

Full Length Research Paper

Drying and reduction in sensitivity to desiccation of *Campomanesia xanthocarpa* seeds

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The aim of this study was to evaluate the level of sensitivity to desiccation of *Campomanesia xanthocarpa* seeds and reduce it by using polyethylene glycol (PEG) and abscisic acid (ABA) to maintain viability. In experiment I, the seeds were desiccated to 35, 30, 25, 20, 15, 10, and 5 ± 2% moisture content by using silica gel (fast drying) and by drying them at room conditions (slow drying; 25 ± 2°C and 35% relative humidity). In experiment II, the seeds were soaked for 0, 60, and 120 h in PEG (-1.48 MPa), with ABA at different concentrations (0, 10⁻³, 10⁻⁴ and 10⁻⁵ µM). Subsequently, they were desiccated to 15% moisture content by fast drying. *C. xanthocarpa* seeds are sensitive to desiccation; however, we showed that slow drying them at 25°C room temperature for 12.5 h to 15% moisture level, and then fast drying by using silica gel for 7 h to 20% water content maintained long-term seed viability. Sowing must be performed immediately to avoid further moisture loss. Osmotic conditioning, irrespective of whether abscisic acid was used, did not reduce the sensitivity to desiccation of *C. xanthocarpa* seeds.

Key words: Abscisic acid, Brazilian Savanna, conservation, drying, Myrtaceae, polyethylene glycol.

INTRODUCTION

The reproductive success of a species depends on several factors, including the number and quality of the seeds produced. The initial life history phase of a plant is considered among the most critical periods, because the establishment of plant populations will depend on the ability of the seeds and seedlings to adapt to adverse or variable conditions (Garcia et al., 2007).

The tropical savanna at the Brazilian Savanna, in Brazil, presents a wide biodiversity of flora. Similarly, the Mato Grosso do Sul holds several species, including

Campomanesia xanthocarpa O. Berg., commonly known as guabiroba, a tree species found in areas ranging from Minas Gerais to Rio Grande do Sul. *C. xanthocarpa* is a heliophytic, hydrophytic, to mesophytic plant, very common in mixed ombrophilous forests, and found frequently on moist soils of alluvial terrains, clumps, and open areas of secondary forests (Reitz, 1983). Its fruits are round and green when young and become yellow and sweet when ripe. The seeds are spread by zoochory, and the fruit attracts mainly birds, especially the *Turdus* sp.,

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Tangara sp., and *Thraupis* sp. (Frisch and Frisch, 2005), contributing to maintain the ecosystem. This tree species is recommended for home orchards, parks, and recovering degraded riparian forests (Silva et al., 2007; Valillo et al., 2008). However, information on seed conservation, cultivation, and seedling planting optimization techniques are scarce.

In recent years, due to the need of restoration and conservation of ecosystems, there was an increase in the number of studies to understand the behavior of seeds of native species during storage (Davide et al., 2003); however, considering the great diversity of flora species and the lack of programs for production of seeds with genetic and physiological quality in sufficient quantities to meet demand (Silva et al., 2014), the information available are still scarce. The biggest challenge to express this progress is related to the best practice for germplasm conservation of fruit species native to Cerrado, through desiccation of seeds.

Specific technologies are required to conserve and maintain the viability of seeds that are not tolerant to desiccation. The main technique still used to date is based on the reduction of the seed's metabolism, either by reducing the moisture content or cooling (Kohama et al., 2006). In addition to moisture reduction, drying rate can also influence the level of sensitivity of the seeds to desiccation (Marcos Filho, 2005; Berjak and Pammenter, 2008). This depends on inherent seed characteristics, such as the nature of the seed coating, overall size, and stage of development (Berjak and Pammenter, 2008). The ability to tolerate desiccation is considered an adaptive strategy to allow seed survival under unfavorable environmental conditions, enabling the dissemination of the species (Medeiros and Eira, 2006).

Other than reducing the seed's metabolism, subjecting the seeds to osmotic treatment with polyethylene glycol (PEG) and abscisic acid (ABA) has been shown to induce or increase tolerance to desiccation in recalcitrant (Beardmore and Whittle, 2005; Andreo et al., 2006; Faria, 2006) and orthodox seeds (Buitink et al., 2003; Faria et al., 2005). PEG and ABA can be used in isolation, together, or in combination with other stress conditions, such as drying. Reducing the level of sensitivity to desiccation involves maintaining seed viability while reducing moisture content to the maximum.

