Storage of *Simarouba amara* Aubl. seeds

Armazenamento de sementes de *Simarouba amara* Aubl.

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Abstract: Prior knowledge about the physiological behavior of seeds under storage conditions allows the use of appropriate techniques to maintain viability. The goal of the present study was to classify the behavior of *Simarouba amara* seeds regarding their physiological potential during storage. The seeds were obtained from mature fruits, collected from five parent plants located at the Joanes-Ipitanga Environmental Protection Area (EPA) (Simões Filho, Bahia) in January 2018. Two experiments were carried out in a completely randomized design. In the first one, seed desiccation tolerance was evaluated with seven moisture contents: 5%, 7.5%, 10%, 12.5%, 15%, 25% and 35.6% (initial moisture). A germination test was carried out to evaluate the seeds' vigor. In the second experiment, the seeds were stored with moisture contents of 7.5%, 12.5% and 35.6% at temperatures of 8 °C, 15 °C and 20 °C) for two storage periods, two and four months, and samples were taken to determine moisture content and conduct the germination test. Germination tests were performed in BOD chambers at 30 °C and with 12-hour light photoperiod. Daily counts were performed for the number of germinated seeds. We measured germination percentage, mean germination time, uncertainty index, percentage of normal seedlings, and percentage of unviable seeds. *Simarouba amara* seeds tolerate desiccation up to 7.5% moisture content, but lost viability when stored under this condition. At four months of storage all seeds lost viability. *Simarouba amara* seeds are recalcitrant and lose viability before reaching two months of storage.

Keywords: Desiccation tolerance. Seed conservation. Recalcitrant.

Resumo: Objetivou-se classificar o desempenho das sementes de *Simarouba amara* quanto ao potencial fisiológico durante o armazenamento. As sementes foram obtidas de frutos maduros, coletados de cinco matrizes localizadas na Área de Proteção Ambiental (APA) Joanes-Ipitanga (Simões Filho, Bahia) em janeiro de 2018. Foram realizados dois experimentos em delineamento inteiramente casualizado. No primeiro, foi avaliada a tolerância à dessecação, com sete teores de umidade: 5%, 7,5%, 10%, 12,5%, 15%, 25% e 35,6% (umidade inicial). Foi realizado teste de germinação para avaliação do vigor. No segundo, as sementes foram armazenadas com umidades de 7,5%, 12,5% e 35,6%, nas temperaturas de 8 ºC, 15 ºC e 20 ºC, por dois períodos de armazenamento (2 e 4 meses), sendo retiradas amostras para teste de umidade e germinação. Os testes de germinação foram realizados em germinadores tipo BOD, à temperatura de 30 ºC e fotoperíodo de 12 horas/luz. Foram realizadas contagens diárias para o número de sementes germinadas e avaliados a porcentagem de germinação, o tempo médio de germinação, o índice de incerteza, a porcentagem de formação de plântulas normais e o percentual de sementes inviáveis. As sementes de *S. amara* toleraram a dessecação até 7,5% e são recalcitrantes.

Palavras-chave: Tolerância à dessecação. Conservação de sementes. Recalcitrantes.

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INTRODUCTION

Irregular production of seeds of most native forest species, associated with the difficulties found in storing them, challenges seed conservation and seedling production activities (Souza *et al*., 2011). The seed is the main propagation pathway of most native tree species, and its longevity often is low (Martins & Pinto, 2014), making it difficult to propagate these species.

Seed storage is one of the techniques used for the conservation of plant genetic resources, considered a low-cost alternative that allows the viability of these propagules to be maintained for long periods (Costa, 2009), depending on the storage potential of the species. *Ex situ* conservation via storage seeks to preserve the physical, physiological and sanitary qualities of seeds, aiming at future use for obtaining healthy seedlings, for the formation of commercial plantations, restoration of degraded areas and even for the formation of native forest gene banks (Floriano, 2004).

