

Sensitivity to drying and storage of *Syzygium cumini* (L.) Skeels Myrtaceae seeds

Sensibilidade à secagem e armazenamento de sementes de Syzygium cumini (L.) Skeels (Myrtaceae)

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ABSTRACT

Syzygium cumini seeds are recalcitrant, thus cannot tolerate drying and storage. The aim of this study was to evaluate the longevity of these seeds under different storage conditions and to assess the effect of osmotic treatment with polyethylene glycol (PEG) and abscisic acid (ABA) on reducing sensitivity to desiccation and increasing the longevity of these seeds. Seeds were desiccated until they reached preestablished moisture contents (40, 35, 30, 25 and 15%) and allowed to germinate. With 25% moisture content, germination was approximately 40%, and, with 15%, germination did not occur anymore. In the treatments aimed at reducing sensitivity to desiccation, seeds were incubated for 15 days in a solution of PEG (-1.88 MPa) or PEG (-1.88 MPa) + ABA (10^{-4} M), desiccated until they reached the preestablished moisture contents mentioned above, and then allowed to germinate. Five conditions were tested for storage: plastic bag in a cold room (8-10 °C; 45% RH); plastic bag in an air-conditioned room (20 °C; 60% RH); paper bag at room temperature; PEG solution at -1.88 MPa at 20 °C; and PEG solution at -1.88 MPa + ABA 10^{-4} M at 20 °C. Germination tests were carried out after 15, 30 and 90 days of storage. The best storage condition was in a plastic bag in an air-conditioned room, which was capable to keep a germinate rate close to 100% for up to 90 days. Under the conditions tested, osmotic treatment with or without ABA did not reduce sensitivity to desiccation and did not prolong seed longevity.

KEYWORDS: osmotic treatment; seed storage; forest species; polyethylene glycol; abscisic acid; seed desiccation.

RESUMO

As sementes de *Syzygium cumini* são recalcitrantes, portanto não toleram secagem e armazenamento. O objetivo deste estudo foi avaliar a longevidade dessas sementes sob diferentes condições de armazenamento e avaliar o efeito do tratamento osmótico com polietilenoglicol (PEG) e ácido abscísico (ABA) na redução da sensibilidade à dessecação e no aumento da longevidade dessas sementes. As sementes foram dessecadas até atingirem teores de umidade pré-estabelecidos (40, 35, 30, 25 e 15%) e colocadas para germinar. Com 25% de umidade, a germinação foi de aproximadamente 40% e com 15% a germinação não ocorreu mais. Nos tratamentos que visaram reduzir a sensibilidade à dessecação, as sementes foram incubadas por 15 dias em solução de PEG (-1,88 MPa) ou PEG (-1,88 MPa) + ABA (10^{-4} M), dessecadas até atingirem os teores de umidade pré-estabelecidos mencionados acima e colocadas para germinar. Foram testadas cinco condições de armazenamento: saco plástico em câmara fria (8-10 °C; 45% UR); saco plástico em sala climatizada (20 °C; 60% UR); saco de papel

em temperatura ambiente; solução de PEG a -1,88 MPa a 20°C; e solução de PEG a -1,88 MPa + ABA 10^{-4} M a 20 °C. Os testes de germinação foram realizados após 15, 30 e 90 dias de armazenamento. A melhor condição de armazenamento foi em saco plástico em sala climatizada, capaz de manter taxa de germinação próxima de 100% por até 90 dias. Nas condições testadas, o tratamento osmótico com ou sem ABA não reduziu a sensibilidade à dessecação e não prolongou a longevidade das sementes.

PALAVRAS-CHAVE: tratamento osmótico; armazenamento de sementes; espécies florestais; polietileno glicol; ácido abscísico; dessecação de sementes.

INTRODUCTION

The genus *Syzygium* (Myrtaceae) comprises approximately 1,200 species of trees and shrubs that grow naturally in the subtropical and tropical regions of Africa, Asia, and Oceania (COCK & CHEESMAN 2018).

The species investigated in this study, *Syzygium cumini* (L.) Skeels are popularly known as black plum and are native to Bangladesh, India, Pakistan, Sri Lanka, Malaysia, the Philippines, and Indonesia (DISSANAYAKE et al. 2022). It produces large quantities of berry-like, fleshy, and edible fruit every year, containing a single polyembryonic seed (CAVALCANTI & PERNAMBUCO 2010) with recalcitrant behavior (NAIR et al. 2020). This species has medicinal uses against dysentery, hemorrhage, and diabetes (BIJAULIYA et al. 2018, CHHIKARA et al. 2018). It is also used as an ornamental tree (SANTIAGO et al. 2016), in agroforestry systems (GAUTAM et al. 2016), and for ecological restoration (MISHRA et al. 2013).

