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Article

Impacts of storage on germination and development of red ginger seeds: a tropical ornamental species

Impactos do armazenamento na germinação e desenvolvimento de sementes de alpinia-vermelha: uma espécie ornamental tropical

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Abstract: Ornamental species exhibit variability in seed size, shape, color, and reserve tissue composition. These variations often affect seed germination and seedling development. Proper seed storage is essential for maintaining seed quality. Although seed quality is not improved during storage, seeds can be preserved under favorable conditions. The objective of this study was to assess the effects of storage temperature and period on the germination of *Alpinia purpurata* seeds. The experiment was conducted in a germinator and in a greenhouse, using a completely randomized design with four replications, in a 2x3 factorial arrangement consisting of two temperature conditions (ambient temperature at 25 °C; refrigeration temperature at 5 °C) and three storage periods [zero (control), 30, and 60 days]; each experimental unit consisted of 25 seeds. The evaluated variables were seed moisture content, percentages of germination and emergence, germination speed index, mean germination and emergence times, and seedling characteristics (shoot and root lengths and number of leaves). The results showed that the storage period negatively affected germination and the emergence and development of *A. purpurata* seedlings.
Keywords: *Alpinia purpurata*, floriculture, temperature, tolerance to desiccation.

Resumo: Sementes de espécies ornamentais apresentam variabilidade no tamanho, forma, cor e composição dos tecidos de reserva. Essas variações, muitas vezes, influenciam diretamente no processo de germinação e, nas etapas da produção de mudas. O armazenamento adequado também desempenha um papel fundamental na qualidade das sementes, no entanto, a qualidade não pode ser melhorada durante o armazenamento, mas pode ser preservada quando as condições de conservação são favoráveis. Objetivou-se com este estudo avaliar os efeitos da temperatura e do tempo de armazenamento na germinação das sementes de *Alpinia purpurata*. O experimento foi conduzido em germinador e casa de vegetação. Delineamento inteiramente casualizado em um esquema fatorial 2x3, duas condições de temperaturas (ambiente e refrigerado) e três períodos de armazenamento (controle, 30 e 60 dias) com quatro repetições com 25 sementes por unidade experimental. As variáveis avaliadas foram o teor de água, porcentagem de germinação e emergência, índice de velocidade de germinação, tempo médio de germinação e de emergência. E as características da plântula tais como comprimento da parte aérea e da raiz e número de folhas e diâmetro foram avaliados. Os resultados revelaram que o tempo de armazenamento das sementes influenciaram negativamente na germinação, emergência e desenvolvimento de plântulas de *A. purpurata*.
Palavras-chave: *Alpinia purpurata*, floricultura, temperatura, tolerância a dessecação.

Introduction

Floriculture is a significant economic activity in Brazil, continuously growing and expanding across the country (Souza et al., 2020). In this sense, various plant species have been identified with ornamental potential for cut flower production, including *Alpinia purpurata* (Zingiberaceae), known as red ginger. This species is recognized worldwide for its lush inflorescences, long vase life, and continuous blooming, making it highly valued in flower markets (França et al., 2019). The inflorescences of this species are characterized by a terminal spike shape, reaching up to 30 cm in length, composed of brightly colored bracts ranging from red to pink and tubular flowers attached to the inflorescence peduncle (França et al., 2020).

Seeds of ornamental plants produced through sexual propagation exhibit variability in size, shape, dormancy, color, and reserve tissue composition. These variations generally affect the germination process and several stages of seedling production (Senar, 2018). Although seed propagation offers the advantage of producing a large number of seedlings, obtaining high-quality seeds for ornamental plants is often costly and limited to a few companies (Senar, 2018). Additionally, the variability in seed quality results in different responses to the germination environment, hindering seedling uniformity and increasing cultivation costs (Cardoso and Vendrame, 2022). This highlights the importance of investigating methods that optimize seed germination and seedling development, focusing on improving production efficiency and supporting breeding programs that depend on genetic variability and availability of viable seeds.

Deterioration during storage is another crucial aspect that affects seed germination and viability, influenced by seed chemical composition, seed coat structure, reserve quantity, and abiotic variables, including temperature and humidity (Rosa et al., 2023; Guariz et al., 2022). Seeds of perennial tropical species such as *A. purpurata* are classified as recalcitrant, as they have exhibit moisture content and are not desiccation-resistant, challenging long-term storage (Roberts, 1973). This characteristic, combined with seed variability, poses challenges to obtaining uniform seedlings, increasing costs and impacting production efficiency.

