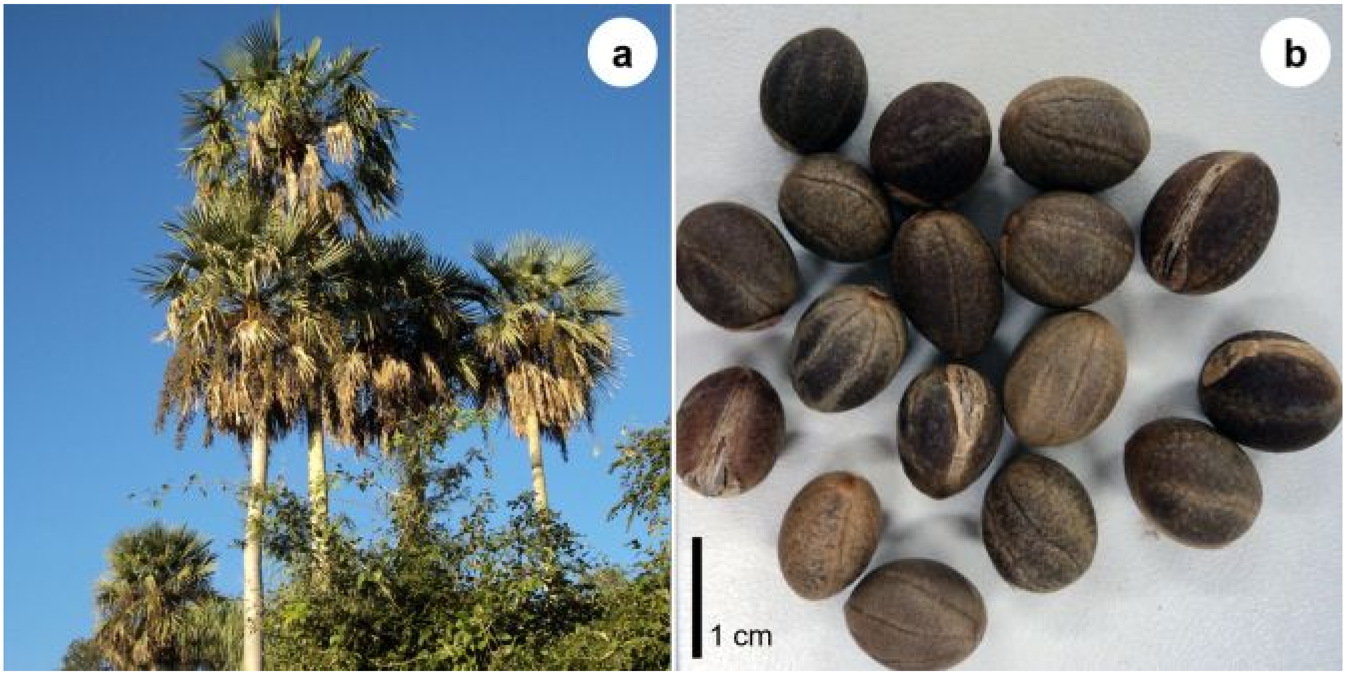

Figure 1

(a) Individuals of Copernicia alba from a natural population (locally named carandazal) occurring in a natural area of Pantanal, Mato Grosso do Sul, Brazil. (b) Seeds of C. alba after cleaning under laboratory conditions. Photos by the first author