The unquestionable value of this multifunctional tree led to the recent sequencing of its genome (ABHISEK et al. 2023), highlighting the importance of studies on the conservation of seeds, not only to meet the needs of tree nurseries, but also to develop germplasm conservation programs.

In the conservation of genetic biodiversity, seeds are expected to have a high storage capacity (AMORIM et al. 2023), meaning that most of the species present in seed banks are those that produce orthodox seeds, i.e., those that tolerate desiccation and storage for long periods under low temperatures and air humidity (BEWLEY & BLACK 1994, MARCOS-FILHO 2015, SMOLIKOVA et al. 2020).

Recalcitrant seeds, on the other hand, do not acquire desiccation tolerance, do not go through the drying phase, are dispersed with high moisture content, and are metabolically active. In addition, the recalcitrant seeds of many species, especially those of tropical origin, cannot tolerate temperatures below 15 °C (BERJAK et al. 1989, PAMMENTER & BERJAK 1999). These seeds are sensitive to desiccation and have a short storage life, ranging from a few days to several months (BARBEDO 2018, BHARUTH & NAIDOO 2020, CHANDRA et al. 2021).

One of the possible causes of the loss of viability of stored recalcitrant seeds is the fact that their metabolic activity is permanently active during storage, requiring additional moisture, which is obviously not supplied, resulting in greater water stress (PAMMENTER et al. 1994). Therefore, one possible way to increase the longevity of these seeds during storage is to reduce their metabolic rates, which can be achieved by storing them in osmotic media, with or without the addition of germination inhibitors such as abscisic acid (ABA) (BERJAK & PAMMENTER 2003, DRESCH et al. 2017). ABA acts on the synthesis of LEA (Late Embryogenesis Abundant) proteins, which are related to desiccation tolerance in seeds (LEPRINCE et al. 2017, CHEN et al. 2020).

One of the most commonly used products in osmotic treatments is polyethylene glycol (PEG), which is a chemically inert and non-toxic solute that is not absorbed by seeds because of its large molecules (VILLELA et al. 1991, ANDRÉO et al. 2006).

In addition to reducing metabolism, osmotic treatment and ABA, when applied alone or in combination with other stress agents, such as drying and heat shock, can induce or increase desiccation tolerance not only in recalcitrant seeds (ANDRÉO et al. 2006, PEREIRA et al. 2020, WANG et al. 2023), but also in germinated orthodox seeds (MASETTO et al. 2015, KOUR & ZHAWAR 2018), *Medicago sativa* L. somatic embryos (ALWAEL et al. 2017), and the development of orthodox seeds (KERMODE & FINCH-SAVAGE 2002, LEDUC et al. 2012).

Techniques that reduce the sensitivity to desiccation and increase the storage capacity of recalcitrant seeds are important for the conservation of genetic resources. The hypothesis of this study was that osmotic treatment with PEG, with or without ABA, can reduce sensitivity to desiccation and extend the storage life of *Syzygium cumini* seeds. The objectives of this study were to characterize the behaviors of these seeds in terms of desiccation and storage under different conditions and to verify the effects of PEG and ABA on desiccation sensitivity and longevity.

MATERIALS AND METHODS

Ripe dark purple fruits of *Syzygium cumini* were obtained by shaking tree branches of trees and collecting the fallen material. The trees were on the campus of the Federal University of Lavras (UFLA), Lavras, Brazil. The seeds were processed by pulping them in a steel mesh sieve under running water until all fruit residue was removed. The seeds were then placed in a single layer in an air-conditioned room at 20°C and 60% relative humidity until they lost their surface water (approximately two hours) and were then used for germination, desiccation, and storage studies. This work was carried out at the Forest Seed Laboratory of the Forest Sciences Department at UFLA.

The moisture content of the seeds was determined in an oven at 105 ± 3 °C for 24 h in accordance with the Rules for Seed Analysis (BRASIL 2009). Four replicates of five seeds were used, and the results are expressed as a percentage of the seeds' wet weight.

Germination tests were carried out on seeds that had been washed for 10 min in a 1% sodium hypochlorite solution for disinfection and then rinsed under running water. To determine the ideal germination temperature, tests were carried out according to a completely randomized experimental design in germination chambers with four replicates of 25 seeds per treatment between sand in plastic trays at temperatures of 20, 25, 30, and 35 °C, under constant light. The germination criterion was the protrusion of the radicle (visible germination). The results are expressed as the germination percentage and the germination speed index (GSI), calculated according to MAGUIRE (1962).

