



Effect of storage on the germination of *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos seeds

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Keywords

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Palavras-chave

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Abstract: *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos is a tree species of high ecological, economic, and landscape value. Its seeds show significant variations in quality during storage, which poses difficulties in the development of propagation techniques. The aim of this work was to study the physiological behavior of *H. chrysotrichus* seeds during storage by varying the degree of seed humidity and storage temperature. The seeds were stored in paper bags in two storage environments: a refrigerator (7 °C) and a controlled laboratory environment (25 ± 2 °C). The stored seeds were evaluated monthly for moisture content, germination percentage, and germination speed index (GSI) for up to 12 months. *H. chrysotrichus* seeds are orthodox but have a low longevity under natural conditions (25 ± 2 °C), remaining viable in the environment for less than six months. The best conditions for the preservation of the seeds of this species were obtained by storing them in the refrigerator at 7 °C, which maintained the physiological quality of the seeds unchanged for 12 months.

Resumo: *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos é uma espécie arbórea de alto valor ecológico, econômico e paisagístico. Suas sementes apresentam variação significativa na qualidade durante o armazenamento, o que representa dificuldades no desenvolvimento de técnicas de propagação. O objetivo do trabalho foi estudar o comportamento fisiológico das sementes de *H. chrysotrichus* durante o armazenamento pela variação no grau de umidade das sementes e na temperatura de armazenamento. Para tanto as sementes foram acondicionadas em sacos de papel em dois ambientes de armazenamento, mantidas em ambiente de geladeira (7 °C) e em ambiente controlado de laboratório (25 ± 2 °C). As sementes armazenadas foram avaliadas mensalmente em até 12 meses quanto ao grau de umidade, a porcentagem de germinação e o Índice de Velocidade de Germinação (IVG). As sementes de *H. chrysotrichus* são ortodoxas, mas de baixa longevidade em condições naturais (25 ± 2 °C), pois se mantêm viáveis no ambiente por menos de seis meses. A melhor condição para a conservação de sementes dessa espécie foi obtida com o armazenamento na geladeira (7 °C), que manteve inalterada a qualidade fisiológica das sementes por 12 meses.



Introduction

Interest in the propagation of native forest species has intensified due to the emphasis on environmental issues, highlighting the need to recover degraded areas and restore landscapes (BISPO; VIEIRA, 2022). In this context, knowledge of the biology of native species is fundamental for the implementation of such projects, since these species are the most suitable for restoration projects. They make the ecosystem closer and more balanced than the existing one. Among various species used in reforestation programs, the genus *Handroanthus* stands out, whose main propagation method is by seed (LORENZI, 2020). Among various species used in reforestation programs, the genus *Handroanthus* stands out, whose main propagation method is by seed (LORENZI, 2020).

The *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos (Bignoniaceae) is a deciduous (leaf abscission at a certain time of the year), heliophytic (needs sunlight), hygrophytic (tolerates humid environments) tree species native to Brazil and characteristic of the rainforest. The species is found in the states of Minas Gerais, Rio de Janeiro, and Rio Grande do Sul, mainly in the Atlantic rainforest of plains and floodplains that are humid and partially soaked during summer rains (ARRUDA et al., 2017; LORENZI, 2020). It also occurs in the floodplains of the broadleaf forest of the Paraná Basin and the Cerrado, where it is found in both primary forest and secondary formations (LORENZI, 2020; BISPO; VIEIRA, 2022), playing an important ecological role in pollination, biodiversity conservation, and the recovery of degraded ecosystems. In addition to these characteristics, *H. chrysotrichus* also has a high ornamental and economic value, as its wood is moderately heavy and resistant, and it is a species widely used in urban afforestation due to its striking yellow flowers (SAMPAIO; COSTA; MENDES-RODRIGUES SAMPAIO, 2013; LORENZI, 2020; SIMÕES et al., 2021).

Thus, the growing demand for seedlings of native species has highlighted the need to develop protocols that improve the production of high-quality seedlings capable of meeting the requirements in the field for future plantations (LEÃO et al., 2015; FARIA et al., 2017; REZENDE et al., 2023), and it is essential to develop further studies with native forest species to contribute information on germination tests and seed storage conditions, since the vegetation in these environments is in a critical state and vulnerable to constant climate change, deforestation, and land use (ARMENTERAS et al., 2021; BISPO; VIEIRA, 2022).

