ISSN 1807-1929
Revista Brasileira de Engenharia Agrícola e Ambiental
Brazilian Journal of Agricultural and Environmental Engineering
v.27, n.6, p.457-462, 2023
Campina Grande, PB – http://www.agriambi.com.br – http://www.scielo.br/rbeaa

DOI: http://dx.doi.org/10.1590/1807-1929/agriambi.v27n6p457-462

Storage potential of African mahogany seeds under different environmental and packaging conditions[1]

Potencial de armazenamento de sementes de mogno africano em diferentes ambientes e embalagens

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HIGHLIGHTS:

Polyethylene packaging is favorable for African mahogany seed storage.
Storing African mahogany seeds in a cold chamber improves its physiological quality.
Storage period directly affects the germination capacity and vigor of African mahogany seeds.

ABSTRACT: African mahogany (*Khaya grandifoliola*) is a forest species with excellent wood quality. Due to the increasing demand for viable seeds in forest production programs, the storage capacity of this species must be evaluated. Therefore, this study aimed to determine the appropriate environmental and packaging conditions for the storage of African mahogany seeds. Initially, the water content of the seeds, germination rate, and seedling length were determined in two environments (cold chamber and laboratory), two packages (polyethylene and glass), and three storage periods (72, 144, and 216 d) as well as in additional treatment without storage. The variables analyzed during storage were water content, germination capacity, germination speed index, and seedling length. The experiment was conducted in a completely random design with four repetitions in a split-plot scheme and an additional treatment of 2 × 3 × 2 + 1. African mahogany seeds stored in a cold chamber (6 °C and 72% relative humidity) in a polyethylene packaging maintained their physiological quality for 216 d.

Key words: *Khaya grandifoliola*, seed conservation, germination, vigor

RESUMO: O mogno africano (*Khaya grandifoliola*) é uma espécie florestal que apresenta excelente qualidade madeireira. Com a crescente demanda por sementes viáveis para atender programas de produção florestal, tem-se observado a necessidade de estudos sobre a capacidade de armazenamento dessa espécie. O objetivo deste trabalho foi determinar as condições adequadas de ambiente e embalagem para o armazenamento de sementes de mogno africano. Inicialmente, o grau de umidade das sementes; a germinação e o comprimento de plântulas foram determinados. Foram testados dois ambientes - câmara fria e laboratório; duas embalagens (polietileno e vidro); e três períodos de armazenamento (72, 144 e 216 dias). As variáveis analisadas no armazenamento foram: grau de umidade, porcentagem de germinação, índice de velocidade de germinação (IVG) e comprimento de plântulas. O experimento foi realizado em delineamento inteiramente casualizado com quatro repetições em parcelas subdivididas e tratamento adicional 2 × 3 × 2 + 1. As sementes de mogno africano armazenadas em ambiente de câmara fria (6 °C e 72% UR) em embalagem de polietileno mantiveram a qualidade fisiológica por 216 dias.

Palavras-chave: *Khaya grandifoliola*, conservação de sementes, germinação, vigor

• Ref. 265646 – Received 24 Jul, 2022
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• Accepted 27 Dec, 2022 • Published 11 Feb, 2023
Editors: Ítalo Herbet Lucena Cavalcante & Walter Esfrain Pereira

Introduction

Khaya grandifoliola C. DC. (African mahogany) is an excellent species for the timber industry owing to its desirable characteristics, such as high quality and durable reddish tone wood, mainly used in the manufacture of high-value products (Ribeiro et al., 2017; Ferraz Filho et al., 2021; Souza et al., 2022).