Environmental conditions during storage may accelerate the seed deterioration process, and the choice of these conditions depends on the ability of seeds to tolerate desiccation and low storage temperatures. Roberts (1973) separated the species into orthodox and recalcitrant, and Ellis *et al.* (1990) incorporated the term intermediate. Seeds with orthodox behavior tolerate desiccation at moisture levels below 5% and can be stored at low temperatures, for example at -20 °C, for long periods (Roberts, 1973; Hong & Ellis, 1996). Others are called intermediate because they tolerate dehydration at moisture contents of 8 to 10%, but are damaged when exposed to temperatures near or below 0 °C (Ellis *et al*., 1990; Hong & Ellis, 1996). Seeds that are not able to tolerate water removal, losing viability when they reach 12-31% moisture and storage at low temperatures, are classified as recalcitrant (Roberts, 1973; Hong & Ellis, 1996).

The seeds may come from subtropical climate, tending to tolerate lower temperatures; however, due to the moisture level, do not tolerate sub-zero temperatures (Farrant *et al*., 1988). Moderately and highly recalcitrant seeds do not tolerate so much water loss. However, in the absence of additional water, germination is slow enough to maintain viability for several weeks if moisture content is kept high. These species are believed to be from tropical forests or wetlands, where the environment is favorable for germination throughout the year (Farrant *et al*., 1988).

For the proper classification of species regarding storage potential, it is necessary to apply specific protocols, such as those developed by Hong & Ellis (1996). For many Brazilian native species there is no information on seed behavior during storage, and species are often classified on this issue without applying the protocols recommended for this purpose, which makes these studies inconclusive.

Simarouba amara is a tree species found in tropical forests with timber potential and is used in the manufacturing of crates, wooden ceilings, matches, frames, plywood and musical instruments (Loureiro *et al*., 1979). In addition, the species is indicated for medicinal use and for the recovery of degraded areas. There is no consensus in the literature regarding the classification of the species; for instance, Corbineau & Côme (1989) classify it as recalcitrant and, on the other hand, for Cruz & Corrêa (2016) *S. amara* is intermediate. In this context, the objective was to classify *S. amara* seeds regarding physiological behavior during storage.

MATERIAL AND METHODS

The experiments were carried out at the Ecology and Forest Restoration Laboratory of the Department of Forest Engineering of the Federal University of Recôncavo da Bahia, in the campus of Cruz das Almas, Bahia.

The fruits of *S. amara* were harvested from five parent plants located in the Joanes-Ipitanga Environmental Protection Area (EPA) in Simões Filho, Bahia (12° 47' 04" S, 38° 24' 14" W), in January 2018. Fruits went through manual processing, and their pulp was removed using sieves. After processing, seed moisture content was determined using four replicates with four grams of seeds each, using the oven method at 105 ± 3 °C for 24 hours (Brasil, 2009).

EVALUATION OF THE DESICCATION TOLERANCE

After determining the initial moisture content (35.6%), the seeds were put to dry using blue gel silica (3 mm) and conditioned in a room with controlled temperature of 20 °C, according to the methodology proposed by Hong & Ellis (1996). The seeds were desiccated to various moisture contents: 5%, 7.5%, 10%, 12.5%, 15% and 25% (Hong & Ellis, 1996).

Seeds were arranged on three germination paper sheets, moistened with distilled water with a volume corresponding to 2.5 times the paper weight (Brasil, 2009) wrapped in the form of a roll, placed in plastic bags and put into a Biochemical Oxygen Demand (BOD) chamber, under temperature of 30 °C and 12-hour light photoperiod (Brasil, 2013). The experiment was carried out in a completely randomized design with seven treatments, corresponding to the desiccation levels of 5%, 7.5%, 10%, 12.5%, 15%, 25% and 35.6% (initial moisture), with four repetitions of 25 seeds each. Daily counts were performed for 66 days, adopting the criteria of root emergence for germination and the first pair of leaves for normal seedling (Brasil, 2009). We waited ten days after the last radicle emergence to determine the end of the count.