Storage is maintaining the quality of seeds until they are used for sowing. This process is fundamental for species whose seeds quickly lose their physiological quality and cannot be sown immediately after harvest, and is important for the conservation of genetic resources through germplasm banks (MARCOS FILHO, 2015; BARBOSA; PASSOS; DUARTE, 2024). It should be noted that deterioration is a natural and inevitable process of physical destruction and loss of physiological capacity, but it can be controlled, and this is the essence of storage, which is one of the *ex situ* conservation strategies. The longevity of stored seeds is mainly influenced by their initial quality, the degree of humidity, the time elapsed between harvest and storage, the phytosanitary and thermal treatments applied, the type of packaging, and the temperature and relative humidity of the storage environment (OLIVERA et al., 2012; CIPRIANI; GARLET; LIMA, 2019).

Additionally, seed storage should start at physiological maturity, and the main challenge is to ensure that the seeds are still of high quality after a certain period, prolonging their longe-

vity by controlling the degree of humidity, temperature, and conditions of the storage environment (CIPRIANI; GARLET; LIMA, 2019; BARBOSA; PASSOS; DUARTE, 2024).

Therefore, considering the above, the aim of this study was to evaluate the germination behavior and the degree of humidity of *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos seeds stored in two environments for 12 months.

Material and Methods

The research was carried out at the Seed and Plant Tissue Culture Laboratory of the Universidade Federal de Santa Maria (UFSM).

The fruits of the *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos were collected from mother trees in the UFSM area in November 2022 and brought to the laboratory for seed extraction. The seeds were stored for 365 days (12 months) in permeable paper packages inside airtight plastic bags in two different storage conditions: 7 °C (refrigerator environment) and 25 ± 2 °C (laboratory environment). The following experiments were performed monthly during the 12-month period:

Moisture content: The moisture content of the seeds was determined immediately after collection and every 30 days (up to 12 months), using the oven method at 105 °C for 24 hours (BRASIL, 2009), on two 1 g samples per treatment, with the results expressed as a percentage according to the following formula:

$$U (\%) = \frac{100 \times (P-p)}{P - t}$$

Where: P = initial weight, p = final weight, t = tare (weight of the container).

Germination test: 200 seeds were used (20 replicates of 10 seeds) in gerbox plates (11 x 11 x 3.5 cm) lined with *germitest* paper and kept in a growth chamber at 25 ± 2 °C (BRASIL, 2009; MARTINS; LAGO; SALES, 2009) with 12 hours of light per day. The paper was moistened with a volume of distilled water equal to three times its weight without hydration (BRASIL, 2009). Evaluations, performed daily for 14 days after the test was set up, provided data on the percentage of germination (BRASIL, 2009). A seedling established as normal was considered germinated according to the formula below and the methodology of Brasil (2009).

$$\%G = (N/100) \times 100$$

Where: N = number of seeds germinated at the end of the test.

Germination Speed Index: obtained by counting the daily number of emerged seedlings in the germination test and calculating the Germination Speed Index (GSI) following the procedures described by Maguire (1962), according to the formula below:

$$GSI = (G1/N1) + (G2/N2) + \dots + (Gn/Nn)$$

Where: GSI = Germination Speed Index; G1, G2, Gn= number of seeds germinated in the first, second, and last count; N1, N2, Nn= days after sowing on the first, second, and last counts.

The experimental design was entirely randomized, in a factorial scheme, involving 2 treatments (storage temperature) over 12 months of evaluation, with 20 replicates of 10 seeds each being used for each treatment, giving a total of 200 seeds. The data were submitted to analysis of variance, and when the treatments showed a significant difference ($p < 0.05$), the means were compared using the Scott-Knott test at 5% probability.

Results and Discussion

Handroanthus chrysotrichus seeds had a variable moisture content depending on the storage conditions and over the period of the experiment, with the freshly harvested seeds having a moisture content of about 12.6% (Table 1).

Table 1 - Moisture content (%) of *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos seeds in two storage conditions during 12 months of treatment

Time (months)	Laboratory environment (25 ± 2 °C)	Refrigerator environment (7 °C)
0	12,60 aA*	12,60 aA
1	8,72 cB	10,01 bA
2	8,32 cB	9,18 bA
3	8,60 cB	9,49 bA
4	9,02 bA	8,34 cA
5	9,40 bA	7,78 cB
6	8,20 cA	7,21 dB
7	8,27 cA	7,06 dB
8	9,88 bA	7,48 dB
9	9,60 bA	7,75 cB
10	9,06 bA	7,13 dB
11	9,55 bA	7,75 cB
12	8,25 cA	6,91 dB

*Averages followed by the same lowercase letter in the column and uppercase letter in the row do not differ significantly by the Skott-Knott test at 5% probability.

Source: Authors (2024).