African mahogany propagates via recalcitrant seeds (Okere & Adegeye, 2011). Some alternatives have been reported for the conservation and propagation of this species via embryo preservation and in vitro plant regeneration, but these techniques are laborious and expensive (Okere & Adegeye, 2011; Ballesteros et al., 2021). Therefore, determining the best form of storage is necessary for the logistics, commercialization, and conservation of genotypes, especially African mahogany, in germplasm banks for research purposes and to prevent inconsistent seed production (Carvalho et al., 2016; Silva et al., 2020). One viable alternative is the preservation of seeds in a controlled environment in a suitable packaging, which acts as a barrier for gas exchange and helps to maintain the seed quality (Marcos-Filho, 2015; Gibbert et al., 2019).

The best form of storage has been evaluated in the seeds of forest species, *Myrcianthes pungens*, *Schinus terebinthifolius*, and *Caesalpinia ferrea*, and polyethylene and glass packaging have been determined to improve the maintenance of the physiological quality of seeds; these combined with controlled temperature and humidity have been reported to prolong the maintenance period up to six months for *C. ferrea* (Oliveira et al., 2018; Gibbert et al., 2019, Silva et al., 2019). Therefore, we aimed to determine the appropriate environmental and packaging conditions for the storage of African mahogany seeds in the present study.

Materials and Methods

African mahogany (*K. grandifoliola* C. DC.) seeds were collected from the fruits of six trees (approximately 50-years-old) in Rio de Janeiro (RJ; latitudes: 22° 45' 37.7", 22° 45' 36.5", 22° 45' 35.7", 22° 45' 20.8", 22° 45' 19.5", and 22° 45' 19.6"; longitudes: 43° 41' 09.7", 43° 41" 08.8", 43° 41" 08.1", 43° 39" 57.6", 43° 39" 56.8", and 43° 39 55.5"").

Freshly harvested seeds were sent to the laboratory in polyethylene containers, and the experiments were started two days later. The water content of the seeds was determined using the oven method at 105 ± 3 °C for 24 hours, with four sub-samples of 12 seeds each (4.5 ± 0.5 g) (Brasil, 2009). The results were expressed as percentages on a wet basis.

Next, the germination test was performed with four repetitions of 50 seeds in acrylic plastic boxes (29.0 × 23.0 × 9.0 cm) containing 330 g of vermiculite medium grain size substrate and 300 mL of water, leaving the substrate with 60% field capacity. Every two days, 15 mL of water was added in each repetition. The seeds were placed to germinate in the subsurface of the substrate with the embryo facing upwards (Carvalho et al., 2016). The containers were placed in a germination chamber (B. O. D.) at 25 °C, with a 12 hours photoperiod, as described by Carvalho et al. (2016). Daily monitoring was performed to determine the first and last counts of germination tests. The criterion adopted for evaluation was the formation of normal seedlings, and the results were expressed as percentages (Brasil, 2009).

After the last count of the germination test, the lengths of 20 normal seedlings taken from each repetition were evaluated using a graduated ruler. The results were expressed in centimeters (cm).

The seeds were stored in two environmental conditions (cold chamber: 6 °C and 72% relative humidity [RH] and laboratory: 16 °C and 73% RH) in two types of packaging (polyethylene bags [thickness of 0.15 mm with adequate size to accommodate the seeds] and glass jars) for four storage periods (0, 72, 144, and 216 d). Environmental conditions of the storage room during the evaluation period (from February to October, 2021) were measured using a digital temperature and RH sensor.

A completely randomized design with four repetitions in a split-plot scheme with an additional treatment of 2 × 3 × 2 + 1 was adopted and represented by two environmental conditions (cold chamber: 6 °C and 72% RH and laboratory: 16 °C and 73% RH), two packages (polyethylene and glass), and three storage periods (72, 144, and 216 d) as well as an additional treatment without storage.

The physiological potential of the seeds during storage was evaluated as follows:

Germination speed index (GSI): It was determined via the germination test, where evaluations were made every two days, from the appearance of the first normal seedling until the stabilization of germination. GSI was calculated as described by Maguire (1962) using Eq. 1:

$$GSI = \frac{G_1}{N_1} + \frac{G_2}{N_2} + ... + \frac{G_n}{N_n} \quad (1)$$

where,

G_1, G_2, G_n - number of seedlings germinated in the first, second, and last counts, respectively

N_1, N_2, N_n - number of days elapsed from sowing to the first, second, and last counts, respectively.