Therefore, identifying optimal seed storage conditions, including temperature and seed moisture content, is essential to preserve seed viability and minimize the incidence of fungi and other microorganisms during storage (Santos et al., 2022; Bianchini et al., 2021). In this context, the objective of this study was to assess the effects of storage conditions in terms of temperature and storage period on the germination of *Alpinia purpurata* seeds, thus contributing to the advancement of breeding programs and the efficiency of seedling production for this ornamental species.

Material and Methods

Study area and plant material

The study was conducted at the experimental area of the State University of Mato Grosso (UNEMAT), in Tangará da Serra, Mato Grosso (MT), Brazil (14°39'S, 57°25'W, and altitude of 321 meters), using seeds from *Alpinia purpurata* plants from an active germplasm bank of tropical

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flowers. The region's climate is tropical, with a dry season from May to September and a rainy season from October to April. The mean annual rainfall depth is 1,830 mm (Dallacort et al., 2011).

Seeds were produced from September to November 2022 through manual cross- pollination between the cultivars Kimi (donor) and Jungle King (recepient) *A. purpurata* cultivars. After pollination, inflorescences were protected with organza bags to prevent contamination by external pollinators. Fruits were harvested 70 days after pollination, at which point they were considered mature based on their purple coloration and the change in seed color from white to brown (Fig. 1A and B).

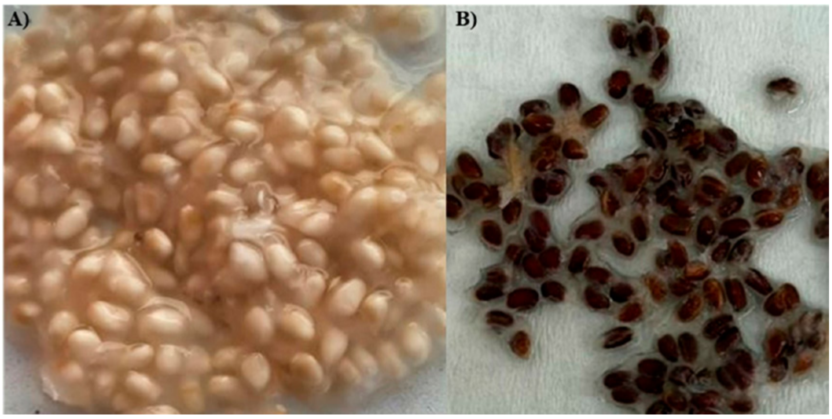

Fig. 1. Immature (A) and mature (B) *Alpinia purpurata* seeds.

Seeds were manually extracted and washed in running water to remove mucilage and part of the aril, followed by drying at ambient temperature for 24 hours before storage and evaluation. Seed moisture content was assessed by weighing the samples on a precision balance and drying them in an oven at 105 °C for 24 hours, following the *Regras Para Análise de Sementes* of Brazil (Rules for Seed Analysis) (Brasil, 2009).

After drying at ambient temperature for 24 hours, the seeds were processed, divided into equal portions to ensure each lot contained the same quantity of seeds, and packaged in kraft paper bags. The seeds had moisture content of 24.4% at the time of storage. Some seed samples were stored under ambient temperature (25 °C) and others were refrigerated (5 °C) for 30 and 60 days, according to the experimental treatments.

Seed germination and seedling emergence tests

Germination was assessed by placing the seeds on a paper towel moistened with distilled water at a ratio of 2.5 times the weight of the dry paper (Brasil, 2009). Four 25-seed replications were maintained in a germinator chamber (Mangelsdorf model) set to a temperature of 25 ºC and a 12-hour photoperiod. Germinated seeds were counted daily for 30 days to determine the germination speed index, as described by Maguire (1962). The number of normal seedlings was counted after 30 days; normal seedlings were defined as those with well-developed root systems and shoots, with primary green leaves, as described in Brasil (2009).

Seedling emergence was determined using four 25-seed replications sown in 23×16×4 cm trays containing a commercial substrate composed of peat moss, perlite, vermiculite, and dolomitic limestone; the substrate was moistened to 70% of field capacity. The trays were placed in a greenhouse with 50% shade. They were irrigated using overhead sprinklers, with three one-minute intervals in the morning and three two-minute intervals in the afternoon to maintain substrate moisture.

Seedling emergence was monitored daily for 60 days after sowing (DAS) to calculate the emergence speed index (Maguire, 1962). The daily number of emerged seedlings in each replication was summed and divided by the number of days since sowing. The number of emerged seedlings was used to determine the percentage of emergence, and the mean emergence time was assessed (Labouriau, 1983). Normal seedlings were measured at 60 DAS for shoot and root lengths (cm) and number of leaves.

