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# Water immersion and one-year storage influence germination of the pyrenes of *Copernicia alba* Morong, a palm tree from a neotropical wetland

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**Water immersion and one-year storage influence the germination of the pyrenes of *Copernicia alba* Morong, a palm tree from a neotropical wetland**

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Título reduzido: Water immersion and one-year storage influence the germination of the pyrenes of *Copernicia alba*

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**ABSTRACT** – (Water immersion and one-year storage influence germination of the pyrenes of *Copernicia alba* Morong, a palm tree from a neotropical wetland). Palm seeds are known for displaying dormancy and a thick endocarp that delays germination and embryo growth; but water treatments may accelerate their germination process. Additionally, *ex-situ* conservation can cause seed viability loss over time. Data on seed dormancy and storage have been neglected for many native palm species in Brazil. We investigated the effect of water treatments and one-year storage on the germination of *Copernicia alba* Morong, a palm tree from the Brazilian Pantanal wetland. To address our aim, fresh and stored pyrenes were immersed in water (at room temperature for 24, 48, and 72h) and in hot water (~75°C for 5- and 10-min). Fresh pyrenes germinated up to 84% in control, reaching 100% after water immersion for 48 and 72h. In contrast, one-year storage reduced germination by almost 50%, but water immersion slightly increased the germination of stored pyrenes. On the contrary, hot water decreased germination for both fresh and stored pyrenes. Seeds of *C. alba* may be classified as orthodox seed storage behavior. Taken all together, water treatments at room temperature improved the germination of the pyrenes. In contrast, long-term seed storage and hot-water treatments may jeopardize germination.

**Keywords:** caranday palm, flooding, hot water, Pantanal, seed storage

**RESUMO** – (A imersão em água e o armazenamento de um ano influenciam a germinação dos pirênios de *Copernicia alba* Morong, uma palmeira de um bioma alagado neotropical). As sementes de palmeiras geralmente apresentam dormência e um endocarpo espesso que retarda a germinação e o crescimento do embrião, contudo, tratamentos com água podem acelerar o processo de germinação das suas sementes. Além disso, a conservação de sementes *ex-situ* pode causar perda de viabilidade das sementes ao longo do tempo. Dados sobre dormência e armazenamento de sementes têm sido negligenciados para muitas espécies de palmeiras nativas no Brasil. Nós investigamos o efeito da imersão em água e armazenamento de um ano sobre a germinação de sementes de *Copernicia alba* Morong, uma palmeira do Pantanal brasileiro. Para testar nosso objetivo, pirênios recém-colhidos e armazenados foram imersos em água a temperatura ambiente por 24, 48 e 72h e água quente (~75°C por 5 e 10 min). Pirênios recém coletados germinaram 84% no controle, atingindo 100% após imersão em água por 48 e 72h. Em contraste, o armazenamento de um ano reduziu germinação em ~50%; contudo, a imersão em água promoveu a germinação dos pirênios armazenadas. Por outro lado, a água quente diminuiu a germinação dos pirênios frescos e os armazenados. Sementes de *C. alba* podem ser classificados em ortodoxas. Em geral, a imersão em água à temperatura ambiente aumentou a germinação dos pirênios. Entretanto, armazenamento for longa duração e tratamentos com água quente podem prejudicar a germinação.

