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Scientific Article

EX SITU CONSERVATION: SEED STORAGE STRATEGIES FOR Monimiaceae (Laurales) SPECIES IN THE ATLANTIC FOREST

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ABSTRACT

The Atlantic Forest is one of the world's hotspots, being a center of diversity for several plant groups, including Monimiaceae, however, this high diversity is under intense pressure. The species *Grazielanthus arkeocarpus* and *Mollinedia ovata* of the Monimiaceae are found in the Biological Reserve of Poço das Antas, in Rio de Janeiro state, where fires threaten the existence of these individuals. Furthermore, *G. arkeocarpus* is critically endangered and microendemic. Therefore, implementing initiatives aimed at the ex situ conservation of these species is of utmost importance. The present study's objective was to analyze seed storage behavior of the species to conserve in seed banks. Mature fruits were collected and benefited, and the seeds were conducted for completely randomized experiments. The seeds were divided into three batches: fresh seeds (control), with 30%, and 10% water content (WC). Then, each batch was stored for five days at temperatures of -20, 10, and 20 ºC. Germination tests were conducted at temperatures of 20 and 15/25 ºC for 30 days, and the viability of ungerminated seeds was checked. Statistical analyses were performed using GLM with the Binomial model in R Software. Fresh seeds of *G. arkeocarpus* and *M. ovata* have a water content of 57% ± 2.5 and 58% ± 2.5, respectively. The *G. arkeocarpus* batch with 10% WC presented zero germination at all storage temperatures, but the viability percentage was above 80% when stored at -20 and 10 °C, indicating the presence of primary dormancy. In contrast, the *M. ovata* batches with 30% and 10% WC obtained relatively high percentage germination, above 60%. The data demonstrate that the seeds of both species are orthodox and can be stored in seed banks for the ex situ conservation of these species.

Keywords: Seed banks, Germination, Endemism, Orthodox

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CONSERVAÇÃO EX SITU: ARMAZENAMENTO DE SEMENTES DE ESPÉCIES DE Monimiaceae (Laurales) NA MATA ATLÂNTICA

RESUMO A Mata Atlântica é um hotspot mundial, abrigando diversos grupos de plantas, incluindo Monimiaceae. *Grazielanthus arkeocarpus* e *Mollinedia ovata* são espécies de Monimiaceae encontradas na Reserva Biológica de Poço das Antas - RJ, onde incêndios ameaçam a existência desses indivíduos. Além disso, *G. arkeocarpus* é microendêmica da área e criticamente ameaçada de extinção. Portanto, é de suma importância implementar iniciativas que visem à conservação ex situ dessas espécies. O objetivo do presente estudo foi analisar o comportamento de armazenamento das sementes de ambas as espécies para conservá-las em bancos de sementes. Frutos maduros foram coletados, beneficiados e as sementes foram conduzidas para experimentos inteiramente casualizados. As sementes foram divididas em três lotes: sementes frescas (controle), 30% e 10% de teor de água (TA). Em seguida, cada lote foi armazenado por cinco dias em temperaturas de -20, 10 e 20 ºC. Os testes de germinação foram conduzidos em temperaturas de 20 e 15/25 ºC por 30 dias e a viabilidade das sementes não germinadas foi verificada. As análises estatísticas foram realizadas usando GLM com o modelo Binomial no Software R. As sementes frescas de *G. arkeocarpus* e *M. ovata* têm um teor de água de 57% ± 2,5 e 58% ± 2,5, respetivamente. O lote de *G. arkeocarpus* com 10% de teor de água apresentou germinação igual a zero em todas as temperaturas de armazenamento, mas a viabilidade foi superior a 80% quando armazenadas em -20 e 10ºC, indicando possível dormência primária. Em contraste, os lotes de *M. ovata* com 30% e 10% TA apresentaram viabilidade relativamente alta, todas acima de 60%. Os dados demonstram que as sementes de ambas as espécies são ortodoxas e podem ser armazenadas em bancos de sementes.