Seedling length: After the last count of the germination test, the lengths of 20 normal seedlings from each repetition were measured using a graduated ruler. The results were expressed in centimeters.

Water content was determined using the oven method at 105 ± 3 °C for 24 hours, with four sub-samples of 12 seeds each (4.5 ± 0.5 g) (Brasil, 2009). The results are expressed as percentages on a wet basis.

Seed weights of 1000 seeds were determined using eight sub-samples of 100 pure seeds, according to the Rules for Seed Analysis (Brasil, 2009), and expressed in grams.

Seed biometric characterization: Using a digital pachymeter, the length, width, and thickness of 50 seeds were measured.

Statistical analysis was conducted using generalized linear models, in which the responses (germination, GSI, water content, and seedling length) were evaluated under different distributions consistent with the characteristics of the data to obtain the best-fitting model using Akaike's information

criterion. Means were compared using Tukey's test at $p \leq 0.05$. According to the significance of the effects determined using the aforementioned method, a regression was obtained between the response variables and storage periods, and the coefficients were evaluated using the t-test. R software (version 4.2.0) was used for statistical analysis.

Results and Discussion

Average weight of 1000 seeds of *K. grandifoliola* C. DC. was 433.16 g, and the initial water content of the seeds was 6.8%. Biometric characteristics of the seeds are presented in Table 1; the average length, width, and thickness were found to be 40.7, 27.3, and 2.13 mm, respectively. Nunes et al. (2019) classified the African mahogany seeds based on size (length x width) into three groups: small (2.34 × 1.55 cm), medium (3.08 × 2.01 cm), and large (3.67 × 2.35 cm). Consequently, the seeds can be classified as large seeds.

In relation to the percentage of normal seedlings obtained by the day of evaluation in the germination test, 7% normal seedlings were observed 14 d after sowing (in relation to the total number of seeds sown). A peak was observed with 42% of normal seedlings after 19 d, and after 21 d, 32% of normal seedlings were verified. After 22 d of sowing, the number of normal seedlings remained constant. Thus, the evaluation of the first count in the germination test of African mahogany seeds should be performed 19 d after sowing and that of the final count should be performed 21 d after sowing.

African mahogany seedling development, illustrated in Figure 1, can be divided into three stages: root emission (Figure 1A), onset of aerial part development (Figure 1B), and normal seedling formation (Figure 1C). The root system is pivotal; with a developed taproot, the seeds exhibit no dormancy, and the first two leaves are opposite and simple (Reis et al., 2019).

Figure 2 illustrates the normal and abnormal seedlings of African mahogany plant. As shown in Figure 2A, the seedling has complete, well developed, and healthy essential parts, with a well-formed root system, aerial part, and expanded leaves. Figure 2B and 2C show the abnormal seedlings with undeveloped, short, thick, and twisted aerial parts.

Average RH and the maximum, minimum, and average temperatures during the storage period in each environment are shown in Figure 3A and 3B. Laboratory room (Figure 3A) had an average temperature of 16 °C and RH of 73%, while the cold chamber had an average temperature of 6 °C and RH of 72%.