**Palavras-chave:** água quente, alagamento, armazenamento de sementes, carandá, Pantanal

## Introduction

Palm seeds often display morphophysiological dormancy due to underdeveloped embryos (Baskin & Baskin 2014) and a thick endocarp that delays germination and embryo growth (Pérez 2009, Ribeiro *et al.* 2011, Oliveira *et al.* 2013, Carvalho *et al.* 2015, Oliveira *et al.* 2015, Soares *et al.* 2021). Water treatments may accelerate the germination process of palm seeds by facilitating oxygen absorption and loosening mechanical restrictions of the surrounding tissues (e.g., Bovi 1990, Martin *et al.* 1996, Ferreira & Gentil 2006, Fava & Albuquerque 2011, Rubio Neto *et al.* 2012, Pinto *et al.* 2012, Goudel *et al.* 2013, Ferreira *et al.* 2021). 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 has been associated with seed viability loss over time (Broschat 1994, Orozco-Segovia *et al.* 2003, Nascimento *et al.* 2010, Jacob & Decruse 2015, Oliveira *et al.* 2015, Felix *et al.* 2017, Beltrame *et al.* 2018, Bastos *et al.* 2021). Several external factors influence the viability of tropical palm seeds during storage, such as temperature and time, and intrinsic factors like seed moisture content, seed maturation, and desiccation sensitivity (recalcitrance) (Roberts 1973, Orozco-Segovia *et al.* 2003, Baskin & Baskin, 2014, Sano *et al.* 2015). Recalcitrant seeds do not tolerate water loss - typically below a threshold <12% - thus rapidly fading in laboratory storage (Hong & Ellis 1996) and showing a low potential for forming soil seed banks (Oliveira *et al.* 2015). On the other hand, orthodox seed storage behavior can be maintained *ex situ* over a long-term in suitable environments (Hong & Ellis 1996). 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* (Jacq.) Lodd. Mart.) from the *Cerrado* savannas (Ribeiro *et al.* 2012) and *Copernicia alba* Morong from Brazilian wetlands (Masetto *et al.* 2012).

Knowledge of seed biology, such as dormancy, germination, and storage, is crucial to aid efforts to improve seed conservation techniques (Ellis *et al.* 2007, Hay & Probert 2013, Costa *et al.* 2017) and to use for restoration programs (Merritt & Dixon 2011, León-Lobos *et al.* 2012, Kildisheva *et al.* 2016, Vittis *et al.* 2020). Therefore, to conserve seeds of a species safely is necessary to assess germination requirements and the category of seed storage behavior to find out the most satisfactory storage environment and the duration of successful storage (Hong & Ellis 1996, Teixido *et al.* 2017). Unfortunately, data on seed dormancy and longevity and storage behavior have been neglected for

several wild native species in Brazil (Ribeiro *et al.* 2016). Such studies have also been limited to a relatively small number of palm species (Jagannathan 2021).

*Copernicia alba* Morong is a palm tree (Arecaceae family) native to tropical and subtropical ecosystems in South America, occurring in the Chacos of Argentina, Paraguay, Bolivia, and the Pantanal in Brazil (Lorenzi *et al.* 2004). This species is commonly named “carandá” or caranday palm, often forming monodominant populations in the Pantanal wetland, which is periodically subjected to flooding followed by dry periods (Pott & Pott 1994). These areas can remain flooded for up to eight months per year (Pott & Pott 1994, Nunes-da-Cunha & Junk 2011, Figure 1 a). In the natural areas, *C. alba* exhibits early successional characteristics, can tolerate fires, and its fruit provide food for wildlife such as macaws, parrots, and fishes (Pott & Pott 1994). The economic relevance of *C. alba* regards its wood durability and its uses in rural constructions such as corrals and fences (Lorenzi *et al.* 2004, Pivetta *et al.* 2011).

Previous studies pointed out that this palm species may display dormancy, and water immersion treatments can enhance germination (Fava & Albuquerque 2011, Masetto *et al.* 2012). Besides, seed storage behavior knowledge is still insufficient (but see Masetto *et al.* 2012). Several species, including palms species that dominate wetlands ecosystems may show fruit/seed dispersal and germination strategies related to the water pulse (Orozco-Segovia *et al.* 2003, Fabri 2018, Pires *et al.* 2018, Bao *et al.* 2018, Oliveira *et al.* 2019, Elias & Viera 2020). Therefore, this study aimed to evaluate the pretreatment in water immersion (~ 25 and 75°C) and the one year of storage on seed germination of *C. alba*, and addressed the following questions: i) do these seeds show dormancy?; ii) what is the effect of water pretreatments on germination of the caranday pyrenes?; iii) what is the classification of germination behavior after one year of storage?; and iv) do the pretreatments in water immersion enhance or reduce the germination of stored seeds? The thick endocarp can hinder germination; thus, water immersion treatments are expected to improve germination. However, we anticipated that seed viability might decrease after the relatively prolonged storage period.