Palavras-Chave: Bancos de sementes, Germinação, Endemismo, Ortodoxas

1. INTRODUCTION

The Atlantic Forest is one of the biomes with the highest species richness globally, with many of them being endemic and highly threatened with extinction, making it a priority for global conservation efforts (Mayrinck et al., 2019). The Atlantic Forest has frequent criminal fires and expansion of deforested areas, and the climate increased 3ºC in some areas in the last 60 years (Coelho, 2024). This scenario creates an urgency to collect and store seeds of plants for future reintroduction programs and species conservation (Palomeque et al., 2020). However, knowledge regarding the optimal conditions for seed storage is still limited (Palomeque et al., 2020).

A group with a high number of threatened species in the Atlantic Forest is the family Monimiaceae. Besides, the Atlantic Forest is also one of the diversity centers of Monimiaceae, with about 20% of its species endemic to this biome (Lírio et al., 2020, 2023). On the other hand, only one seed germination study has been conducted with this family until now, which investigated the seeds germination potential of *Mollinedia clavigera* Tul. (Schneider et al. (2019). Consequently, germination studies are necessary for conservation actions for Monimiaceae species to prevent their extinction and provide material for the restoration and enrichment of tropical forests, including ex situ conservation (Sommerville et al., 2021).

Seed banks are one of the most effective ex situ conservation strategies to prevent species extinction (Mayrinck et al., 2019; Sommerville et al., 2021; Andrade et al., 2021). These banks play a crucial role in conserving species, aiming to maintain seeds alive and secure for extended periods, provided they are subjected to ideal storage conditions (Mayrinck et al., 2019; Andrade et al., 2021). The first step to succeed in using this strategy is to understand seed desiccation tolerance, as it is a key factor in maintaining high viability (Daws et al., 2006; Mayrinck et al., 2019; Palomeque et al., 2020). When subjected to storage, seeds undergo a natural aging process, leading to reduced quality and, eventually, a loss of germination capacity if storage conditions are inadequate (Vitis et al., 2020). Temperature, humidity, and oxygen concentration are environmental factors directly affecting seed deterioration (Vitis et al., 2020). Reducing seed water content is one of the methods used to extend viability during storage (Palomeque et al., 2020).

Seed desiccation tolerance is the most crucial characteristic in seed bank conservation, defined as an organism's ability to restore metabolic functions after drying (Silva et al., 2019; Ley-López et al., 2023). There is no well-defined limit regarding seed storage behavior and desiccation tolerance, but it is known that orthodox seeds tolerate moisture levels below 7% and temperatures close to -20°C, allowing them to be stored for long periods, while recalcitrant seeds cannot withstand desiccation and cannot be stored in seed banks (Palomeque et al., 2020; Wawrzyniak et al., 2020). There is also a third group classified as suborthodox, which tolerate desiccation between 7% and 10% and storage at low temperatures (Silva et al., 2019; Vitis et al., 2020; Paul et al., 2025). Tropical species may also exhibit dormancy, a stage in which seeds do not germinate even when all ideal conditions for the process are present, leading to variations in seed germination rates during storage (Qin et al., 2010; Baskin & Baskin 2014; Andrade et al., 2021; Ley-López et al., 2023). However, such classification is important for establishing a foundation for the conservation of threatened plants and understanding the seed storage capacity of species in this biodiversity hotspot through seed responses to desiccation and storage at different temperature ranges (Mayrinck et al., 2019; Vitis et al., 2020). In addition, scientific interest has been aroused to understand the storage behavior of forest seeds in the face of climate change to conserve native species (Silva et al., 2019).

Within this context, investigating storage behavior becomes essential, as this characteristic emerges as a crucial adaptive strategy for species survival in challenging environments. Thus, this study analyzed and compared the storage behavior of two species of Monimiaceae seeds to promote ex situ conservation through seed banks: *Grazielanthus arkeocarpus* Peixoto & Per.-Moura and *Mollinedia ovata* Ruiz & Pav. It is noteworthy that, to date, there are no records in the scientific literature on the germination of these two species, which reinforces the importance and originality of this work.