Gamma distribution was best fitted and the interactions among different environment, packaging, and storage period factors had significant effect ($p \leq 0.05$) on the water content; therefore, one regression was performed for each treatment. The initial water content of the seeds was 6.8% (Figure 4A and 4B), which varied during the storage period, depending on the environmental and packaging conditions. In the laboratory room (Figure 4A), the water content percentages were 8.76, 12.33, and 12.13% for the polyethylene package and 7.80, 8.74,

Table 1. Biometric characterization of African mahogany seeds

Variables (mm)	Minimum	Mean	Maximum	Standard deviation	CV (%)
Length	32.9	40.7	47.7	2.6	6.37
Width	22.5	27.3	31.7	1.9	7.08
Thickness	1.38	2.13	2.8	0.41	19.42

CV, coefficient of variation. n = 50

Figure 1. Germination process of African mahogany seeds. (A) Root emission 13 d after sowing. (B) Onset of aerial part development 19 d after sowing. (C) Normal seedling formation 25 d after sowing

Figure 2. Seedlings of African mahogany. (A) Normal seedlings. (B) and (C) Abnormal seedlings

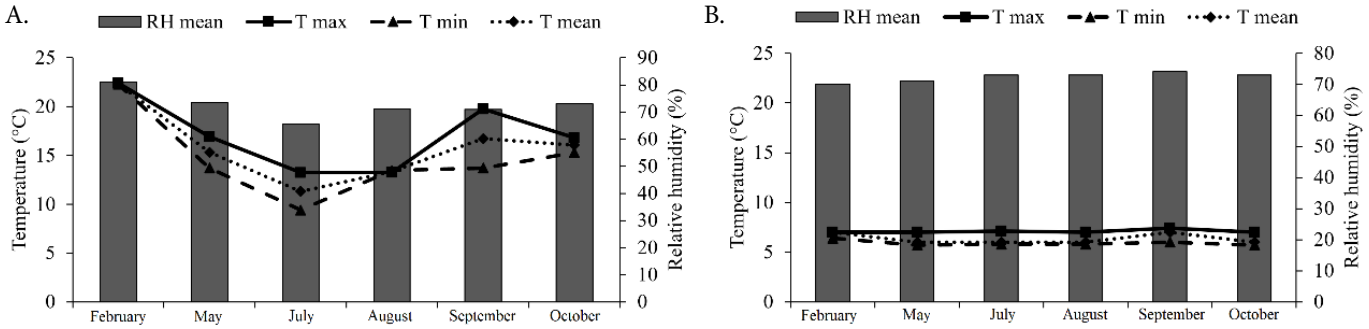

Figure 3. Temperature (T) and relative air humidity (RH) in the two environmental conditions during the storage of African mahogany seeds. (A) Laboratory. (B) Cold chamber