The experiment was conducted in a completely randomized design with four replications, using a 2x3 factorial arrangement consisting of two temperature conditions (ambient temperature at 25 °C; and refrigeration temperature at 5 °C) and three storage periods [zero (control), 30, and 60 days]; each experimental unit consisted of 25 seeds.

The obtained data were evaluated for normality using the Shapiro-Wilk test at a 5% significance level and met the assumptions for analysis of variance. Analysis of variance was conducted based on the significance of the F-test, and means were compared using the Tukey's test at 5% significance level. All statistical analyses were performed using the Sisvar® software (Ferreira, 2011).

Results and Discussions

Analysis of variance showed that only the storage period had a significant effect on seed germination. Storage of *Alpinia purpurata* seeds resulted in reductions in seed moisture content compared to non-stored seeds (Fig. 2). The moisture reduction during storage is a crucial factor, as it is directly connected to the maintenance of seed viability. Seeds with low moisture content have low metabolic activity, which can contribute to the preservation of their germination capacity. However, the critical point at which moisture loss could compromise seed viability should be investigated, mainly in the case of recalcitrant seeds (Cécel and Barbedo, 2023).

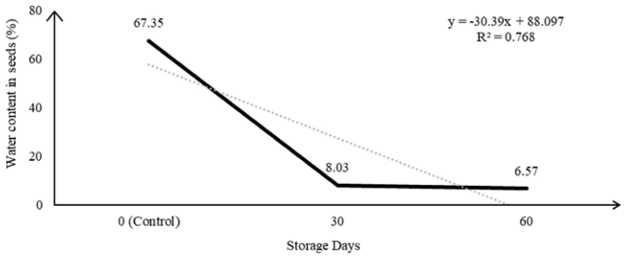

Fig. 2. Moisture content in *Alpinia purpurata* seeds stored for zero (control), 30, and 60 days in kraft paper bags.

Storage conditions, species-specific characteristics, and the chemical composition of seed reserves directly affect the preservation period of seed physiological quality, i.e., seed deterioration rate (Ohse, 2022), particularly for recalcitrant seeds, such *A. purpurata* seeds. Assessing seed longevity is important to identify the period during which seeds can be stored and remain viable under controlled environmental conditions (Vitis et al., 2020). A study on *Alpinia malaccensis* showed that seed moisture content reached up to 35% and recommended further investigations to determine seed viability after storage (Rival et al., 2015).

Considering that moisture content is a limiting factor for seed germination and seedling stability in the field, water availability is a determinant of plant distribution within ecosystems (Leal et al., 2020; Rodrigues et al., 2019). *A. purpurata* seeds stored for 30 and 60 days exhibited a significant decrease in germination percentage, at 48% and 28.5%, respectively, compared to the control (Fig. 3A). This reduction may be attributed to moisture loss, indicating a desiccation intolerance in the seeds.

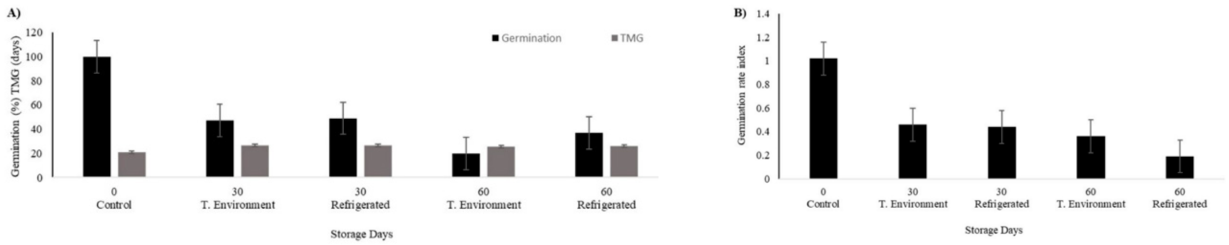

Fig. 3. Germination percentage, mean germination time (A), and germination speed index (B) of *Alpinia purpurata* seeds stored under different temperature conditions for zero (control), 30, and 60 days.

Furthermore, the longer storage period negatively affected seed viability, resulting in a higher number of ungerminated seeds and abnormal seedlings (Table 1).

Germination is a complex process that transform a quiescent embryo into a metabolically active organism that develops well-differentiated tissues, essential for establishing a healthy seedling (Nautiyal et al., 2023). The successful germination of *A. purpurata* seeds directly depends on the efficient activation of these metabolic processes, which can be compromised by prolonged storage periods that reduce seed moisture content and, consequently, negatively affect seedling development.

