

## 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 1200 tree and shrub species that naturally grow in the subtropical and tropical regions of Africa, Asia and Oceania (COCK & CHEESMAN 2018).

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

The undoubtedly value of this multipurpose tree led to the recent sequencing of its genome (ABHISEK et al. 2023), highlighting the importance of studies on the conservation of its seeds, not only for meeting the needs in the tree nurseries, but also for germplasm conservation programs.

In the conservation of genetic biodiversity, seeds are expected to have a high storage capacity (AMORIM et al. 2023), meaning that the majority of 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 a high moisture content and are metabolically active. In addition, recalcitrant seeds of many species, particularly 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 a few 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, thus requiring additional moisture, which is obviously not supplied, resulting in increased water stress (PAMMENTER et al. 1994). Therefore, one possible way to extend the longevity of these seeds during storage is by reducing 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), a chemically inert and nontoxic solute that is not absorbed by seeds due to the large size of its 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 (ALWAEEL et al. 2017), and developing orthodox seeds (KERMODE & FINCH-SAVAGE 2002, LEDUC et al. 2012).

Techniques that provide less sensitivity to desiccation and increase storability of recalcitrant seeds would be highly important for the conservation of genetic resources. The hypothesis of the present 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 behavior of these seeds in terms of desiccation and storage under different conditions and to verify the effect of PEG and ABA on desiccation sensitivity and longevity.

## MATERIAL AND METHODS

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

The moisture content of the seeds was determined in an oven at  $105 \pm 3$  °C for 24 hours 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 wet weight of the seeds.

Germination tests were carried out with seeds that were previously washed for 10 minutes in a 1% sodium hypochlorite solution for disinfection and then rinsed in running water. To determine the optimum germination temperature, the tests were carried out in accordance with 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 germination speed index (GSI), calculated according to MAGUIRE (1962).

To characterize the sensitivity to desiccation, the 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 preestablished 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 preestablished moisture level was reached, samples of the seeds were removed from the drying boxes and set to germinate, as described above.

To study the possible effect of PEG and PEG + ABA on reducing seed sensitivity to desiccation, two treatments were tested: 1) incubating the seeds in a PEG solution (-1.88 MPa) and 2) incubating the seeds in a PEG solution (-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 the solutions were poured over them in enough quantity to cover them slightly.

The trays were covered with plastic film to prevent excessive evaporation of the solution and kept at  $20 \pm 2$  °C in the dark. As a control, a third sample of seeds was placed in a plastic bag and kept under the same temperature and light conditions. After 15 days, the incubation was interrupted and the seeds were washed in running water until all the residue was removed. The seeds were then superficially dried on paper towels and dried in drying boxes, as described above until they reached the same target moisture contents 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. Samples were taken after 15, 30 and 90 days, washed (those that were in PEG or PEG + ABA) and used for moisture content determination and germination tests, as described above.

All the experiments were performed in accordance with a completely randomized design. Data were submitted to the normality Shapiro-Wilk test and to the chi-squared test of homogeneity and subjected to analysis of variance (ANOVA) using the F test at 5% probability and the Statistical Assistance Software ASSISTAT (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%, very similar to the value (46.2%) found by BAXTER et al. (2004) for seeds of the same species. Moisture contents above 45% are common in recalcitrant seeds by the time of dispersal (MAYRINCK et al. 2016).

Seed germination was high (90-100%) at temperatures between 20 and 35 °C (Figure 1), similar to what was 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. At 15 °C, germination began on the 11th day and lasted until the 47th day.

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

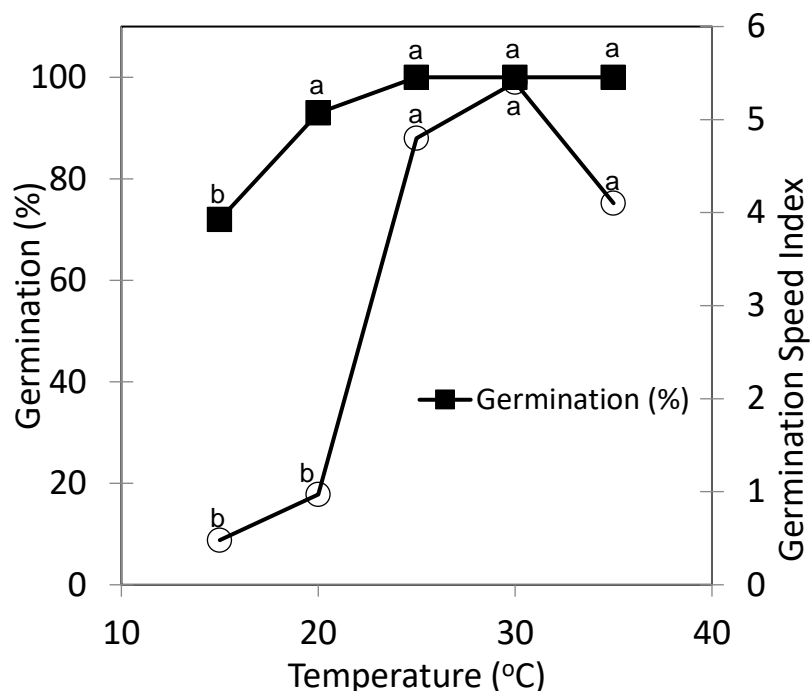


Figure 1. Final germination percentage and germination speed index of freshly harvested *Syzygium cumini* seeds at different temperatures. Averages followed by equal letters within each parameter evaluated are not significantly different according to Tukey's test at 5% probability.