## Material and Methods

Seed collection and benefiting - We harvested fresh, mature, black fruits directly from 15 matrices of *Copernicia alba* Morong from a natural population at the Carandazal trail (Fig 1a), located in a natural area of the Miranda subregion of the Pantanal wetland, in Mato Grosso do Sul State, Brazil (GPS coordinates 19°48'30.1''S and 57°10'13.5''W) in July 2011. The black exocarp was considered the criterion of maturation for fruit harvesting. Harvesting involved cutting the outer branches and dislodging the fresh fruit bunches with a tree pruner, and then fruits fell freely on the plastic wrap

placed on the ground. We took the fruits to the Seed Laboratory at Universidade Federal do Mato Grosso do Sul, Campo Grande, MS, where the experiments were carried out. The exocarp and mesocarp of the fruits were removed by manual friction; then, the pyrenes were inspected for fungal infection and insect attack. All seeds were mixed in a lot. Mature fruit is a dry, fibrous drupe with an ovoid shape and black color, which consists of an exocarp, a fibrous mesocarp, and a stony endocarp that encloses the seeds, called pyrenes with 1-2 cm in length (Figure 1 b-d). Since a thick (rigid) endocarp surrounded the seeds, we used the whole pyrenes as germination units in our study. The initial moisture content of the pyrenes was determined by fresh-weighing two samples of 15 pyrenes and then re-weighing the samples after oven-drying at  $105 \pm 3^\circ\text{C}$  for 24h, according to the Brazilian rules for seed testing (Brasil 2009). After benefiting and initial assessment of moisture content, a subplot of cleaned pyrenes were placed in a paper bag and stored in a dry cold room at  $19^\circ\text{C}$  ( $45 \pm 5\%$  relative humidity) for one year to evaluate viability through germination test after the storage period.

### Experimental factors and germination trials

Germination of pretreated pyrenes - Freshly harvested pyrenes were used to determine the effect of soaking pyrenes in water compared to the control (untreated/non-soaked pyrenes) under laboratory conditions. For the pretreatments, pyrenes were immersed in water at room temperature ( $\sim 25^\circ\text{C}$ ) for 24h; 48h; and 72h; and in hot water at  $75^\circ\text{C}$  for 5 min and 10 min. Plastic trays (20 x 30 x 9 cm) were used to maintain the seeds immersed in water under constant fluorescent light for the desired times, with daily water replacement.

After water immersion treatments (at room temperature and hot water), germination trials were carried out. Seeds were placed in rolls of germitest® paper pre-moistened with distilled water at 2.5 times dry the paper weight (Brasil 2009). All rolls of pretreated and untreated pyrenes were kept inside plastic bags to prevent water loss and placed in incubator set to 12 h photoperiod of fluorescent light (4 x 20 W) and alternating temperatures of 20-30°C, based on optimal germination condition for *C. alba* (Fava & Albuquerque 2011). Rolls were rotated within the incubators four times each week. We daily performed germination checks for up to 45 days with protrusion of the cotyledonary petiole  $\geq 2$  mm as the criterion for germination (Janick & Paull 2008, Baskin & Baskin 2014).

Effect of storage and pretreatments on germination of the stored pyrenes - After one year of storage, stored pyrenes were used to assess the viability through a germination test and categorized the seed storage behavior. Seed-bearing species are, in most cases, recognized in three categories based on Roberts (1973) and Ellis et al. (1990): orthodox or desiccation-tolerant (seed can be maintained ex-situ over the long-term using conventional genebank protocols); recalcitrant or desiccation intolerant

(seeds tolerate short-term storage under well-defined and well-controlled environments); and intermediate or partly desiccation-tolerant (seed can be maintained in medium-term storage).