The development of techniques aimed to maintain *C. xanthocarpa* seed viability will promote the use of this species in the restoration of degraded habitats. However, species from the genus *Campomanesia* usually produce desiccation and storage intolerant seeds. The lack of knowledge in relation to drying and shelf life makes it more difficult to use this genus in the restoration of degraded areas. Here, we hypothesized that, under artificial conditions, the seeds of *Campomanesia* may undergo a small reduction in the moisture level, irrespective of the drying speed. Thus, our objective was to evaluate the drying conditions and reduction of desiccation sensitivity by using PEG and ABA for

maintaining *C. xanthocarpa* seed viability.

MATERIALS AND METHODS

In experiment I, fruits harvested in 2012 were manually processed to remove the skin and the pulp. The seeds were subsequently washed and placed over Germitest® paper for 40 min at room temperature ($25 \pm 2^\circ\text{C}$, 35% relative humidity [RH]). Following, the seeds were dried using activated silica gel (8% RH) (fast drying) and a separate group of seeds was dried at room temperature ($25 \pm 2^\circ\text{C}$ and 35% RH) (slow drying). The silica gel dried seeds were placed inside a "gerbox" with silica on the bottom, and the silica gel was replaced immediately after the superficial layer turned pink, fading from its original blue color. For the slow drying method, the seeds were placed inside two open plastic recipients and left at room temperature ($25 \pm 2^\circ\text{C}$ and 35% RH). The seeds were weighed every hour until they reached the pre-established limits of 35, 30, 25, 20, 15, 10 and $5 \pm 2\%$ moisture content, according to Sacandé et al. (2004). After achieving the desired water content for both the drying methods, the seeds were pre-moistened for 24 h in a moisture box (100% RH at 25°C under constant white light) to avoid damage due to soaking. Subsequently, a series of characteristics were determined to evaluate their physiological potential. The water content was determined at $105 \pm 3^\circ\text{C}$ for 24 h using the greenhouse method with three replicates of 5 g of seeds each, and the results were expressed on a wet basis.

Protrusion of the primary root was measured on Germitest® paper rolls with four replications of 25 seeds each, germinated with B.O.D. (Biochemical Oxygen Demand) at 25°C under continuous white light. Assessments were conducted daily, and the root was considered protruded when it reached a length of 5 mm. The results were expressed in percentages (%).

The percentage of normal seedlings was determined in Germitest® paper rolls with four replications of 25 seeds each, which were germinated with BOD at 25°C under continuous white light. Evaluations were performed thirty days after sowing (Herzog et al., 2012) and the results were expressed in percentages (%).

Seedling length was obtained by measuring the lengths of the primary root, aerial part and total plant using a millimeter ruler. The results were expressed in centimeters (cm). The total dry mass was obtained from seedlings that had been dried in an oven at 60°C for 48 h using an analytical balance (0.0001 g), with the results expressed in grams (g). The design was a completely randomized factorial (2 drying x 5 water content). Data were subjected to analysis of variance and regression analysis at 5% probability were performed using the SISVAR.

In experiment II, the fruits harvested from a degraded area of the Cerrado region in 2013 had their pulps removed. The seeds, together with the pulp, were placed inside a transparent plastic bag (0.25 mm thick), and stored at 5°C for 15 days. After this period, all seeds were separated from the pulp and placed on Germitest® paper for superficial drying. One sample was used as a control (that is, this sample was not subjected to osmotic or ABA treatment). The remaining seeds were soaked in PEG (-1.48 MPa, as recommended by Dresch, 2013) for different periods (0, 60, and 120 h). In addition, these samples were exposed to different concentrations of ABA (0 , 10^{-3} , 10^{-4} , and 10^{-5} μM) and maintained in a biochemical oxygen demand (BOD) incubator at 25°C . Following the osmotic treatment, the seeds were washed in running water for 5 min, to remove any trace of PEG and ABA, and subsequently, placed on a paper towel and left to dry for 10 min at room temperature ($25 \pm 2^\circ\text{C}$, 32% RH). The seeds were then dried using activated silica gel (Dresch, 2013), until the pre-established water content ($15 \pm 2\%$ RH) was achieved, following Sacandé et al. (2004). Afterward, the seeds were allowed to germinate, to evaluate the physiological potential of the seeds, as described in experiment I