2. MATERIAL AND METHODS

2.1 Study area

The seeds were collected in the Biological Reserve of Poço das Antas (RBPA), located in the municipality of Silva Jardim – Rio de Janeiro (22º30`S, 42º15` W). This area is a fragment of the Atlantic Forest located in Rio de Janeiro state, Brazil, where *G. arkeocarpus*, endemic to RBPA and Critically Endangered, and *M. ovata*, classified as Least Concern (Lírio et al., 2023; Peixoto et al., 2023), occur. This location is frequently affected by human-induced fires, jeopardizing the existence of various species of fauna and flora (Peixoto et al., 2023). Originally established for the protection of the habitat of the golden lion tamarin (*Leontopithecus rosalia* L.), the reserve comprises fragments of submontane, alluvial, and Dense Ombrophilous Forest, occasionally affected by river floods in some sections (Vieira & Pessoa, 2001; Pereira et al., 2008). It spans approximately 5000 hectares, bordered by the São João River and the Juturnaíba reservoir. The region's climate is defined as "Aw," Tropical with a Dry Season, according to the Köppen-Geiger climate classification (Kottek et al., 2006). The municipality has an average annual rainfall of 1177.6 mm concentrated in the months of October to March and an average annual temperature of 25°C, varying between averages of 18°C to 32°C throughout the year (INMET, 2010).

2.2 Seed collection

To assess seed storage behavior fruits from the two species were collected at RBPA based on availability in June 2021. In the field, mature fruits were non-systematically collected from eight individuals of *G. arkeocarpus* (Figure 1a – c), a limited number due to the rarity of the species, and 26 individuals of *M. ovata*, species which the most individuals available (Figure 1b - d). *Grazielanthus arkeocarpus* is a species with ovaries that develop in fruits enclosed in a receptacle that split open during the maturation, showing the fruits, and

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Mollinedia ovata possesses calyptrate female flowers, i.e., the calyptra falls after pollination, exposing the ovaries that develop in fruits outside the receptacle (Lírio et al., 2025).

Subsequently, the seeds (Figure 1c - d) were placed in plastic bags and homogenized in a single batch per species to obtain the best sampling of the population group in the area. In the laboratory, we manually removed the fruit pulp and washed the seeds under running water. After superficial drying with paper towels, we placed the seeds in sealed impermeable plastic bags and stored in a cold chamber (10ºC and 18% RH) until the experiments were set up (Andrade & Pereira, 1997).

2.3 Storage treatments

Determining the water content (WC) of fresh seeds (control) and the weight of 1000 seeds was according to MAPA (2009). We divided the seeds into three batches and placed them in Kraft paper envelopes for desiccation. The first lot remained with the water content of fresh seeds, while the other two batches were subjected to the drying room (18ºC, 18% RH) until reaching 30% and 10% water content. To achieve 10% water content at the end of drying, we used a drying chamber (0.5 m³) at a controlled temperature of 22°C, using active silica gel (Andrade & Pereira, 1997; Nery et al., 2014). After drying, we stored each sub-lot in sealed impermeable polyethylene packaging at 10°C

Figure 1. Field image of the multiple syconiform fruit with mature drupes of *Grazielanthus arkeocarpus* Peixoto & Per.-Moura (a); and branch with multiple cupuliform fruits and green drupes of *Mollinedia ovata* Ruiz & Pav. (b). Seed of *G. arkeocarpus* (c). Seed of *M. ovata* (d)

Figura 1. Imagem de campo do fruto syconiforme múltiplo com drupas maduras de *Grazielanthus arkeocarpus* Peixoto & Per.-Moura (a); e ramo com frutos cupuliformes múltiplos e drupas verdes de *Mollinedia ovata* Ruiz & Pav. (B). Semente *G. arkeocarpus* (c). Semente *M. ovata* (d) for 24 h to balance the water content between the seeds (Andrade & Pereira, 1997). Finally, we stored the three lots at temperatures of -20, 10, and 20°C for five days (Andrade & Pereira, 1997).

2.4 Germination experiments

We conducted the germination experiment in gerbox-type boxes containing a 3 cm layer of previously sterilized fine vermiculite, moistened with distilled water at twice the substrate weight (MAPA, 2009). Vermiculite was selected because it provides greater contact between moisture and the seed surface, given that the seeds of both species are oval, smooth and relatively large or germination on paper substrate. Furthermore, both species are annual forest with low seed productivity. Therefore, sterilized vermiculite provided the most suitable conditions for germination, ensuring adequate water availability and greater moisture stability within the germination boxes (MAPA, 2009).