Prior the experiments were set, two replicates of 15 stored pyrenes were used to estimate the water content (Brasil 2009) (see seed collection and benefiting subsection). Pretreatments by soaking the pyrenes in water (*see* Germination of pretreated pyrenes) were also applied to the stored pyrenes, before the germination tests, compared to a control (untreated/non-soaked stored pyrenes) under laboratory conditions. Then, stored pyrenes (pretreated and untreated) were set to germinate in rolls of germitest® paper as described above.

At the end of all germination trials, we assessed the germination percentage (G%) and mean germination time (MGT) based on the number of daily germinated pyrenes (Labouriau 1983, Ranal & Santana 2006). Additionally, we calculate the germination speed index according to Maguire (1962). Non-germinated pyrenes were classified as dormant or dead, although a tetrazolium test was not performed to evaluate if the seeds were or were not viable; neither dormant nor dead seeds were included in the data.

A completely randomized factorial experimental design was used with 2 storage conditions (non-stored and stored pyrenes) x 6 pretreatments (control; ~25°C - 24h, 48h, and 72h; and in hot water at 75°C for 5 and 10 min) with four replicates of 25 pyrenes (=100 individual pyrenes) were used for each treatment and control.

Data analysis - Germination variables (G%, MGT, and speed index) were evaluated using analysis of variance with permutations (10,000 iterations) with the *lmPerm* package (Wheeler & Torchiano 2016) in R statistical software (R Core Team 2020). Models compared variable values as a function of water immersion treatments (control, soaking in water for 24, 48, and 72h, hot water for 5 and 10 min), storage period (freshly harvested and stored pyrenes), and the interaction between them (see Supplementary material table S1). The analyses were followed by Tukey HSD tests to address multiple comparisons among all treatment combinations.

## Results and Discussion

*Copernicia alba* Morong seeds displayed no evidence of dormancy in the tested treatments. Freshly harvested pyrenes germinated 84% (Figure 2 a), indicating a high germination percentage. Masetto *et al.* (2012) also found similar findings for the same species collected in a natural area in the Pantanal. Contrasting with our study, Masetto *et al.* (2012) and Fava & Albuquerque (2011) affirmed that the study species exhibits seed dormancy. Genetic factors may affect seed germination by influencing seed viability and quality in the same species from different areas (Soler-Guilhen *et al.* 2020). Initial moisture content of the pyrenes was ~10%, with corresponding results of 10% (Fabri



2018) and 16.6% (Masetto *et al.* 2012) for the same species. In both studies fruits were collected from natural populations of Pantanal wetland in the Mato Grosso do Sul State. The thick endocarp of *C. alba* was permeable to water, not impeding the seeds from germinating. Seeds surrounded by a stony endocarp may block the germination causing its delay for months or years, such as reported to *Butia capitata* Mart.Becc. (Soares *et al.* 2021) and *Syagrus romanzoffiana* (Cham.) Glassman. (Oliveira *et al.* 2015), both species from the Brazilian savanna (Cerrado), and *Bactris maraja* Mart from the Amazon Forest (Rodrigues *et al.* 2014). Fortunately, the lack of seed dormancy of *C. alba* can warrant recruitment in the Pantanal wetlands where seeds/pyrenes are subjected to flooding conditions demonstrated by a rapid flush of seedling emergence in the post-flood environment (Bao *et al.* 2018). Even though many palm seeds may display morphophysiological dormancy related to the undeveloped embryo and/or mechanical restrictions imposed by a thick endocarp (Pérez 2009, Baskin & Baskin 2014), pyrenes of our study species can be easily germinated under laboratory conditions.