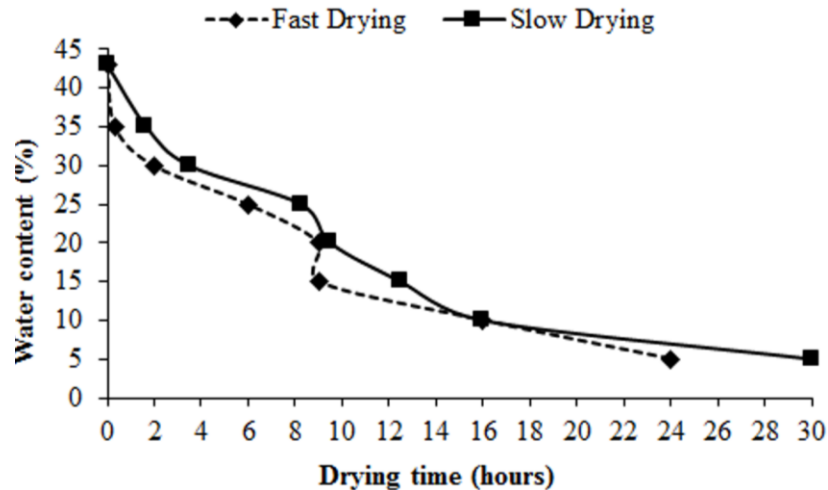


Figure 1. Drying curve on silica (fast) and laboratory conditions (slow) of *C. xanthocarpa* O. Berg. seeds.

RESULTS

C. xanthocarpa seeds could be dried to 5% water content in 30 h by the slow drying method; however, by using the fast drying method (silica gel), the seeds achieved the desired 5% water content within 24 h (Figure 1). For the primary root protrusion and percentage of normal seedlings were observed quadratic adjustments to drying methods, and the water content 36.3 and 39.3%, respectively, for fast drying and 32 and 32.5% respectively in the slow provided close to 100% in different drying values. Desiccating the seeds to 5% moisture level, by using both the methods, completely prevented primary root protrusion, and consequently, none of the seedlings germinated (Figure 2a and b).

Reducing the seeds water content to 15% led to different seedling percentages depending on the drying method. Fast drying yielded 46.7% of normal seedlings while slow drying yielded 65.2% (Figure 2b). The germination speed index (GSI) was also influenced by desiccation and drying methods, with lower results obtained for 5% moisture level (Figure 2c). The length of aerial part and of primary root also showed a quadratic relation with the drying methods (Figure 3a and b). The aerial part of the root showed the maximum growth (5.54 cm) after fast drying, with 31.8% water content; on the other hand, slow dried seeds showed 5.38 cm growth of the aerial part of the root, with 30.9% moisture level (Figure 3a). The largest primary root growth was observed for 34.8% moisture level (7.42 cm) after fast drying and for 30% water content (8.19 cm) after slow drying (Figure 3b). Total seedling length was influenced by the final water content attained and the drying method. Fast dried seeds showed the maximum growth at 33.4% water content (12.93 cm); slow dried seeds, on the other hand, showed the maximum growth at 30.3% water

content (13.57 cm) (Figure 4a). Water content and drying methods did not significantly affect the total dry mass, and the maximum biomass was measured at 29.5% water content (0.0399 g) (Figure 4b).

In experiment II, seeds desiccated to 15% moisture and not treated with PEG or ABA showed better results for primary root protrusion, germination speed, and seedling germination (Table 1). Seeds not treated and treated with PEG and ABA (10^{-5} μM) for 60 and 120 h developed the longest aerial parts of the plant. Primary root length and dry biomass did not statistically differ among treatments, except when the seeds were treated with PEG and 10^{-4} and 10^{-3} μM of ABA for 120 h. Germination did not occur under these treatments and significantly influenced seedling growth.

DISCUSSION

C. Xanthocarpa seeds naturally present a mucilaginous layer, allowing them to retain high moisture content, thereby facilitating germination and plant growth whenever adequate conditions are met. However, for such zoochorous seeds, this mucilage is lost after ingestion, while passing through the digestive tract. In such cases, the seeds became highly desiccation-sensitive, causing them to be efficient only for a short period before heavy rainfalls in this region. Here, we showed that if the seeds are exposed to dry conditions for over 24 h with an average temperature of 25°C, germination might be rendered impossible, thus, making it difficult for this species to naturally spread in the region.