We distributed the seeds of each sub-lot (control, 30%, and 10% WC) after the storage described above, in four replicates with 20 seeds and incubated in germination chambers at temperatures 20 and 15/25ºC (temperatures similar to the habitat) and 8/16h photoperiod for 30 days.

After sowing, all gerbox boxes were closed with their lids to minimize substrate moisture losses. We rewetted every two days, ensuring a layer of water formed on the substrate. Germination was assessed every other day considering seeds germinated those with protrusion of the radicle with positive geotropism (Andrade & Pereira 1997; Andrade et al. 2021).

At the end of the germination test, we verified the non-germinated seeds viability by the cutting test with a scalpel (Baskin & Baskin, 2014). Those with rigid coverings and a healthy embryo were considered viable, (Baskin & Baskin, 2014).

2.5 Statistical analyses

Predictors for analyzing seed storage behavior were seed water content (control, 30%, and 10%), storage temperature (-20, 10, and 20°C), and incubation temperature (20 and 15/25ºC). Response variables were germination and viability. We analyzed the data using generalized linear models (GLM, Ime4 package) (Zuur et al., 2009; Bates et al., 2015) with a binomial error structure and a logit link function for germination and viability data. The best model considering all generalized linear models (GLM) was selected using AIC information criterion (Akaike, 1973). We conducted the analyses in the R statistical platform (R Core Team 2022).

3. RESULTS

The water content of fresh seeds of *G. arkeocarpus* was 57% (± 2.5) and the weight of 1,000 seeds was 127 g. Seeds from the control treatment showed low germination percentages at all storage temperatures (-20, 10, and 20°C) under incubation conditions (20 and 15/25°C) (Figure 2, Table 1). Storage of the control batch at -20ºC presented 70% of not viable seeds, which was significant (estimated value = 0.6591, $p < 0.001$) (Figure 2, Table 1). The treatment applied to seeds with 30% water content also showed a significant reduction in viability when stored at −20°C, with non-viability ranging from 25% to 60% (estimate = −1.31, $p < 0.0144$). In contrast with the previous results, seeds treated with 10% water content showed a significant 5% increase in germination compared to the control treatment (0%) (estimate = 1.64, $p = 0.036$). These seeds also exhibited consistently higher viability across all storage temperatures (estimate = 0.78, $p < 0.001$) (Figure 2, Table 1). Predictors treatment (control, 30%, and 10% water content) and storage temperature were significant in promoting germination (Table 1).

For *M. ovata*, the water content of fresh seeds was 58% ± 2.5 and the weight of 1000 seeds was 106g. Seeds from the control treatment showed germination above 75% when subjected to storage temperatures 10º and 20ºC (Figure 3, Supplementary material 2). Storage of the control batch at -20ºC presented 75% of not viable seeds when incubated at 20ºC, which was significant (estimated value = 3.967, $p = 0.001$) and 90% at 15/25 ºC (value = 4,00, $p = 0.001$) (Figure 3, Table 1). Compared with the control batch, seeds with a 30% water content exhibited a significant 60% reduction in germination across all storage and incubation temperatures (estimated value = −4.0111, p =