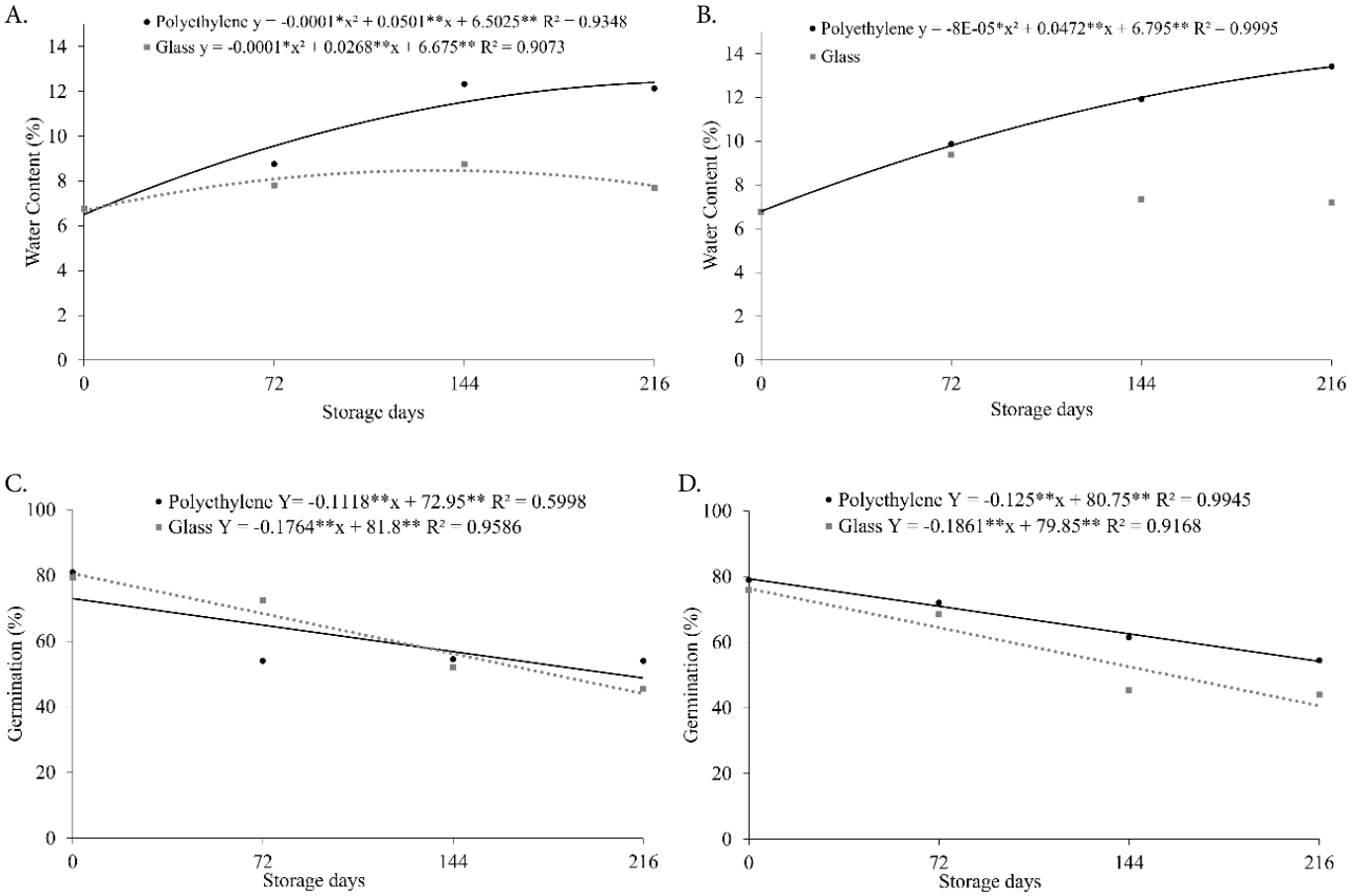

Figure 4. Water content of African mahogany seeds stored in the (A) laboratory room and (B) cold chamber. Germination capacity of seeds stored in the (C) laboratory room and (D) cold chamber based on the storage period

7.69% for the glass package after 72, 144, and 216 d of storage, respectively. In the cold chamber (Figure 4B), the regression did not fit properly for the glass package ($y = -0.0001x^2 + 0.0277x + 7.097$, $R^2 = 0.475$). The water content percentages were 9.87, 11.92, and 13.42% for the polyethylene package and 9.38, 7.34, and 7.19% for the glass package after 72, 144, and 216 d of storage, respectively.

The water content of the seed influences its physical and biochemical characteristics and is one of the main factors responsible for seed deterioration that affects its viability and vigor; thus, it is extremely important to monitor the water content in seeds during storage (Carvalho & Nakagawa, 2012; Lamichhane et al., 2018).

Although the average relative air humidity was similar in both environments, 73% in the laboratory and 72% in the cold chamber, there was an increase in the water content of seeds stored in polyethylene packaging in both environments, indicating absorption of moisture by the seed from the environment due to the permeability of this packaging. The storage of seeds in glass containers allowed for smaller fluctuations in water content in both environments, as glass is an impermeable container that does not allow water exchange (Martins et al., 2009). Similar results were observed in recalcitrant *M. pungens* forest seeds stored for 10 months in polyethylene packaging, as the water content of the seeds was increased by 6% over the storage period because of the semi-permeability of polyethylene packaging (Gibbert et al., 2019).