To characterize desiccation sensitivity, seeds were desiccated at 20 °C in a closed drying box with internal ventilation. The relative humidity inside the box was approximately 18.5%, provided by a saturated solution of sodium hydroxide (NaOH) in a plastic tray at the bottom of the box. The seeds were placed in a single layer on a plastic sheet 20 cm above the NaOH solution and kept until they reached or approached the pre-established moisture contents (40, 35, 30, 25 and 15%). To monitor the reduction in moisture content, the seeds were weighed periodically and the current moisture content was calculated based on the reduction in the fresh weight of the seeds. When each pre-established moisture level was reached, seeds were removed from the drying boxes and placed to germinate, as described above.

To study the possible effect of PEG and PEG + ABA on reducing the sensitivity of seeds to desiccation, two treatments were tested: 1) incubation of the seeds in a solution of PEG (-1.88 MPa) and 2) incubation of the seeds in a solution of PEG (-1.88 MPa) + ABA (10^{-4} M). The PEG solution was prepared according to MICHEL & KAUFMANN (1973). The seeds were placed in a single layer in plastic trays, and solutions were poured over them in sufficient quantity to cover the entire tray.

The trays were covered with plastic wrap to prevent excessive evaporation of the solution and were kept at 20 ± 2 °C in the dark. As a control, a third sample of seeds was placed in a plastic bag and maintained under the same temperature and light conditions. After 15 days, the incubation was stopped and the seeds were washed in running water until all residue was removed. The seeds were then surface-dried on paper towels and dried in drying boxes, as described above, until they reached the same moisture content as before. Germination tests were carried out as described above.

To study the storage of freshly processed seeds, five conditions were tested: 1) plastic bag in a cold room (8-10 °C; 45% RH); 2) plastic bag in an air-conditioned room (20 °C; 60% RH); 3) paper bag at room temperature (laboratory bench); 4) storage in PEG solution (-1.88 MPa) in plastic trays at 20 °C; and 5) storage in PEG solution (-1.88 MPa) + ABA (10^{-4} M) in plastic trays at 20 °C. The samples were taken after

15, 30, and 90 days, washed (those in PEG or PEG + ABA), and used for moisture content determination and germination tests, as described above.

All experiments were carried out according to a completely randomized design. The data were subjected to the Shapiro-Wilk normality test and the chi-square homogeneity test and submitted to analysis of variance (ANOVA) using the F-test at 5% probability and the ASSISTAT statistical aid software (SILVA & AZEVEDO 2016). The means were compared using the Tukey test at 5% probability.

RESULTS AND DISCUSSION

The initial moisture content of the seeds was 49%, which is similar to the value (46.2%) reported by BAXTER et al. (2004) for seeds of the same species. Moisture contents >45% are common in recalcitrant seeds at the time of dispersal (MAYRINCK et al. 2016).

Seed germination was high (90-100%) at temperatures between 20°C and 35 °C (Figure 1), similar to that observed by FIOR et al. (2010) and GUARDIA et al. (2020) for seeds of the same genus. With regard to the germination speed index (GSI), the highest values were observed at temperatures ranging from 25 to 35 °C (Figure 1), with germination beginning four days after the test was set up and lasting until the 13th day. Germination began on the 11th day at 15°C and lasted until the 47th day.

Because there was no significant difference in germination or GSI values between the 25 and 35 °C treatment groups, the 25 °C temperature was used for the other germination tests in this study.