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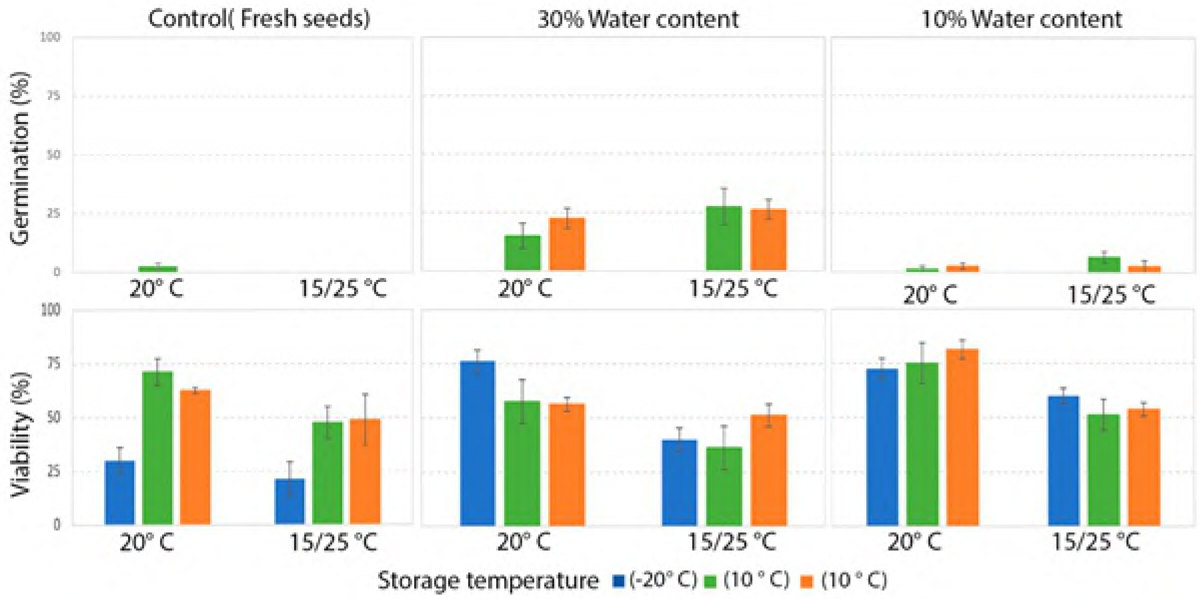

Figure 2. Germination and viability of seeds *Grazielanthus arkeocarpus* Peixoto & Per.-Moura. The fresh seeds (Control) and with 30% and 10% water content subjected to storage at temperatures of -20, 10, and 20 ºC for five days and incubated at temperatures of 20 and 15/25 ºC
Figura 2. Germinação e Viabilidade de sementes de *Grazielanthus arkeocarpus* Peixoto & Per.-Moura. Sementes frescas (controle), com 30% e 10% de teor de água, submetidas ao armazenamento nas temperaturas de -20, 10 e 20 ºC por cinco dias e incubadas nas temperaturas de 20 e 15/25 ºC

Table 1. Results from the binomial GLM for germination and viability in response to temperature of storage treatment (-20, 10, and 20°C), water content (Control, 30%, and 10%), and incubated temperatures (20 and 15/25 ºC) *Grazielanthus arkeocarpus* Peixoto & Per.-Moura and *Mollinedia ovata* Ruiz & Pav.
Tabela 1. Resultados do GLM binomial para germinação e viabilidade em resposta à temperatura do tratamento de armazenamento (-20, 10 e 20°C), teor de água (Controle, 30% e 10%) e temperaturas de incubação (20 e 15/25ºC) *Grazielanthus arkeocarpus* Peixoto & Per.-Moura e *Mollinedia ovata* Ruiz & Pav.

G. arkeocarpus	**Germination**			**Viability**		
	d.f	Deviance (X^2)	P-valor	d.f	Deviance (X^2)	P-valor
Water content	2	113556	<0.001*	2	34003	<0.001*
Storage	2	75429	<0.001*	2	7268	0.0264*
Temperature	1	3323	0.0683	1	54677	<0.001*
Water content × Storage × Temp.	13	10205	0.6771	12	53294	<0.001*
M. ovata	**Germination**			**Viability**		
	d.f	Deviance (X^2)	P-valor	d.f	Deviance (X^2)	P-valor
Water content	2	550.49	<0.001*	2	445.73	<0.001*
Storage	2	408.44	<0.001*	2	24.86	<0.001*
Temperature	1	3.06	0.08	1	4.05	0.0442*
Water content × Storage × Temp.	14	23.02	0.06	12	18.22	0.1091