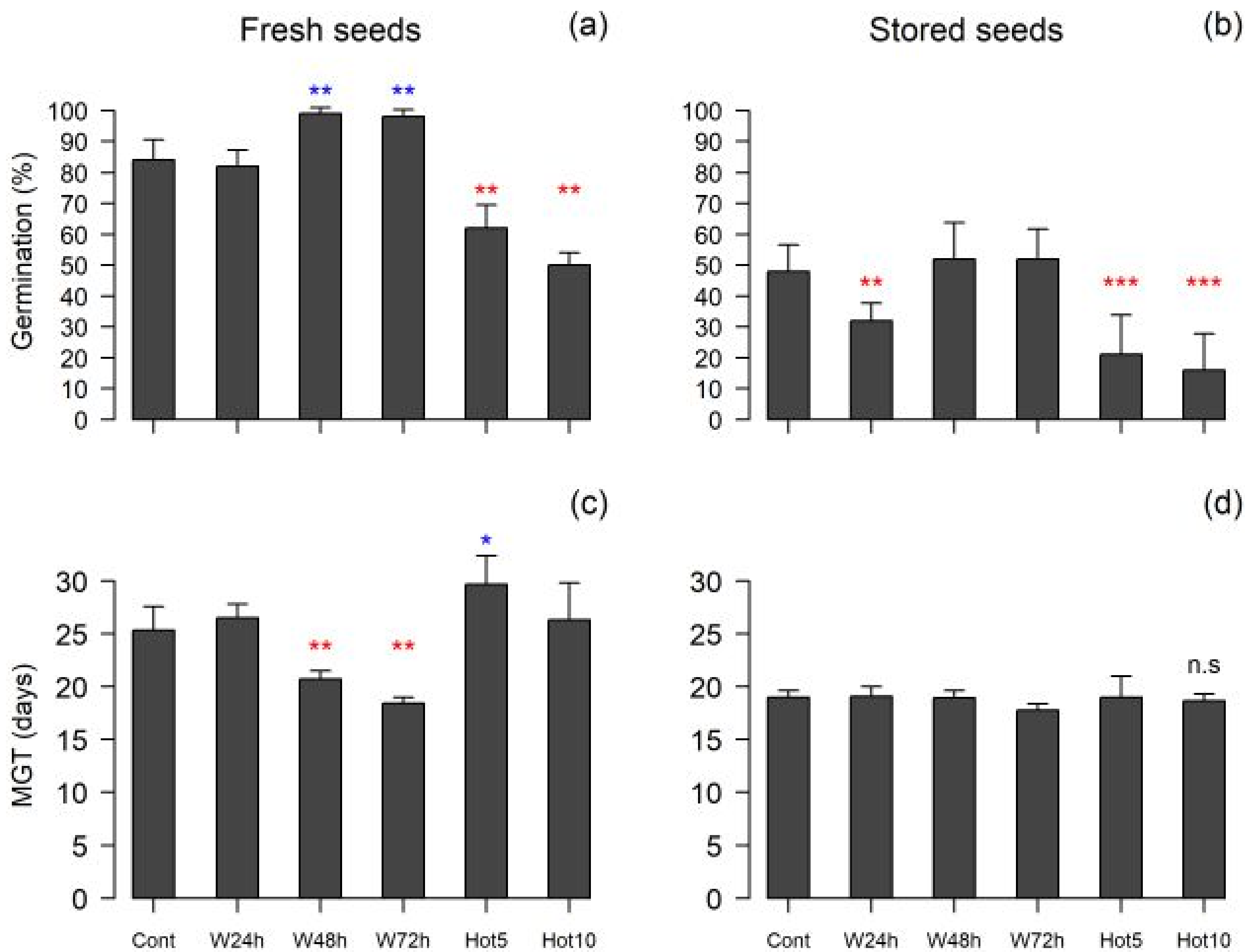

Figure 2

Germination percentage (%) and mean germination time (MGT, days) of fresh-harvested (a) and one-year stored seeds (b) of Copernicia alba, from a natural population, under water immersion (W) treatments for 24, 48, and 72 h (at room temperature), and hot water (Hot, 75°C) for 5 and 10 minutes of exposure compared to their respective controls. Asterisks indicate significant differences (blue refers to a significant increase, red to a significant decrease, always compared to controls). * = P <0.05; ** = P <0.01; *** = P <0.001; n.s = non-significant; Cont = control