Table 1. Mean and standard deviation of germination and seedling emergence percentages of *Alpinia purpurata* seeds.

	Germination			Emergence		
Days of storage	Normal seedlings	Abnormal seedlings	Ungerminated seeds	Normal seedlings	Abnormal seedlings	Ungerminated seeds
0	21.00 ± 2.58^{a}	0.00^{c}	3.75 ± 2.75^{c}	22.5 ± 1.29^{a}	1.25 ± 1.5^{b}	1.25 ± 0.5^{c}
30	5.00 ± 0.40^{b}	7.00 ± 1.22^{a}	13.00 ± 1.47^{b}	10.87 ± 2.92^{b}	4.62 ± 1.77^{a}	7.62 ± 2.49^{b}
60	4.00 ± 1.08^{b}	2.62 ± 0.85^{b}	17.75 ± 1.55^{a}	4.12 ± 2.28^{c}	6.25 ± 1.65^{a}	14.62 ± 0.85^{a}

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

The mean germination time found for the control treatment was of 21 days, significantly shorter compared to seeds stored for 30 and 60 days, which had means of 27 and 26 days, respectively, with no significant difference between them (Fig. 3A). This delay in the germination of stored seeds may be attributed to physiological changes during the storage period. The storage of recalcitrant seeds, such as *A. purpurata*, can result in changes in metabolism, such as reduced cellular respiration and mobilization of energy reserves, which are essential for germination (Berjak and Pammenter, 2013). Additionally, non-stored seeds showed higher germination speed indices and better seedling establishment (Figure 3B), indicating that seed freshness is crucial for optimal performance.

Seeds of *Elettaria cardamomum*, also from the family Zingiberaceae, have required treatments to overcome dormancy, including the use of mechanical, physical, and chemical methods, to reach up to 90% germination (Dahanayake, 2014). This reinforces the diversity of germination responses in Zingiberaceae species and highlights the importance of understanding the specific factors affecting germination for each species.

According to the analysis of variance, the percentage of emergence and the emergence speed index were significantly affected by the factors (storage period and temperature). Mean germination time and the establishment of normal seedlings were significantly affected by the storage period factor. The high moisture content in freshly harvested seeds and the intolerance to storage observed in *A. purpurata* seeds reinforce their recalcitrant nature. These results are consistent with the descriptions by Medeiros and Eira (2006), who highlighted that recalcitrant seeds do not tolerate moisture losses below critical levels (generally between 15% and 50%) and are sensitive to storage temperatures below 15 °C (Rossetti et al., 2023).

The percentage of seedling emergence under greenhouse conditions was 90% for non-stored seeds (Fig. 4A). However, this percentage decreased to 51% and 30% after 30 and 60 days of storage at ambient temperature, respectively (Fig. 4A). The mean germination time in the control treatment was 38 days (Fig. 4A), indicating that prolonged storage significantly impacts seed viability and vigor, and consequently, successful seedling establishment.

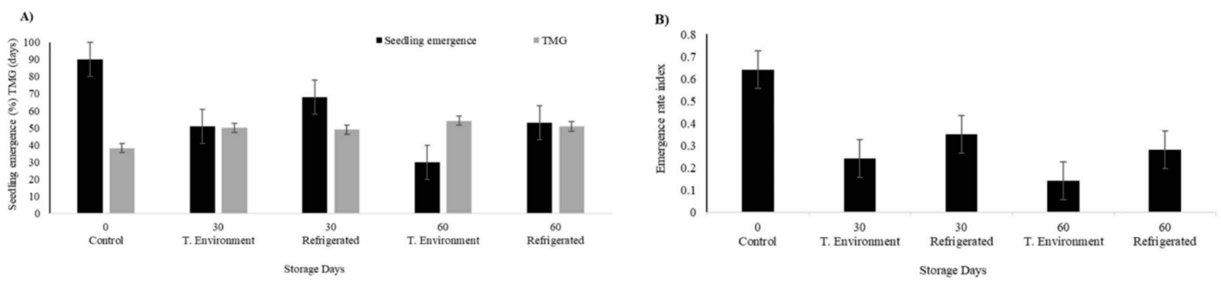

Fig. 4. Percentage of seedling emergence, mean emergence time (A), and emergence speed index (B) of *Alpinia purpurata* seeds stored under different temperature conditions for zero (control), 30, and 60 days.