The storage period reduced the germination performance of the study species. The germination percentage of stored pyrenes decreased to  $\leq 50\%$  (Figure 2 b). Germination times varied from 18 to 33 days in freshly harvested, and around 18 days in the stored pyrenes (Figure 2 c, d). Speed index followed the same patterns of G% (Figure 2 e, f). This germination parameter can be used as a tool to evaluate the rate of germinated seeds/pyrenes (or emerged seedlings) per day, also applied as a metric for seedling vigor (Maguire 1962, Ranal & Santana 2006). The initial water content of the stored pyrenes decreased to 7% after the one-year period of storage. This study demonstrated that stored seeds of *Copernicia alba* Morong species from the Pantanal wetland, even though the germination capacity was reduced over time, can remain relatively viable and germinable at least over the time frame of this study for one year. However, these results showed a limitation of using only germination studies to assess viability. Viability tests such as tetrazolium and X-ray tests are widely used in conservation programs (Terry *et al.* 2003, Gosling 2004, Riebkes *et al.* 2015) and could have been applied to evaluate whether the non-germinated stored seeds remained viable or undergone some kind of secondary dormancy state (Schlindwein *et al.* 2019). Future studies should assess viability through these methods and test different storage conditions to maintain higher germinability of the seeds of the study species.

Regarding seed behavior storage, we suggest that *C. alba* Morong seeds may be classified as desiccation-tolerant (orthodox) given their low water content ranging from 10 to 7% and the moderate germination percentages maintained after the storage period. Hong & Ellis (1996) define as orthodox those seeds that tolerate desiccation  $\leq 12\%$  moisture content and remain viable during 12 months of subsequent storage. Nevertheless, the authors also pointed out that investigations of seed survival in different storage environments, such as at lower temperatures ( $-20^{\circ}\text{C}$ ), should be tested to confirm if the seeds show intermediate rather than orthodox seed storage behavior. Recent research has shown



that some tropical species of the Arecaceae family produce seeds that exhibits desiccation tolerance after storage (Orozco-Segovia *et al.* 2003, Souza *et al.* 2016). In *Cerrado* ecosystems, the seed of *Acrocomia aculeata* (Jacq.) Lodd. ex Mart. has been identified as desiccation-tolerant and can be stored over one year under low temperatures (10°C, see Ribeiro *et al.* 2012). *Ex-situ* seed conservation is a worldwide concern (CBD 2010, Walters *et al.* 2013); however, longevity patterns remain a matter of inquiry for most tropical palm trees (Broschat 1994, Pivetta *et al.* 2011). The natural habitats where the study species occurs can experience prolonged events of drought in pluriannual dry spells of the Pantanal (Thielen *et al.* 2020), such as those that occurred in 2020; therefore, the desiccation tolerance may play a critical role in maintaining this species in the ecosystem under a set of dry years. Nevertheless, low-temperature conservation and *in vitro* culture propagation methods remain unstudied for seeds of *C. alba*.

Overall, water treatments positively influenced germination. The freshly harvested pyrenes reached 100% of germination after immersion in water for 48 and 72h (Figure 2 a). Stored pyrenes germinated 32% after soaking in water for 24h but reached ~50% after 48 and 72h (Figure 2 b). The water treatments for 48 and 72h also improved MGT of freshly harvested pyrenes (to around 20 days) compared to the control (25 days, Figure 2 c), thus providing faster germination. In contrast, there was no change in the mean germination time of stored pyrenes among treatments (Figure 2 d). Hence, water immersion for two-to-three days improves the germination of freshly harvested pyrenes of *C. alba*, which may accelerate germination and seedling growth for nursery production. The number of germinated seeds per day was improved by water immersion, as indicated by the speed index after 48 and 72h, mostly for the freshly harvested pyrenes (Figure 2 e). Water immersion pretreatments also promoted germination of *Butia capitata* (Mart.) Becc. pyrenes (Arecaceae) from the *Cerrado* (Soares *et al.* 2021); and seed of *Copernicia prunifera* (Mill.) H.E. Moore (carnauba) from the Brazilian Northeast region (Reis *et al.* 2011) that seems to display flood-tolerant seedlings (Arruda & Calbo 2004). Both species occur in flooded environments, suggesting that the enhancement of germination ability after immersion in water can improve the competitive performance following flooding events in their natural habitats.