When looking at the evaluations of seed moisture content carried out after 365 days of storage, there was a decrease in this variable compared to the values initially obtained for seeds stored in a refrigerated environment (7 °C), unlike seeds stored in a laboratory environment (25 ± 2 °C), where the moisture content was significantly higher compared to the treatment stored at 7 °C (Table 1).

The moisture content of the seeds stored at 25 ± 2 °C decreased from 12.6 to 8.20%, a decrease of 65.48%, while the moisture content of the seeds stored at 7 °C decreased from 12.6% to 6.91%, a decrease of 54.8% (Table 1). In a similar study carried out by Barbosa, Passos and Duarte (2024) with *Tabebuia aurea* (Silva Manso) Benth. & Hook. F. ex S. Moore stored in a laboratory environment, the degree of humidity decreased by about 50% after 9 months of storage, with an initial moisture content of 14.9%; however, for seeds stored in a refrigerator, the degree of humidity values were not significant, with a degree of humidity after 9 months of 10%. Marcos Filho (2015) and Peske (2019) state that seeds with a high degree of humidity tend to lose viability more quickly because humidity leads to an intensification of the respiratory activity of the seed, consuming its nutritional reserves, and the higher the moisture content of the stored seed, the greater the number of factors adverse to the conservation of its physiological quality.

Additionally, moisture content is of fundamental importance because it indicates the degree of seed maturity and influences the maintenance of physiological quality during storage. One of the main characteristics that gives orthodox seeds high viability is low moisture content (GUARDIA et al., 2020). According to Marcos Filho (2015), during storage, the temperature and the relative humidity of the air surrounding the seeds are the main factors affecting the preservation of the seeds. Of these two factors, relative humidity is the most important because of its direct relationship with the water content of the seed during storage. Almeida et al. (2010) and Menegaes et al. (2021) point out that storage does not improve the quality of seeds, but rather preserves them; however, depending on the plant species, the loss of viability during storage is intensified depending on the storage temperature and the packaging used.

Additionally, Marcos Filho (2015) and Tonetto et al. (2017), emphasize that seed storage is an important tool for preserving species. However, the germination potential of seeds depends largely on their water content, storage conditions, and storage period. Furthermore, reducing metabolic rates can prolong seed longevity in storage. This can be achieved by lowering the temperature and using packaging that minimizes gas and water exchange with the environment (JEROMINI et al., 2015; BRITO et al., 2020). In a refrigerator environment, metabolic processes, especially cellular respiration, are reduced, which ensures the survival of the species by reducing or inhibiting the reactivation of germinative metabolism.

H. chrysotrichus seeds are considered orthodox seeds (MARTINS; LAGO; SALES, 2009), and according to Marcos Filho (2015), Moraes et al. (2020) and Taiz et al. (2021), orthodox seeds not only tolerate desiccation but also show an increase in longevity by reducing storage temperature and humidity. Supporting this, *Caesalpinia echinata* Lam. seeds germinated even at a humidity level close to 7.6% (BARBEDO; BILIA; FIGUEIREDO-RIBEIRO, 2002). In a study by Pinto Junior et al. (2012), which analyzed the appropriate storage condi-

tions for maintaining the physiological quality of *Jatropha curcas L.* seeds, they found that the moisture content of seeds stored in the refrigerator was around 6.98% and in laboratory conditions was around 9.14%. It is worth noting that in this study, even the seeds with a reduced degree of moisture after collection, with a reduction of about 50%, had a germination percentage of over 85% (Table 2).

Table 2 - Germination percentage (%G) and germination speed index (GSI) of *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos seeds in two storage conditions during 12 months of treatment

Time (months)	Laboratory environment (25 ± 2 °C)		Refrigerator environment (7 °C)	
	G (%)	GSI	G (%)	GSI
0	94 aA*	1,98 aA*	94 aA	1,98 aA
1	91 aA	1,69 bB	93 aA	1,93 aA
2	79 bB	1,25 cB	91 aA	1,77 bA
3	83 bB	1,27 cB	96 aA	1,63 cA
4	48 cB	0,62 dB	94 aA	1,59 cA
5	20 dB	0,31 eB	86 bA	1,41 dA
6	0 eB	0 fB	89 bA	1,39 dA
7	0 eB	0 fB	85 bA	1,37 dA
8	0 eB	0 fB	88 bA	1,43 dA
9	0 eB	0 fB	85 bA	1,45 dA
10	0 eB	0 fB	89 bA	1,47 dA
11	0 eB	0 fB	88 bA	1,41 dA
12	0 eB	0 fB	88 bA	1,42 dA

*Averages for each parameter followed by the same lowercase letter in the column and uppercase letter in the row are not significantly different by the Skott-Knott test at 5% probability.