The evaluated variables were germination percentage, mean germination time, uncertainty index, percentage of normal seedlings, percentage of unviable seeds (dead seeds + abnormal seedlings). The data obtained were submitted to analyses of variance and regression in the R program version 3.1.3 (R Development Core Team, 2019).

STORAGE

In the *S. amara* seed storage experiment, a completely randomized design was adopted, with twelve treatments: T1- control (freshly collected seeds with 35.6% moisture content), T2 (freshly collected seeds, dried to 7.5% of moisture content), T3 (freshly collected seeds dried to 12.5% moisture content), T4 (7.5% moisture content stored at 8 °C), T5 (7.5%/15 °C), T6 (7.5%/20 °C), T7 (12.5%/8 °C), T8 (12.5%/15 °C), T9 (12.5%/20 °C), T10 (35.6%/8 °C), T11 (35.6.5%/15 °C) and T12 (35.6%/ 20 °C). T4 to T12 treatments were evaluated after two storage periods, two and four months.

The seeds were placed in Petri dishes in polystyrene boxes (14.5 x 11.5 x 6 cm), each containing 75 seeds. The boxes were conditioned in BOD - type chambers, with temperature regulation, according to the proposed treatments. In the treatments (T10, T11 and T12), the relative humidity (RH) conditions were simulated by the use of saturated sodium chloride solution (75%) (Medeiros, 2006).

After each storage period, seed moisture content was determined by the oven method at 105 ± 3 °C for 24 hours (Brasil, 2009). Then, germination tests were performed, with four replicates of 20 seeds for each treatment, in which the seeds were arranged on three germination paper sheets, moistened with a volume of distilled water corresponding to 2.5 times the paper weight wrapped in rolls, placed in plastic bags and put into a BOD chamber, at a temperature of 30 °C and a 12-hour light photoperiod (Brasil, 2013). Daily counts were performed for 45 days, adopting the criteria of radicle emergence for germination and the first pair of leaves for normal seedling (Brasil, 2009). We waited ten days after the last radicle emergence to determine the end of the count.

The evaluated variables were germination percentage, mean germination time, uncertainty index, percentage of normal seedlings and percentage of unviable seeds (dead seeds + abnormal seedlings). The obtained data were submitted to analysis of variance and means comparison tests (Tukey, $\alpha = 0.05$), in the R program, version 3.1.3 (R Development Core Team, 2019).

In both experiments, on the first day after the end of the counts, a tetrazolium test was performed, removing the seed coats, which were cut longitudinally through cotyledons and embryonic axis. They were placed in Petri dishes, and totally submerged in 0.5% tetrazolium solution, kept in the dark at 30 °C for 2:30 hours (Silva *et al.*, 2016). After color development, the embryos were washed in running water and left submerged in water until the evaluation according to Moore (1972), to verify the viability of hard seeds.

RESULTS AND DISCUSSION

EVALUATION OF THE DESICCATION TOLERANCE

In the desiccation tolerance tests, the germination was null for seeds with moisture content of 5%, which already makes it possible to infer that *Simarouba amara* seeds are not orthodox. Seeds that do not tolerate desiccation at ≤ 5% moisture content are considered recalcitrant (Roberts, 1973; Hong & Ellis, 1996; Fonseca & Freire, 2003). Recalcitrant seed species are dispersed from the parent plant with high moisture contents. Reduction in seed moisture content, depending on its intensity, can lead to rapid loss of viability and death (Roberts, 1973; Costa, 2012). Therefore, recalcitrant species usually originate from habitats that allow rapid establishment soon after the dispersal event (Farrant *et al*., 1988; Berjak & Pammenter, 2008). The Joanes-Ipitanga EPA, where *S. amara* seeds were collected, is characterized by dense ombrophilous vegetation (IBGE, 2012), with high rainfall (Centro de Recursos Ambientais, 2011), which guarantees the establishment and success of the species in that area.

