Storage of *Calophyllum brasiliense* Cambess. seeds

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Abstract

Calophyllum brasiliense is a species native to Brazil and has potential for use in the timber industry, in the reforestation of degraded areas, besides having medicinal properties. Its propagation is mainly by seeds which, depending on their recalcitrant characteristics, leads to difficulty in conservation, due to changes in its physiological potential during storage. Aiming to contribute to the expansion of its cultivation, rational use and conservation, the objective of this study was to investigate the behavior of *C. brasiliense* seeds during storage. Different packings (paper, aluminum and polyethylene) and environmental conditions (room temperature and cold chamber) were quarterly tested over 12 months, by evaluating germination viability and vigor. Based on the results, it was concluded that packaging in polyethylene and freezer storage provided the best conditions for the conservation of seeds, keeping them viable for a period of nine months.

Keywords: Guanandi, forest species, conservation, longevity, recalcitrance.

Armazenamento de sementes de *Calophyllum brasiliense* Cambess.

Resumo

Calophyllum brasiliense é uma espécie nativa do Brasil e está na lista de espécies recomendadas para o reflorestamento de áreas degradadas, além de possuir propriedades medicinais e madeireiras. Sua propagação se dá principalmente via sementes, as quais, em função das suas características recalcitrantes, apresentam dificuldade de conservação, devido a alterações no seu potencial fisiológico ao longo do armazenamento. Visando contribuir com a ampliação de seu cultivo, uso racional e conservação, objetivou-se investigar o comportamento das sementes de *C. brasiliense* durante 12 meses de armazenamento. Testou-se diferentes embalagens (papel, alumínio e polietileno) e condições ambientais (câmara fria e temperatura ambiente), avaliando-se trimestralmente a germinação, viabilidade e vigor. Com base nos resultados obtidos conclui-se que o acondicionamento em embalagem de polietileno e o armazenamento em câmara fria proporcionou a melhor condição para a conservação das sementes, mantendo essas viáveis por um período de nove meses.

Palavras-chave: Guanandi, espécie florestal, conservação, longevidade, recalcitrância.

1. Introduction

Calophyllum brasiliense is a tree native to riparian forests in Brazil, popularly known as Guanandi (Corrêa, 1978; Oliveira and Joly, 2010; Alho, 2011). It features a wide variety of secondary metabolites isolated from leaves, bark, roots, flowers and fruits, such as xanthones, terpenes, chromanones and flavonoids, which exhibit multiple medicinal properties (Noldin et al., 2006; Bernabé-Antônio et al., 2010; Carvalho et al., 2013). It is also suitable for reforestation, especially to restore the vegetation in locations subjected to periodic short-term floods, as well as areas with a permanently soggy soil (Carvalho, 1994; Souza, 2009).

The natural propagation of this species occurs through seeds, exhibit physical and mechanical dormancy, which must be overcome by the total removal of the endocarp and tegument, reaching 72% germination at a temperature of 30 °C (Silva et al., 2014). However, irregular fructification, occurring at 41-month intervals (Fischer and Santos, 2001), besides the recalcitrance displayed by its seeds, released from the parent plant with a high water content, further hinder its use (Vásquez-Carballo et al., 2004; Carvalho et al., 2006).

The key point is to reduce the speed of seed deterioration by reducing its metabolism through water removal or

lowering storage temperature (Roberts, 1973; Araújo and Cardoso, 2007), without which quality improvement is not possible, even under ideal conditions (Villela and Peres, 2004). Among the conservation systems in artificially controlled environments, the cold chamber is intend to seed conservation under temperatures usually below 10 ºC (Sacandé et al., 2004; Zonta et al, 2014). The combination of low temperature and kinds of packaging is the reason why the longevity of stored seeds can range (Marcos Filho, 2013), due to moisture exchange (Nery et al., 2014).