There was no significant difference in germination rate between the seeds incubated in PEG or PEG + ABA for 15 days and desiccated to 35% moisture content (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 ( $20 \pm 2$  °C/60% RH) (Table 2). Below 31% moisture content, 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. seeds when PEG and ABA were used, combined or not.

Table 1. Average germination percentage of *Syzygium cumini* seeds after 15 days of osmotic treatment (PEG) with or without ABA at 20 °C, followed by drying to different moisture contents. The control treatment refers to 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 differ statistically according to the Tukey test at 5% probability. \* No germination was observed.

In seeds with a 25% water content, the percentage of those incubated in PEG or PEG + ABA was lower than that of the control and, at 15% moisture content, the percentage of germination was zero in all the treatments (Table 1). Drying below 20% moisture content 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 showed that the treatments tested increased desiccation sensitivity of the seeds, similarly to the results obtained by ARAUJO & BARBEDO (2017) for immature seeds of *Caesalpinia echinata* Lam. The same trend was observed in the GSI results, in which the control treatment with up to 25% moisture content had higher values than did the other treatments (Table 2).

Table 2. Average values of the 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 to different moisture content. The control treatment refers to 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 differ statistically according to the Tukey test at 5% probability. \* No germination was observed.

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

At 90 days, the germination of the seeds stored in plastic bags at 20 °C did not change, while those 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 with *Coccoloba gigantifolia* Melo, Cid Ferreira & Gribel seeds, whose viability was reduced when stored at 8 °C.

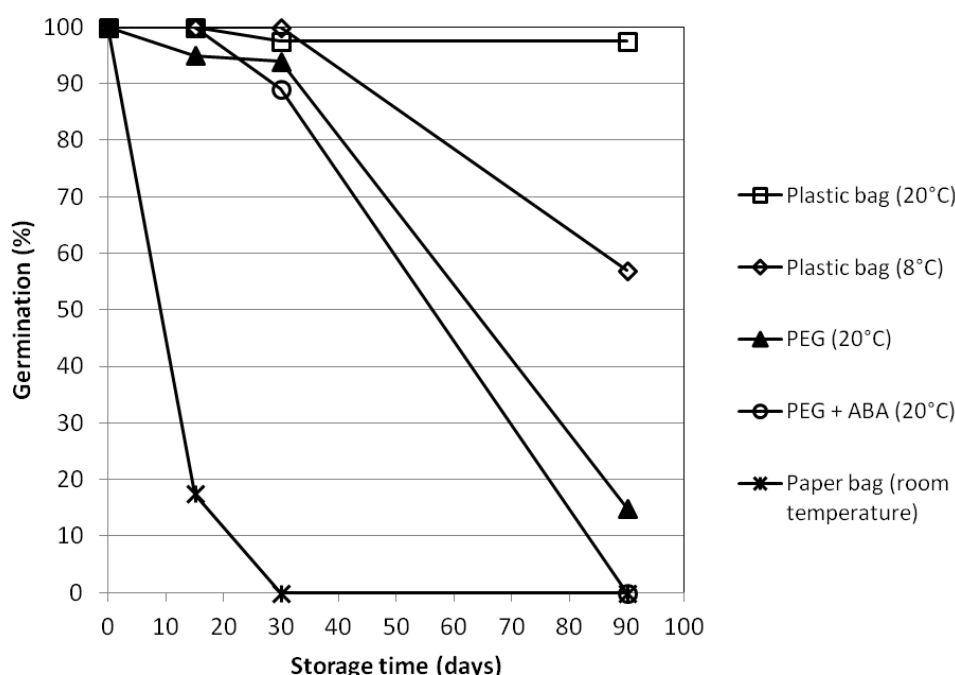


Figure 2. Average germination percentage of *Syzygium cumini* seeds after 15, 30 and 90 days of storage under different conditions.

At 90 days, the seeds stored in PEG had low germination (15%), while those stored in PEG + ABA did not germinate. The possible low oxygenation of the 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 the 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 at maintaining their viability after 540 days although with a low capacity to form normal seedlings.

Another factor that may have contributed to the total lack of germination in seeds stored in PEG + ABA,

is the inhibitory effect of ABA on seed germination (BEWLEY & BLACK 1994). Different results (positive effect) from the use of ABA were found by PELISSARI et al. (2022), who studied the storability of *Magnolia ovata* seeds previously treated with ABA and dried to 5% moisture content.

One of the causes of viability loss of stored recalcitrant seeds is that they remain metabolically active during storage, thereby exhausting the seed's food reserves (FARIA et al. 2006). Although the treatments used in the present work were not effective at prolonging the storability 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). Further studies should therefore be carried out to test other water potentials and ABA concentrations during the storage of *Syzygium cumini* seeds.

## CONCLUSIONS

*Syzygium cumini* seeds completely lose their viability when dried to 15% moisture content. The viability of *Syzygium cumini* seeds stored in plastic bags at 20 °C was 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 storability under the conditions tested.

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