The ability of seeds to survive desiccation is a functional trait that may be related to the ecology of plant regeneration. Pioneer species usually produce orthodox and dormant seeds (Tweddle *et al*., 2003) and non-pioneer species tend to produce recalcitrant seeds (Pammenter & Berjak, 2000; Tweddle *et al*., 2003). Thus, *S. amara* is a non-pioneer species (Lima *et al.*, 2014; Oliveira *et al.*, 2011), which corroborates its recalcitrant behavior.

The percentage of normal seedlings (p-value = 0.33) and the percentage of unviable seeds (p-value = 0.60) were not influenced by moisture content between 7.5% and 35.6%. Between the moisture contents of 7.5% and 35.6% the average percentage of normal seedlings was 89% and the average percentage of unviability was 10%. Corbineau & Côme (1989) found 100% mortality rates for *S. amara* seeds desiccated with 8% moisture. In the work conducted by Corbineau & Côme (1989), the seeds were dried in an open environment under the temperature of 20 °C and 55% humidity. In the present study, the drying was performed using silica gel (Hong & Ellis, 1996). This indicates that the drying method employed in the present study made it possible to further reduce the moisture content of the seeds before they lost viability. According to Nery *et al.* (2014), the seed drying method may influence desiccation tolerance.

The mean germination time and germination synchronization (uncertainty index) were favored in seeds with moisture content of 17% (minimum point) and 18% (maximum point), respectively (Figure 1). These results suggest that desiccation, up to approximately 17% moisture, stimulates seed vigor, since the shorter the germination time and the greater the synchronization, the more vigorous the seed lot (Ribeiro-Oliveira *et al*., 2013).

The satisfactory germination performance at 7.5% moisture content leads to the assumption that *S. amara* seeds can be considered intermediate because, according to Ellis *et al.* (1990), intermediate seeds tolerate desiccation from 8 to 10%. However, to confirm such assumption it is necessary to observe the behavior of seeds stored at different temperatures (Hong & Ellis, 1996). The results of the storage experiment, discussed below, will allow the conclusion about the classification of the species regarding the storage potential. Therefore, the effect of desiccation on the viability of the seeds and their response to the storage environment are determining factors in this classification.

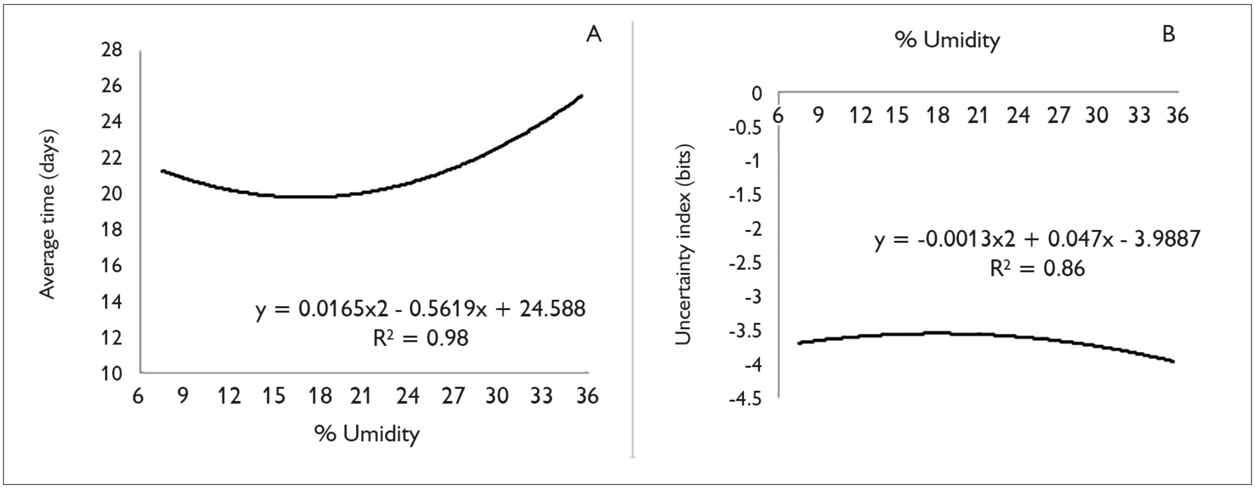

Figure 1. Evaluation of mean germination time (days) (A) and uncertainty index (bits) (B) for *Simarouba amara* Aubl. seeds stored for two months as a function of moisture content.