Therefore, the study about recalcitrant seed storage enables its availability, which is extend for a longer period (Zonetti et al., 2011), essentially to help commercial exploitation for use in seedling production, aiming at recovery programs of degraded areas (Garcia et al., 2006). Due to the scarcity of information on the storage of *C. brasiliense* seeds, this study aimed to analyze the longevity of seeds submitted to different moisture contents, packaging, and storage conditions.

2. Material and Methods

The present study was conducted at Laboratório de Crescimento e Desenvolvimento de Plantas, Department of Biology, at Universidade Federal de Lavras – UFLA), Lavras, MG, Brazil.

Ripe *C. brasiliense* fruits were collected in plants located at Parque Quedas do Rio Bonito (MG- Brazil). Subsequently, the fruits were taken to the laboratory, where they were pulped and subjected to treatments.

To perform the experiment, a part of a batch of freshly harvested seeds with an initial moisture content of 33.7% (on a fresh weight basis) was used, and the other part of the seeds was oven dried with forced air circulation at 35 ºC, until they could reach 31.2% moisture content.

Seeds were treated with the fungicide Carbendazim: 0.1% (w/v) for 15 minutes, stored for 12 months under two conditions: Cold chamber (8 ºC ± 2 ºC /45% M) and room temperature (28 ºC ± 10 ºC), in three different packagings: transparent polyethylene (113.06 g/m^2 weight, 0.1 mm thickness, with 1kg capacity); kraft paper (1 kg capacity), and 2 layers of aluminum foil. Packagings were sealed with adhesive tape.

The seeds were evaluated every three months for germination percentage and germination speed index (GSI), besides the monitoring of seed moisture content throughout storage. Moisture content was determined based on fresh weight, using an oven at 105 ºC ± 3 ºC for 24 hours and four replications of five seeds (with wrap) each, until reaching constant weight (Brasil, 2009).

Germination tests were carried out in B.O.D. chambers at a constant temperature of 30 ºC, in the dark. The substrate was Germitest® paper, moistened with distilled water 2.5-fold the paper weight (Brasil, 2009). Each treatment consisted of 25 unwrapped seeds (manually scarified, with the removal of endocarp and tegument). The evaluation of germination was carried out in 2-day intervals, and the percentage of rootlets 5-mm seeds in each treatment, as well as the Germination Speed Index (GSI), were calculated according to Maguire (1962).

Tests were carried out in a completely randomized design. Germination data were transformed in arcsin $(X/100)^{0.5}$ and GSI data transformed in $(X+0.5)^{0.5}$, subsequently subjected to statistical analysis PROC GLM, using the software SAS®. Means were compared by the Tukey test at 0.05 significance.

3. Results and Discussion

The seeds kept in cold chamber and at room temperature showed ranging moisture levels, especially when considering the type of packaging used (Figure 1).

Under cold chamber conditions, a decrease in the initial seed moisture content packed in polyethylene was observed, compared to other packagings in all studied treatments (Figures 1A and 1B). For the seed batch with 31.2% initial moisture stored in cold chamber, there was a less marked decrease in moisture content, as shown in Figure 1B. Regarding the seed batch with 33.7% initial moisture stored at room temperature and packed in aluminum foil, there was a reduction to 12% in seed moisture content at the end of the 12-month storage period (Figure 1C). In general, the use of polyethylene packaging, which is resistant to water vapor exchanges, was the most suitable for preserving seed moisture content throughout the 12-month period.

In contrast, Souza et al. (2011), studying the storage of *Geoffrea spinosa*, a recalcitrant species from riparian forests (34.16% moisture), reported that cold storage and polyethylene packaging did not favor storage and, in the first three storage periods, there was a gradual decrease, followed by a slight increase in storage to ninety days.

In the initial test, the decreased moisture content from 33.7% to 31.2% did not affect the germination of seeds, which exhibited 83.9% and 89.4% germination, respectively (data not shown). After storage, seeds in cold chamber had higher germination rates, when compared to room temperature, 92.39% and 73.06%, respectively. Among the tested packagings, aluminum provided the best results regarding germination percentage (98.88%), followed by polyethylene (90.31%), and paper (44.90%). For storage period, the best result was found for three-month storage (97.30%), decreasing the percentage over time to 77.53%, 46.04%, and 13.98% germination, at 6-, 9- and 12-month storage, respectively (Figure 2).