Source: Authors (2024).

During the 12-month experiment, the germination percentage of the seeds stored in a laboratory environment, i.e., at a temperature of 25 ± 2 °C, was reduced in the second month of storage, and from the sixth month there was no more germination; in the treatment where the seeds were stored in a refrigerator (7 °C), the seeds proved to be viable with a germination percentage considered satisfactory for the species, and only after 150 days (5 months) of storage there was a reduction in germination, with 86% (Table 2).

There were significant differences in germination percentage between the two storage environments, and from the second month on, there was a reduction in germination when stored at laboratory temperature, 25 ± 2 °C (Table 2). In this treatment, 20% germination was observed up to the fifth month of storage, after which no seed germination was observed, while in the 7 °C refrigerator storage treatment, 86% germination occurred in this fifth month (Table 2). It should be noted that in this storage environment, from this month (fifth month) to the end of the experiment (12th month), no statistical differences were observed in this parameter (Table 2).

The results obtained in this study are confirmed by Pinto Junior et al. (2012), who mention that low storage temperatures are among the ideal conditions for maintaining the physiological quality of seeds since they maintain the embryo with reduced metabolic activity; the same was found by researchers Pontes et al. (2006) with *Caesalpinia peltophoroides* Benth. seeds, and Borba Filho and Perez (2009) with *Tabebuia roseoalba* (Ridl.) Sandwith and *T. impetiginosa* (Mart. ex DC.) Mattos seeds. Souza, Bruno e Andrade (2005) found that *T. serratifolia* (Vahl) Nicholson seeds stored in a laboratory environment (27 ± 3 °C) showed a total loss of vigor after 120 days of storage. According to Moraes et al. (2020), orthodox seeds not only tolerate desiccation, but also show an increase in longevity when storage temperature and humidity are reduced. Seeds dispersed with a degree of humidity around or less than 20% are likely to show orthodox behavior in terms of storage (MARCOS FILHO, 2015; MORAES et al., 2020; TAIZ et al., 2021).

The same behavior observed with the germination percentage was observed with the germination speed index (GSI) shown in Table 2, with statistical differences observed between the two storage environments (7 and 25 °C). In the first month, in the laboratory environment, there was a reduction in GSI compared to the collection period, and for the treatment where the seeds were stored in a refrigerated environment; there was a reduction in GSI over the months of storage (Table 2).

The GSI results observed in this work were also verified in the work of Romanoski et al. (2023), where they tested the storage time and packaging of African mahogany seeds (*Khaya grandifoliola* C. DC.) and showed a reduction in GSI in seeds stored in a laboratory environment, while seeds of this species stored in a cold room environment (6 °C) in polyethylene packaging maintained their physiological quality for 216 days. Furthermore, in the work carried out by Barbosa, Passos and Duarte (2024), evaluating the storage of *Tabebuia aurea* seeds in three storage environments, they observed that in the laboratory environment, the GSI showed a decrease of 1.13 over the storage period, and in the refrigerator and cold room environments, the GSI values showed no significant effect.

According to Marcos Filho (2015), the GSI test is one of the indirect tests of vigor and is classified as a physiological test because it measures the changes in the physiological responses of the seed that occur because of a reduction in its vigor. In addition, Junqueira et al. (2017) and Wylot et al. (2019) emphasized that the increase in germination percentage and rate reflects the individual or joint action of various internal and exogenous factors, which can significantly increase these two parameters in the germination process. In this test, it was shown that external conditions, such as storage temperature, had a negative influence

on the parameters evaluated by physiological effects, especially the intensification of the respiratory activity of the seeds with losses in viability, as emphasized by some researchers (MARCOS FILHO, 2015; ALMEIDA et al., 2010; PINTO JUNIOR et al., 2012; PESKE, 2019; MENEGAES et al., 2021), and even with a humidity close to 6%, the seeds stored in the refrigerator continued with the germination process.

Conclusion

Storage at 7 °C was effective in maintaining the viability of *Handroanthus chrysotrichus* seeds over a 12-month period, resulting in higher germination percentages and germination speed index values compared to storage in a laboratory environment (25 ± 2 °C).

Although moisture content decreased in both treatments, this variable did not significantly influence the evaluated germination parameters. These results support the classification of the species as having orthodox seeds, which tolerate desiccation and benefit from storage at low temperatures to reduce metabolic activity and prolong seed longevity.

Therefore, storage in a refrigerated environment is recommended to maintain the physiological quality of *H. chrysotrichus* seeds, contributing to the conservation and sustainable use of the species.

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