STORAGE

After two months of storage, germination was zero at temperatures of 7 °C and 15 °C for all moisture levels tested. The seeds kept at 7 °C had 100% mortality. The tetrazolium test, applied to non-germinated seeds of all treatments tested, confirmed that these seeds were dead. Seeds with recalcitrant behavior lose viability with desiccation and are sensitive to low temperatures (Nascimento *et al*., 2010; Garcia *et al*., 2015). At 20 °C, germination occurred for seeds stored with moisture content of 35.6% and 12.5%, but with considerable reduction when compared to freshly collected seeds (Table 1). Considering that intermediate seeds tolerate, besides the reduction in moisture content to levels close to 8%, storage at low temperatures (above 0 °C) (Ellis *et al*., 1990), we inferred that *S. amara* seeds are not intermediate and can therefore be classified as recalcitrant. Recalcitrant seeds have reduced viability in a short period, even when stored with high moisture contents (Santos, 2001; Bewley *et al.*, 2013).

Although, by definition, drying of recalcitrant seeds results in declining viability, considerable variation in desiccation sensitivity has been reported in the literature. Recalcitrant seeds form a very heterogeneous group

Table 1. Percentage of germination (% G), percentage of normal seedlings (% Normal), percentage of unviable seeds (% Unviable) for *Simarouba amara* Aubl. seeds, considering moisture content and storage time at 20 °C. Average on the columns, followed by equal letters do not differ statistically according to Scott-Knott test ($\alpha = 0.05$).

Treatments	%G	Normal (%)	Unviable (%)
Fresh collected seeds (35.6% humidity)	92 a	89.0 a	11.3 b
Fresh collected seeds dried at 12.5% humidity	87 a	87.0 a	8.3 b
Fresh collected seeds dried without dissecation (2 months)	25 b	17.0 b	63.0 a
Seeds stored at 12.5% humidity (2 months)	19 b	11.0 b	69.0 a
QME	79.9	38.7	38.6
Significance	4.2E-8	2.3E-10	5.7E-09
CV%	16.05	12.20	16.35

and can be classified from lowest to highest desiccation sensitivity (Pammenter & Berjak, 2000). In this context, Farrant *et al.* (1988) proposed to group seeds into highly, moderately and minimally recalcitrant. Species that have minimally recalcitrant seeds endure greater water loss before their viability is lost.

Guarea kunthiana A. Juss, similar to *S. amara*, showed reasonable tolerance to desiccation and, after storage (for 90 days), under condition 10 °C, lost viability and were classified as recalcitrant (Nery *et al*., 2014).

There was a reduction in the moisture content of the stored seeds for all moisture contents evaluated. For seeds stored with 35.6% and 12.5% moisture contents after two months, there were reductions to 13.5% and 11.2%, respectively. At four months of storage there was no germination and 100% of the seeds were not viable, as confirmed by the tetrazolium test results.

Simarouba amara seeds did not tolerate water loss at ≤ 5% levels and lost viability before 60 days of storage. The physiological mechanisms responsible for intolerance to desiccation in recalcitrant seeds are not yet fully understood, making it difficult to develop adequate protocols for storage and conservation of these seeds. In this context, it emphasizes the need for future studies evaluating the storage of *S. amara* seeds at moisture content of 17%, with the hypothesis that this moisture prolongs their viability during storage. In addition, it is important to evaluate storage for periods shorter than two months, considering that this information is useful for planning the planting of this species. It is also recommended that other propagule storage techniques, such as cryopreservation, be tested for *S. amara*.

CONCLUSION

Simarouba amara seeds have recalcitrant behavior and lose viability before reaching two months of storage maintained at a temperature of 20 °C with moisture contents of 12.5% and 35.6%.

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