Stored seeds showed a mean germination time of 49 and 52 days for the 30- and 60-day storage periods, respectively (Fig. 4A). Contrastingly, non-stored seeds exhibited higher emergence speed indices and more efficient seedling establishment (Fig. 4B). Fresh *A. purpurata* seeds had greater viability, yielding a significantly higher number of normal seedlings (Table 1), indicating that sowing freshly harvested seeds is essential for maximizing seedling production efficiency.

Storage negatively impacted seedling development, resulting in increased percentages of abnormal seedlings and ungerminated seeds (Table 1). This directly compromises seedling quality and can reduce successful plant establishment in the field. Abnormal seedlings tend to have lower vigor and reduced ability to compete with weeds and adapt to adverse conditions, potentially resulting in lower survival rates (Reed et al., 2022).

Seedlings from control seeds exhibited better shoot and root development than those from seeds stored for 30 and 60 days (Fig. 5). Stored seeds resulted in seedlings with reduced vigor and fewer leaves compared to control seeds (Fig. 5). A low number of leaves reduces the available leaf area for light capture, limiting photosynthesis and, consequently, the production of essential carbohydrates needed for root and shoot growth. Moreover, a low quantity of leaves is associated with low vigor, which negatively affects seedling growth and adaptation to the field. The limited root development observed in seedlings from stored seeds compromised water and nutrient absorption, reducing plant vigor and their potential for successful establishment under field conditions.

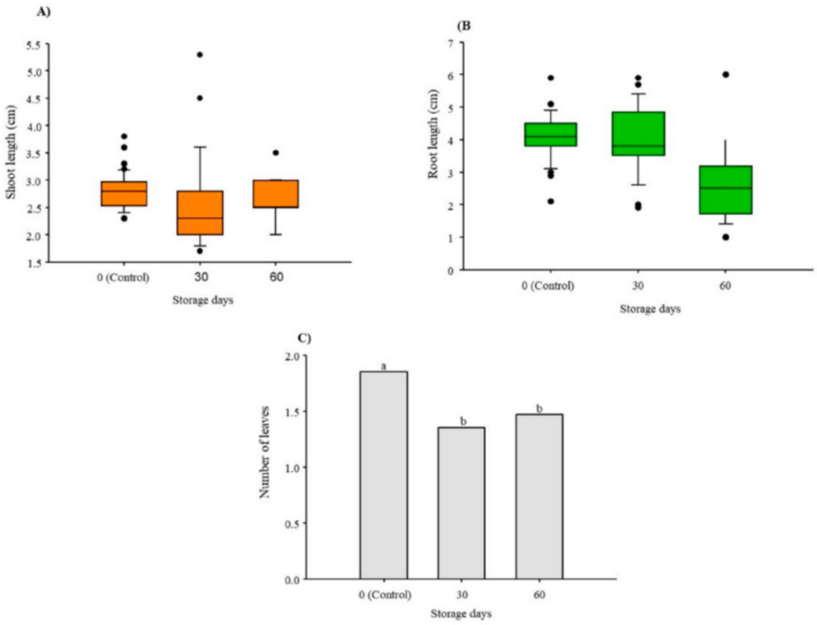

Fig. 5. Shoot and root lengths and number of leaves in *Alpinia purpurata* seedlings from seeds stored for zero (control), 30, and 60 days.

The results of the present study showed that not only storage period and environmental conditions, but also the substrate used significantly impacted the development of *A. purpurata* seedlings. Seedling vigor and root development were directly affected, highlighting the importance of considering not only water availability, temperature, and light, but also the suitability of the substrate to optimize germination and subsequent plant growth (Silva et al., 2024). A study on *Alpinia malaccensis* showed that the use of alternative substrates, such as charcoal and rice husks, resulted in radicle growth up to 14 cm, denoting the positive impact of adequate substrates on root development (Rival et al., 2015). This highlights the importance of investigating alternative substrates to promote the development of healthy and vigorous *A. purpurata* seedlings.

Conclusions

Storage of *Alpinia purpurata* seeds under refrigeration resulted in a higher germination percentage compared to storage at ambient temperature. However, seeds stored for 30 and 60 days resulted in less seedling development than control seeds (zero days of storage), indicating that prolonged storage periods negatively affect seedling vigor.

Although seedling development decreased, storage did not completely inhibit the germination potential of *A. purpurata* seeds, indicating that while seedling vigor and growth were compromised, the retained some viability.

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Author Contribution

RPAF: field work, data collection and analysis, manuscript preparation and review. **MHC**: data analysis. **LSN**: data analysis. **TTA**: data analysis. **WK**: data analysis and interpretation, manuscript review. **CAS**: data analysis and interpretation, manuscript preparation and review.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

All the research data is contained in the manuscript.

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