These responses occurred, mostly, due to seed oxidation during storage, even with no presence of insects or fungi (Jeller et al., 2003). This behavior is justified by higher temperature fluctuations and moisture to which the seeds were submitted under different packaging conditions, since some packagings allow water exchange between seeds and the surrounding environment, such as paper, interfering with the metabolic activity of seeds, reducing their longevity.

Previous studies with *C. brasiliense* storage, showed that the best storage was observed at 15 ºC and 32% seed moisture

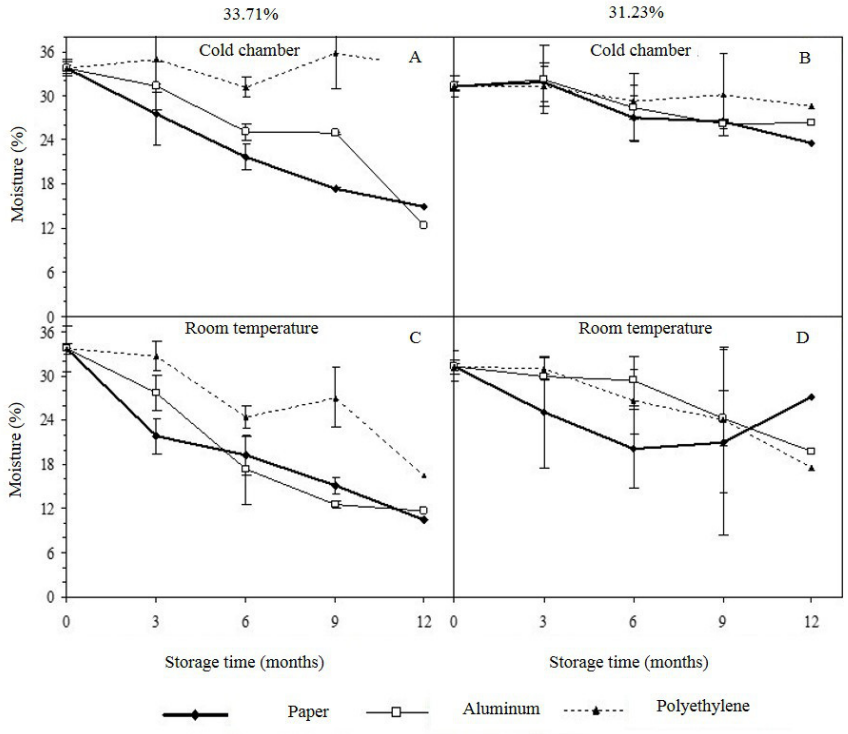

Figure 1. Moisture levels (%) of *C. brasiliense* seeds packaged in different containers (paper packaging, aluminum and polyethylene) and stored for 12 months under cold room conditions (A and B) and room temperature (C and D). Initial moisture levels of 33.7% and 31.2%, respectively.