For germination (Figure 4C and 4D), the binomial distribution was best fitted, and the interactions among factors had a significant effect ($p \leq 0.05$). The storage of seeds in a laboratory room in polyethylene packaging (Figure 4C) quickly reduced the viability of seeds in the short term, with

the initial germination rate dropping from 81 to 54% after 72 d of storage, after which, the germination rate was maintained until 216 d of storage, which was not statistically different from the results obtained with the glass packaging. In cold storage (Figure 4D), there was no significant difference in germination rate between the two packages; however, in the glass package, there was a reduction in viability from day 144 until the end of storage period.

Due to their recalcitrant behavior, the seeds show a sharp drop in germination capacity depending on the environmental and packaging conditions (Silva et al., 2020). However, compared to other recalcitrant species, where dissection sensitivity strongly affects the seed storage capacity and seeds can lose their physiological quality within a few weeks, the African mahogany seeds retained approximately 75% of their initial germination capacity under all storage conditions. This means that either the combination of packaging and environmental conditions was able to maintain the germination capacity of the seeds or the seeds exhibited a lower level of recalcitrance. Based on desiccation tolerance, seeds can be classified into other forms besides orthodox and recalcitrant types, and the degree of recalcitrance can vary based on the genotypic and phenotypic characteristics, such as the degree of maturity at which the seeds are dispersed (Barbedo, 2018; Krzyzanowski et al., 2020)

Pinto Junior et al. (2012) verified the loss of physiological quality in *Jatropha* seeds, which are classified as orthodox during storage in laboratory rooms in polyethylene and glass containers for 180 d. However, Borba Filho & Perez (2009) verified that for seeds of ipê-branco (*Tabebuia roseo-alba*), the best storage conditions were polyethylene packaging in a cold chamber environment (14-20 °C and 74-82% RH). According to Krzyzanowski et al. (2020), storage conditions are distinct among species, and the reduction in germination as a function of storage period is related to the loss of physiological quality.

For GSI (Figure 5A and 5B) and seedling length (Figure 5C and 5D), the gamma distribution was the best fit and the interactions among the factors were found to be significant ($p < 0.05$). In the laboratory (Figure 5A), after 72 d of storage, there was a significant drop in the vigor of seeds stored in polyethylene packaging that was maintained until 216 d, which was not statistically different from that observed in the glass packaging. In the cold chamber (Figure 5B), there was no difference between the packaging conditions and the physiological quality was maintained during the storage period.

Vigor was evaluated based on the length of seedlings (Figure 5C and 5D). In the laboratory room, although there were no significant differences between the packages and the regression in the polyethylene package fit properly ($y = 0.0067x + 19.885$, $R^2 = 0.3285$), a decrease in vigor was observed after 216 d for seeds stored in glass containers. In the cold chamber, there was a difference between the packages for 144 d, as the seeds stored in glass packages showed decreased vigor, but those stored in polyethylene containers maintained their physiological quality. Variations in climatic conditions affect the seedling length more than the seed germination capacity