0.001). The viability remained around 55% at all three storage temperatures tested when seeds were incubated at 20ºC (estimated value = 2.2345, $p < 0.001$) (Figure 3, Table 1). The batch with 10% water content exhibited a significant reduction in germination across all storage and incubation temperatures compared with the control batch (estimated value = -6.4191, $p < 0.001$) (Figure 3, Supplementary material 2). Furthermore, seeds stored at -20 ºC exhibited 50% higher viability than the control batch stored under the same temperature conditions, regardless of incubation temperatures (estimated value = -1.135, $p < 0.227$) (Figure 3, Supplementary material 2). Predictors treatments and storage temperature were important in promoting germination ($X^2 = 550.49$, $p < 0.001$; $X^2 = 408.44$, $p < 0.001$), but the interaction between them was not significant ($X^2 = 23.02$, $p = 0.06$). The interaction between predictors, treatments, storage temperature, and incubation temperature was not significant for viability.

4. DISCUSSION

Seeds of *G. arkeocarpus* and *M. ovata* are orthodox and they are viable (70% and 75%) even with the water content reduced from (57% ± 2.5) to 10% and being stored at low temperatures (-20 and 10 ºC). This information is consistent with what was proposed by Roberts (1973) and contributes significantly to the ex situ conservation of species, as it indicates that these species can be efficiently preserved in seed banks, one of the safest and most cost-effective strategies for preserving genetic resources. The viability maintained under these conditions confirms that these seeds can be stored long-term, which is essential for future ecological restoration and biodiversity protection. In general, seeds are future plants that will form after the germination process and many can be stored for years, making them the primary propagule used in reforestation and species reintroduction programs for threatened species (Udayangani et al., 2020).

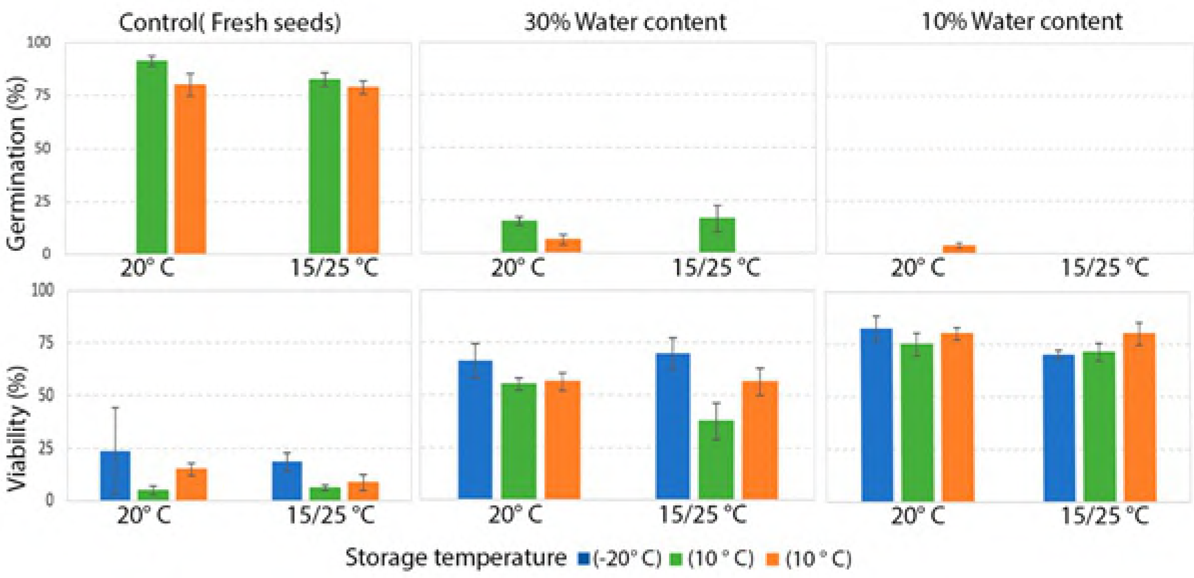

Figure 3. Germination and viability of seeds *Mollinedia ovata* Ruiz & Pav. The fresh seeds (Control) and with 30% and 10% water content subjected to storage at temperatures of -20, 10, and 20 ºC for five days and incubated at temperatures of 20 and 15/25 ºC

Figura 3. Germinação e Viabilidade de sementes de *Mollinedia ovata* Ruiz & Pav. Sementes frescas (controle), com 30% e 10% de teor de água, submetidas ao armazenamento nas temperaturas de -20, 10 e 20 ºC por cinco dias e incubadas nas temperaturas de 20 e 15/25 ºC