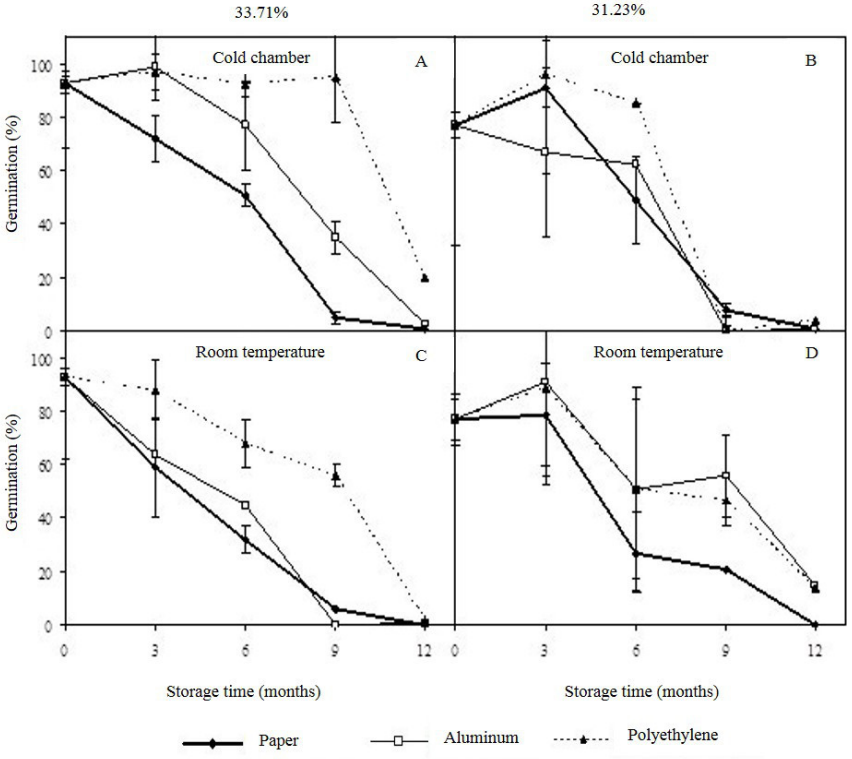

Figure 2. Germination percentage of *C. brasiliense* seeds, stored for 12 months in degrees of initial moisture: 33.7% and 31.2%, under storage conditions: cold chamber (A and B), laboratory environment (C and D) and different packages.

content for three months, reaching up to 70% germination. They still reported that seeds completely lost their viability after six-month storage (Vásquez-Carballo et al., 2004).

Even in species with orthodox characteristics, temperatures during storage significantly influence the achieved longevity and viability, as is the case of *Tabebuia aurea*, with germination under refrigerated conditions (13 °C), which ensured viability for at least 360 days, regardless the packaging used, either paper or plastic (Neves et al., 2014).

Regarding GSI, it was found that there were no significant differences between cold chamber and room temperature, despite a difference for polyethylene packaging at three-month storage. On the other hand, there were significant differences for paper and aluminum packagings throughout the storage period (Figure 3).

In Figure 4A and 4C, it is noteworthy that seeds with 33.7% initial moisture content presented higher GSI when packed in polyethylene, in both temperatures, at three-month storage. After this period, there was a marked decrease in seed vigor storage at room temperature. Only seeds stored at room temperature (31.2% initial moisture content) and packed in aluminum foil exhibited higher GSI, when compared to seeds packed in polyethylene and paper (Figure 4B and 4D).