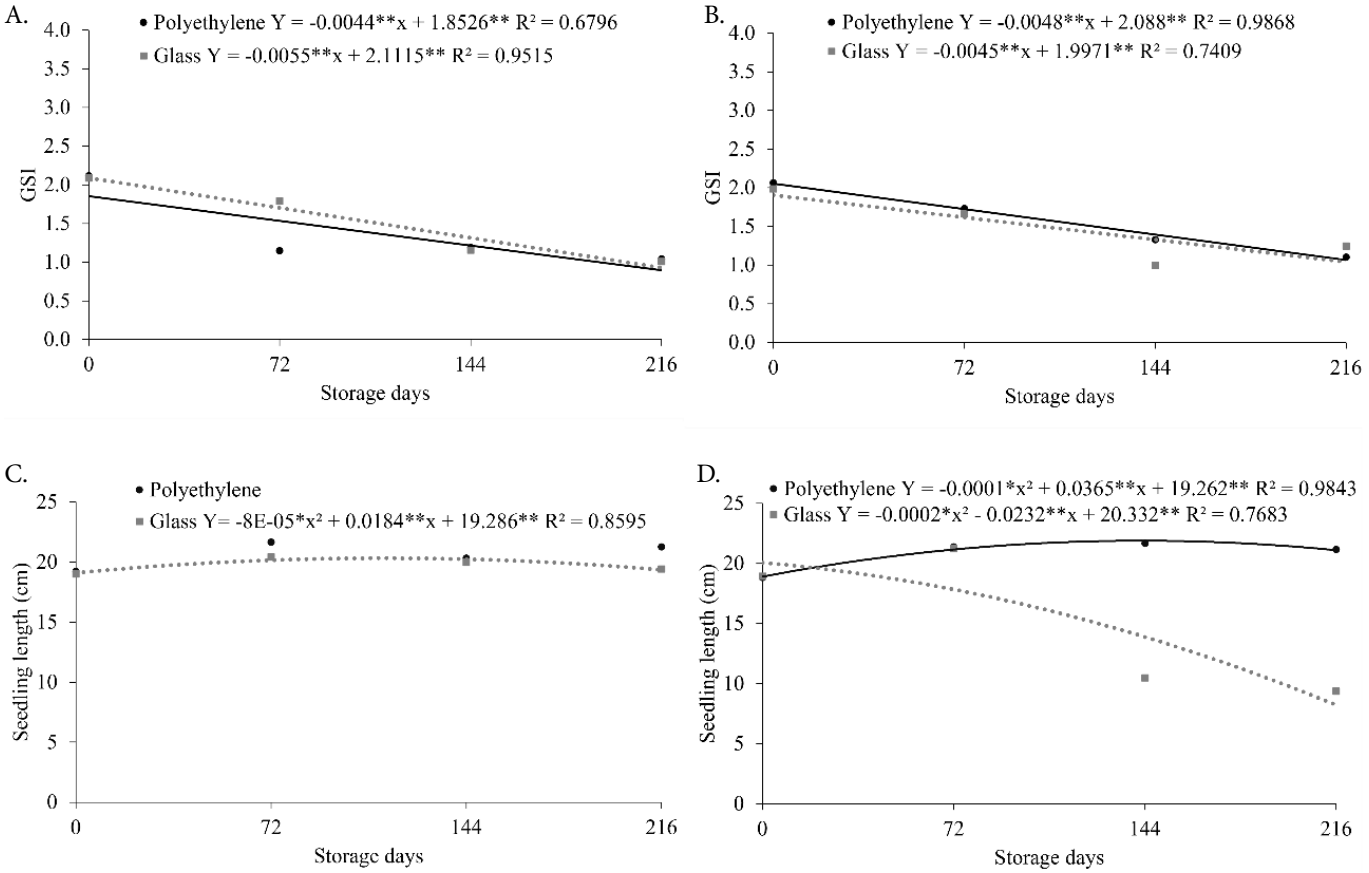

Figure 5. Vigor - germination speed index (GSI) and seedling length of African mahogany seeds stored in different environmental and packaging conditions. (A and C) GSI and seedling length in the laboratory room. (B and D) GSI and seedling length in the cold chamber based on the storage period

or vigor based on seed performance testing (such as GSI) at certain thresholds. Therefore, storage of seeds in a cold room in glass packaging at low temperatures (favors its recalcitrant behavior) may be responsible for the obvious decrease in seed length (Krzyzanowski et al., 2020).

Conclusions

1. Based on germination and vigor, the best conditions for storage of African mahogany seeds for up to 216 d were determined to be the cold chamber environment (6 °C and 72% RH) and polyethylene packaging.

2. The germination percentage and GSI of African mahogany seeds were reduced under laboratory conditions in glass packaging after 144 d of storage.

Acknowledgments

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for granting the scholarship.

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