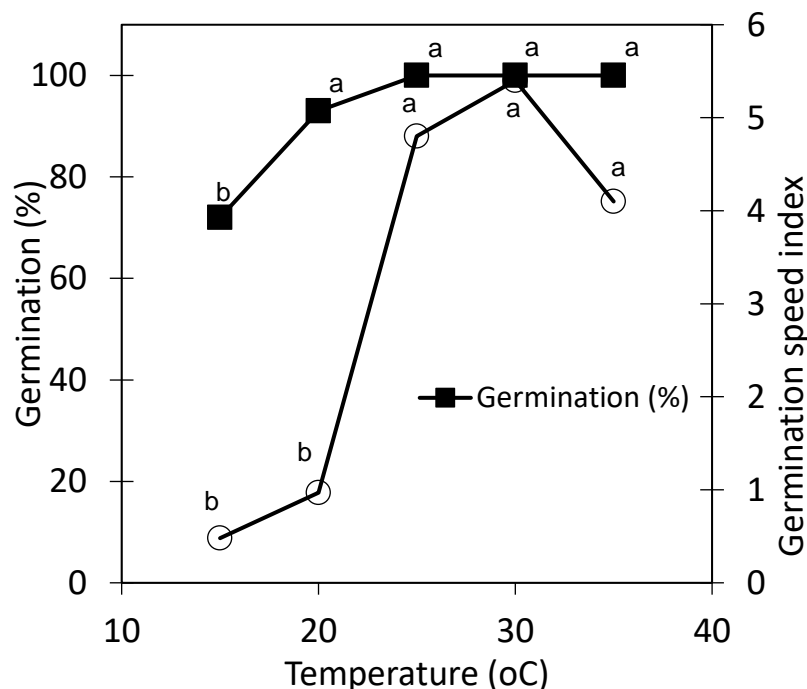


Figure 1. Final percentage germination and germination speed index of freshly harvested *Syzygium cumini* seeds at different temperatures. Averages followed by equal letters within each evaluated parameter are not significantly different according to Tukey's test at 5% probability.

There was no significant difference in the germination rate between the seeds incubated in PEG or PEG + ABA for 15 days and desiccated at 35% humidity (Table 1); however, a decrease in the germination speed index (GSI) was observed in relation to that of the control seeds stored in a plastic bag in an air-conditioned room (20 ± 2 °C/60% RH) (Table 2). Below 31% humidity, there was a decrease in the percentage of germinated seeds in the PEG + ABA treatment group. A reduction in germination was also

observed by PELISSARI et al. (2022) in *Magnolia ovata* (A.St.-Hil.) Spreng. when PEG and ABA were used together or not.

Table 1. Average percentage germination of *Syzygium cumini* seeds after 15 days of osmotic treatment (PEG) with or without ABA at 20 °C, followed by drying for different moisture contents. The control treatment included seeds that were not subjected to osmotic treatment before drying but were kept for 15 days in a plastic bag at 20 °C.

Seed moisture content (%)	Treatment before drying		
	Control	PEG	PEG + ABA
42	94Aa	77Ba	76Ba
35	73Aa	56Ab	57Ab
31	77Aa	58Bab	35Bc
25	43Ab	14Bc	9Bd
15	*	*	*

Averages followed by the same uppercase letter in the row and lowercase letter in the column do not statistically differ according to the Tukey test at 5% probability. * No germination was observed.

In seeds with a water content of 25%, the percentage of those incubated in PEG or PEG + ABA was lower than that of the control, and in seeds with a moisture content of 15%, the germination percentage was zero in all treatments (Table 1). Drying below 20% humidity was also lethal for seeds of *Syzygium zeylanicum* (L.) DC. (SHARANYA et al. 2023) and *Syzygium maire* (A. Gunn.) Sykes et Garn.-Jones (NADARAJAN et al. 2021). These findings show that the treatments tested increased the sensitivity of the seeds to desiccation, similar to the results obtained by ARAUJO & BARBEDO (2017) for immature seeds of *Caesalpinia echinata* Lam. The same trend was observed in the GSI results, where the control treatment with up to 25% humidity had higher values than the other treatments (Table 2).

Table 2. Mean germination speed index (GSI) of *Syzygium cumini* seeds after 15 days of osmotic treatment (PEG) with or without ABA at 20 °C, followed by drying for different moisture contents. The control treatment included seeds that were not subjected to osmotic treatment before drying and were kept for 15 days in a plastic bag at 20 °C.

Seed moisture content (%)	Treatment before drying		
	Control	PEG	PEG + ABA
42	1.45Aa	1.11Ba	1.14Ba
35	0.93Ab	0.71Bb	0.54Bb
31	0.91Ab	0.71Ab	0.29Bc
25	0.26Ac	0.13Bc	0.17Ac
15	*	*	*

Averages followed by the same uppercase letter in the row and lowercase letter in the column do not statistically differ according to the Tukey test at 5% probability. * No germination was observed.

The germination percentage of fresh (undried) *S. cumini* seeds stored in paper bags at room temperature decreased significantly during the first 15 days, from 100% to 17.5%, reaching 0% after 30 days. The seeds stored under other conditions maintained an average germination rate of over 89% for up to 30 days. No spontaneous germination was observed during seed storage under any of the tested conditions.