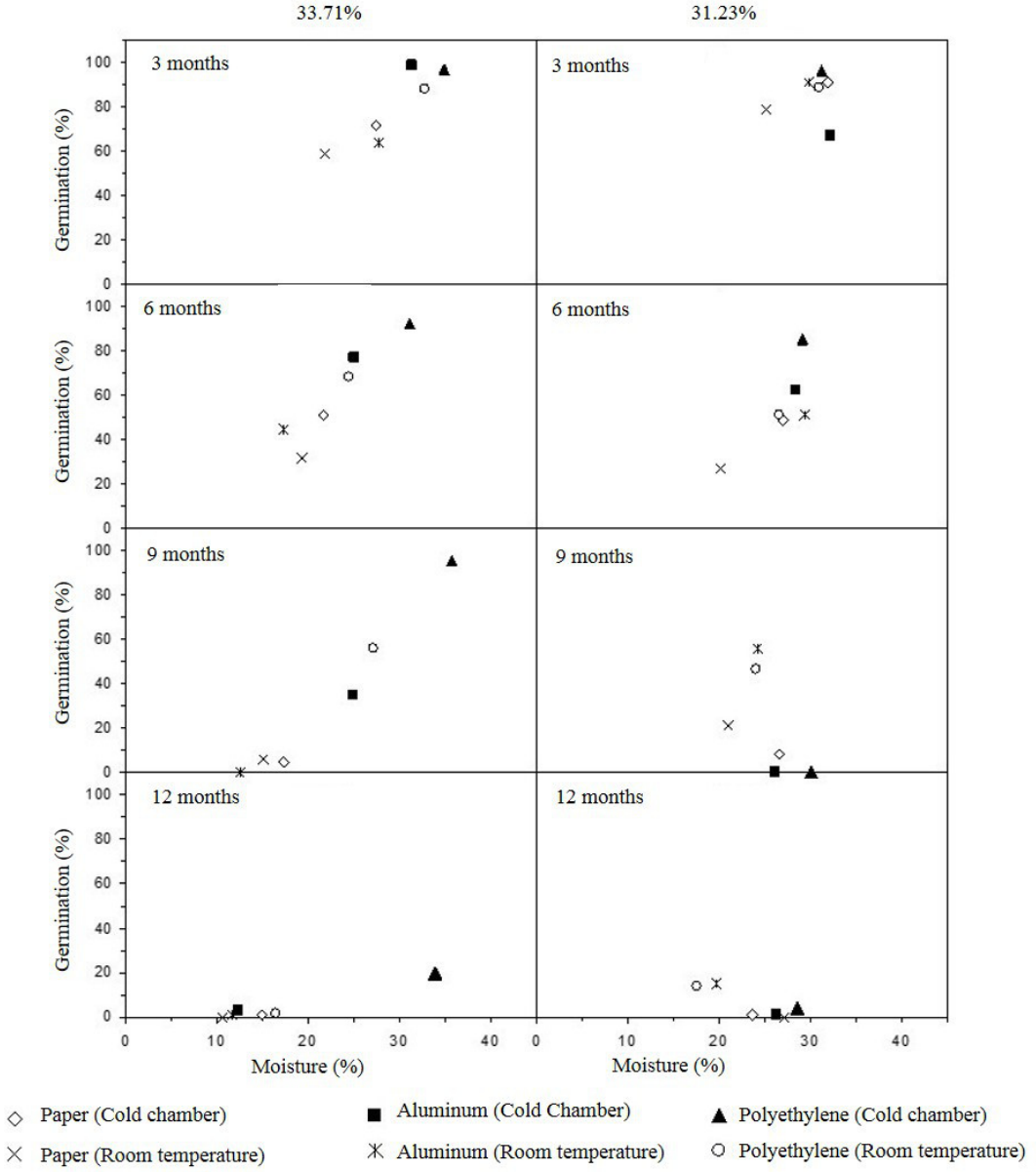

Figure 3. Effect of moisture content (%) on the germination *C. brasiliense* seeds stored for 12 months at initial levels of moisture, storage and packaging conditions.