On the contrary, hot water treatments negatively affected the germination process. The germination percentage (G%) of freshly harvested pyrenes of *C. alba* decreased to 62 and 50% for 5 and 10 minutes of exposure, respectively (Figure 2 a, b). Such treatments also reduced the germination percentage of stored pyrenes to 21% for 5-min and 16% for 10-min of exposure (Figure 2 b). Additionally, the 5-min hot water treatment delayed the mean germination time of freshly harvested pyrenes to 30 days (Figure 2 c), with no effect on MGT after storage (Figure 2 d). Likewise, hot water treatments jeopardized the germination of *Euterpe oleracea* Mart. (Arecaceae) seeds from the Amazon

rainforest (Bovi & Cardoso 1976). Heat shock also did not break the seed dormancy of *A. aculeata* (Jacq.) Lodd. ex Mart. (macaw palm) but caused high seed deterioration rates (Rodrigues-Junior et al. 2016).

The tolerance of *C. alba* seeds/pyrenes to prolonged water immersion should be further investigated to understand if the heightened germination is sustained. High and fast germination after flooding can be a vital trait for maintaining monodominance since most buried propagules are recruited at the end of the seasonal flooding period in the Pantanal wetlands (Bao *et al.* 2018).

### Conclusions

Our study highlights some important considerations for studies involving water immersion to accelerate seed/pyrene germination and the *ex-situ* conservation of a tropical palm species. The results reveal that water imbibition of seeds enclosed by a thick endocarp was not blocked, promoting the seeds germination. *Copernicia alba* Morong seed did not display dormancy, exhibiting a high germination capacity in the control. The pyrenes stored for one year at 19°C under a relative humidity of 45% decreased the water content from 10 (initial) to 7% and germination rates by almost 50%. Therefore, we suggested that our study species should be classified as orthodox. Soaking in water for 48 and 72h improved the germination rate and time of freshly harvested and stored seeds; however, hot treatments reduced the germination capacity delaying the germination time.

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### Conflict of Interest Statement

The authors declare no conflict of interest.

### Credit authorship contribution statement

**Vanessa Couto Soares:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Formal analysis, Writing - original draft, Writing - review & editing, and Visualization.

**Luís Felipe Daibes:** Conceptualization, Formal analysis, Data curation, Writing - review & editing, Visualization.

**Geraldo Alves Damasceno-Junior:** Conceptualization, Resources, Writing - review & editing, Visualization, and Funding acquisition.

**Liana Batista de Lima:** Supervision, Conceptualization, Methodology, Writing - original draft, Validation, Formal analysis, Investigation, Resources, Data curation, and Writing - review & editing, and Visualization.

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### Supplementary material

Supplementary material Table S1. Statistical tests of the analysis of variance with permutation, considering different germination parameters (G%, mean germination time and speed index) and the interactions of water immersion treatments and storage period on the germination of *Copernicia alba* Morong palm tree from the Brazilian Pantanal wetland.

Parameter	Treatments	Sum Sq	F value	P
Germination (%)	Water treatments	12460	37.505	<0.001
	Storage period	21505	323.659	<0.001
	Treat X Storage	411	1.236	0.313
MGT	Water treatments	203.8	14.64	<0.001
	Storage period	398.8	143.22	<0.001
	Treat X Storage	145.3	10.44	<0.001
Speed index	Water treatments	3.1155	52.124	<0.001
	Storage period	2.0917	174.975	<0.001
	Treat X Storage	0.2229	3.728	0.008



Figure 1. a. Adult plants of *Copernicia alba* Morong from a natural population (locally named carandazal) occurring in a natural area of Pantanal wetland, in Miranda, Mato Grosso do Sul State, Brazil. b. Mature fruits. c. Fruits with exposed mesocarp. d Pyrenes (seeds surrounded by the rigid endocarp). Photos by V.C. Soares.