At 90 d, the germination of seeds stored in plastic bags at 20 °C was unchanged, whereas seeds stored at 8 °C showed a significant decrease in germination (Figure 2). This reduction may have occurred due to the sensitivity of these seeds to low temperatures, since recalcitrant seeds, especially those of tropical species, can be sensitive to lower temperatures (BERJAK et al. 1989), as shown by FERREIRA et al. (2021), in a study of *Coccoloba gigantifolia* Melo, Cid Ferreira & Gribel seeds, whose viability was reduced when stored at 8 °C.

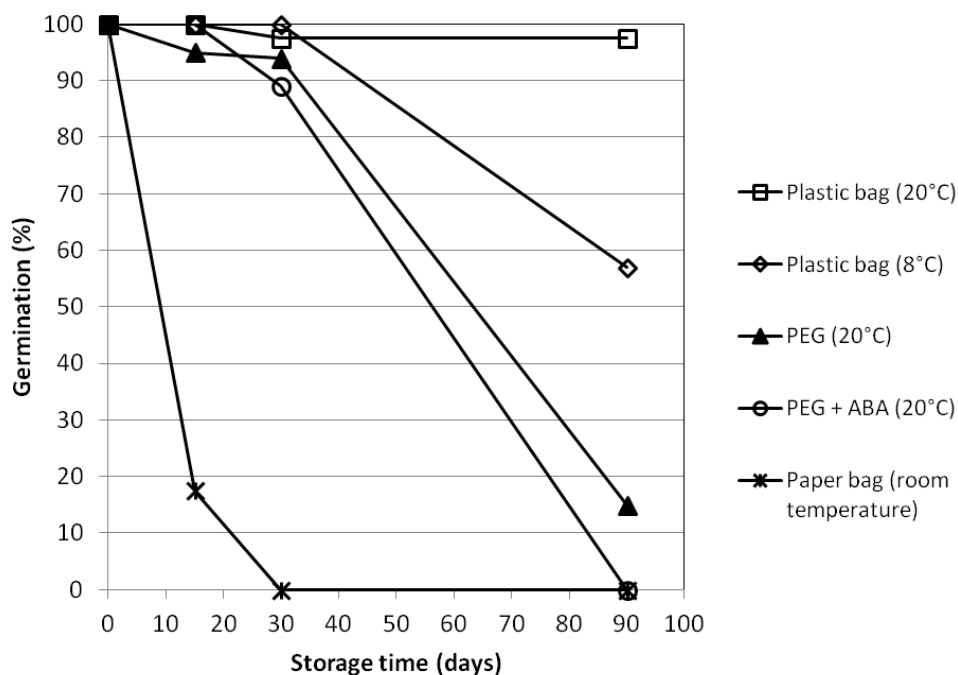


Figure 2. Average germination percentage of *Syzygium cumini* seeds after 15, 30, and 90 days of storage in different conditions.

At 90 days, seeds stored in PEG showed low germination (15%), whereas those stored in PEG + ABA did not germinate. The possible low oxygenation of seeds under these storage conditions, in which they were immersed in solution, may have contributed to the rapid decrease in viability. These results differ from those of a study by CECEL & BARBEDO (2023), in which the storage of recalcitrant *Eugenia brasiliensis* Lam. seeds in PEG solution at 1.0 and 2.0 MPa was effective in maintaining their viability after 540 days, although they had a low capacity to form normal seedlings.

Another factor that may have contributed to the total lack of germination of seeds stored in PEG + ABA was the inhibitory effect of ABA on seed germination (BEWLEY & BLACK 1994). Different results (positive effect) from the use of ABA were reported by PELISSARI et al. (2022), who studied the storage capacity of *Magnolia ovata* seeds previously treated with ABA and dried at 5% humidity.

One of the causes of the loss of viability of stored recalcitrant seeds is their metabolic activity during storage, which depletes the seed's food reserves (FARIA et al. 2006). Although the treatments used in this study were not effective in prolonging the storage capacity of the seeds, the technique is promising, as has already been reported for *Inga vera* embryos stored in PEG solution, which maintained germination above 80% for 90 days (ANDRÉO et al. 2006) and above 90% for 200 days (PEREIRA et al. 2020). Therefore, further studies should be conducted to test other water potentials and ABA concentrations during the storage of *Syzygium cumini* seeds.

CONCLUSIONS

Syzygium cumini seeds lose their viability when dried at 15% humidity. *Syzygium cumini* seeds stored in plastic bags at 20 °C were maintained for up to 90 days.

Osmotic treatment of the seeds with PEG or PEG + ABA did not reduce their sensitivity to desiccation or improve their storage capacity under the tested conditions.

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