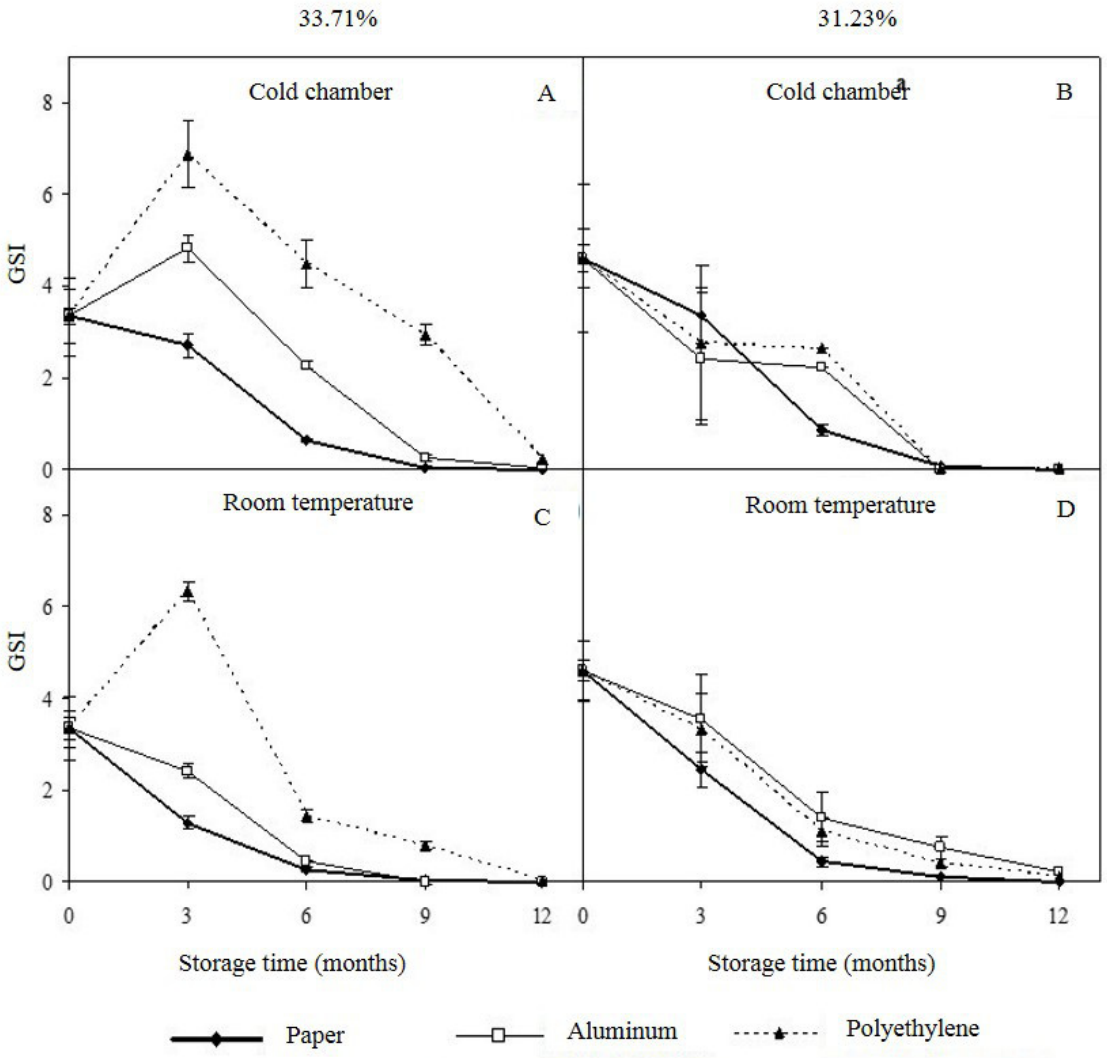

Figure 4. Germination speed index (GSI) of *C. brasiliense* seeds stored under conditions of cold chamber (A and B) and laboratory environment (C and D) for 12 months, with initial moisture contents of 33.7% and 31.2%, packed in paper, aluminum and polyethylene.

As the storage period advanced, the decrease in germination speed was also observed by Silva et al. (2012), while studying the longevity of *Moringa oleifera* L. seeds at different room temperatures. The seeds stored for 27 months in plastic packaging in cold chamber showed higher GSI.

Among the survey studies on *C. brasiliense* behavior classification during storage, stand Vásquez-Carballo et al. (2004) and Carvalho et al. (2006), thus proposing a recalcitrant behavior of *C. brasiliense*.

However, the application of different packagings and temperatures to increase the storage period proposed in this study was satisfactory, as *C. brasiliense* seeds presented an intermediate behavior when stored, standing for low temperatures at a nine-month period, with no meaningful loss of viability and vigor.

4. Conclusions

The behavior of *C. brasiliense* seeds shows that, even though the species is classified as recalcitrant, it supports polythene packaging and can be stored in a cold chamber for up to nine months, which prevents moisture accumulation and accelerated seed deterioration.

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