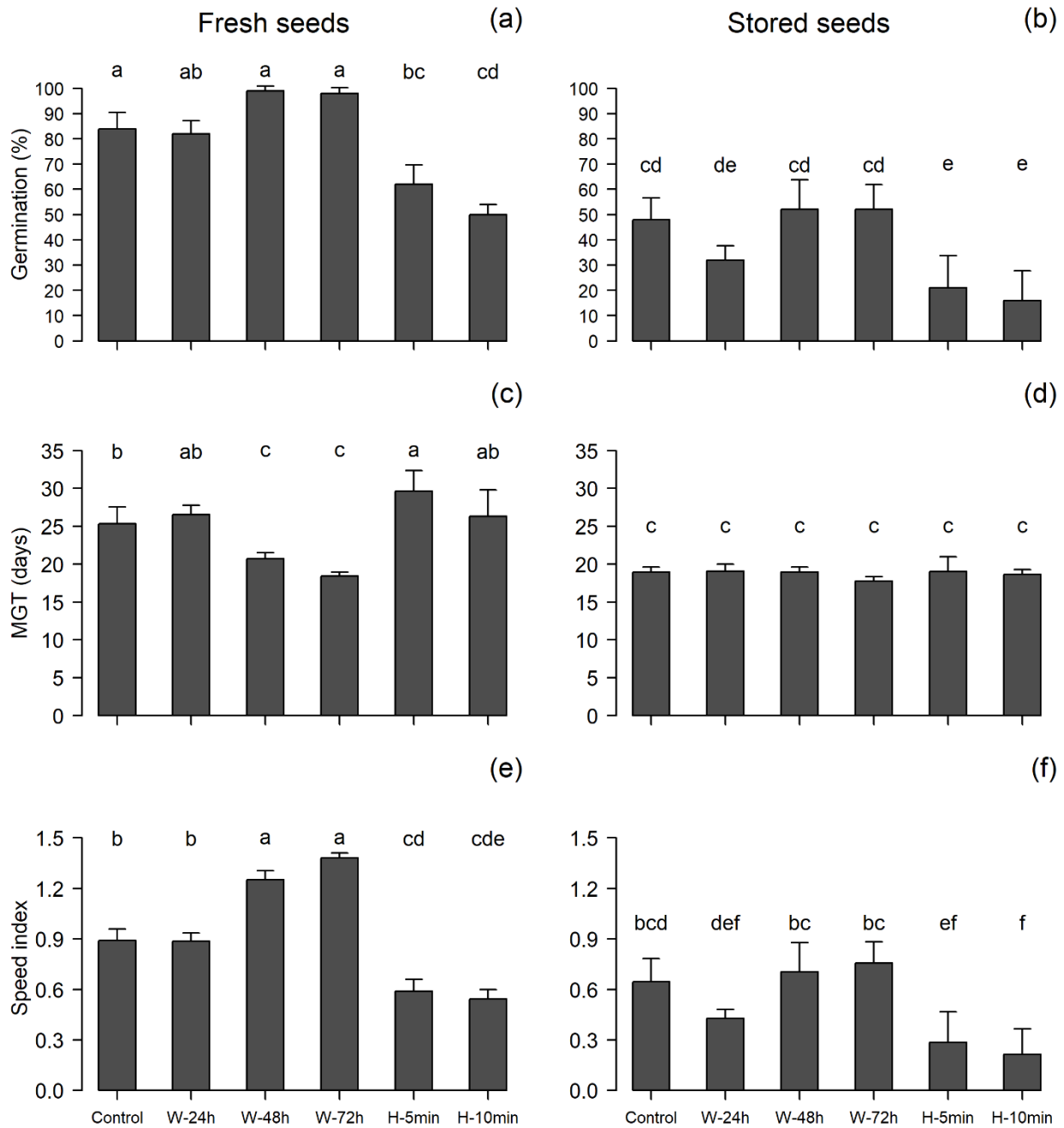


Figure 2. a-b. Germination percentage. c-d. Mean germination time. e-f. Speed index of freshly harvested (right panels) and one-year stored pyrenes of *Copernicia alba* Morong (left panels) from a natural population from the Mato Grosso do Sul State, Brazil, under water immersion (W) treatments for 24, 48, and 72 hours (at room temperature); and hot water (H, 75°C) for 5 and 10 minutes of exposure. Different letters indicate significant differences among treatments for each germination parameter.

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