Fresh seeds (control) of *G. arkeocarpus* and *M. ovata* showed low viability and germination when stored at -20ºC. This may have occurred because the combination of the high-water content (57% ± 2.5 and 58% ± 2.5) inside the seeds, combined with the negative temperature (-20ºC) during storage, causes several complications such as damage to the cell membrane system and loss of viability due freezing, reducing germination (Corbineau, 2024). Seed store studies are crucial for the conservation of plant species, especially those threatened with extinction. Understanding how factors such as water content and temperature affect seed viability allows the development of appropriate long-term preservation techniques, ensuring the maintenance of genetic diversity and the possibility of recovering natural populations in the future. This knowledge is essential for seed banking programs, ecological restoration, and food security.

Seeds from batches with 10% water content showed viability above 80% when stored at temperatures between -20º and 10ºC, agreeing with Mayrinck et. al (2016)

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when they say that the drying process in orthodox seeds can contribute to the development process, also influencing their germination. For *G. arkeocarpus* seeds, the data suggest that they may present dormancy, as the control lot containing 57% ± 2.5% moisture content showed zero germination in all treatments, but viability was around 50% when stored at 10 and 20°C for both incubation temperatures. The partial desiccation (30% WC) was not sufficient to prevent damage under freezing conditions, and the results show a decrease in the viability of these seeds, indicating a need for greater desiccation. Meanwhile, *M. ovata* seeds showed germination above 70% in the control batch at storage temperatures of 10º and 20ºC, confirming the absence of dormancy (Baskin & Baskin 2014). However, *M. ovata* batches with 30% and 10% moisture content showed a 60% reduction in germination compared to the control batch. Even so, viability remained relatively high, above 60% in seeds with 30% moisture content and around 70% in those with 10% moisture content, suggesting the occurrence of secondary dormancy. It is possible to divide seed dormancy into two types: primary dormancy, which is part of the natural maturation process of the species, being a genetic characteristic, and secondary dormancy, which develops through induction, triggered by an environmental stimulus (Lima, 2022). Environmental factors during storage, such as oxygen concentration, temperature, and humidity, can induce physiological changes and secondary dormancy in seeds that were not dormant previously and/or break an existing dormancy (Andrade et al., 2021).

Fruit morphology influences the germination and physiology of forest species seeds, as characteristics such as size, pericarp, and texture determine water penetration and interactions with animal dispersers. Fleshy fruits adapted to specific disperser appetites (birds, mammals) favor dormancy breaking and dispersal efficiency. In the case of *Grazielanthus arkeocarpus*, its fleshy drupe favors zoochoric dispersal, enhancing germination after passage through the digestive tract. The floral morphology of pistillate flowers, in turn, impacts pollination by specialized insects, influencing the production of viable fruits and, consequently, the quality of germinable seeds (Leal & Koski, 2023).

5. CONCLUSION

The seeds of *G. arkeocarpus* and *M. ovata* are orthodox and can be preserved in seed banks at -20°C, provided their water content is reduced to approximately 10%. This ex situ conservation strategy can complement the in-situ actions already carried out in the Poço das Antas Biological Reserve. However, for seedling production, *G. arkeocarpus* seeds require more than 30 days of incubation due to secondary dormancy induced by storage at low temperatures. To enable efficient and low-cost seedling production, especially for post-fire restoration, further studies are needed to characterize the germination mechanisms, the maximum storage time tolerated for each species, and the type of dormancy that occurs after storage.

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AUTHOR CONTRIBUTIONS

Farias, R.G.S.: Conceptualization, Project Administration, Data Curation, Investigation, Formal analysis, Visualization, Writing original draft, review & editing; Lírio, E.J.: Conceptualization, Validation, Supervision, review; Silva, W.B: Conceptualization, Project Administration, Data Curation, Visualization, Review & editing; Andrade, L.G.: Conceptualization, Data Curation, Software, Review.

DATA AVAILABILITY

The entire dataset supporting the findings of this study has been published within the article.

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