The differences found in drying time usually relate to the seeds desiccation sensitivity (Berjak and Pammenter, 2008). In the case of *C. adamantium*, its seeds have been shown to tolerate desiccation to approximately 21%

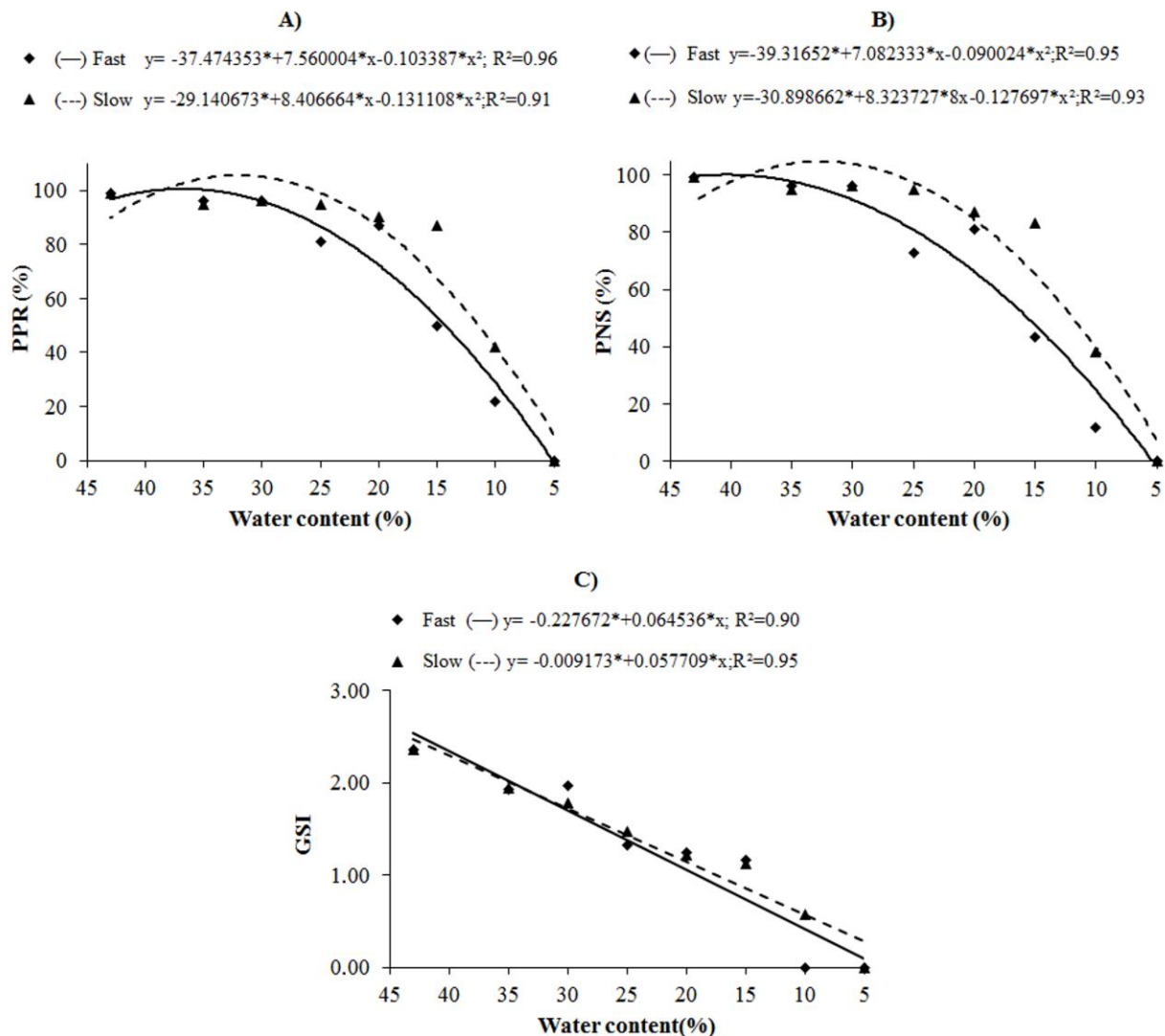


Figure 2. Protusion of the primary root (PPR) (a) (%), percentage of normal seedlings (PNS) (b) (%) and germination speed index (GSI) (c) of *C. xanthocarpa* O. Berg. seedlings depending of fast and slow drying different water contents.

moisture content after 7 h drying in silica gel, and 17% moisture content after 10 h drying under normal ambient conditions (Dresch, 2013). Desiccation to 13% moisture content (360 min drying in a greenhouse at 30°C) allowed for the development of normal seedlings in *C. pubescens* seeds. However, when the water content was reduced to 5%, after 750 min of drying, only 8% of the seedlings germinated, suggesting some level of desiccation sensitivity (Dousseau et al., 2011).

Maintenance of the seeds physiological potential is directly related to the level of tolerance to moisture loss and the desiccation speed. Desiccation, combined with the drying method, influenced the germination process, suggesting different sensitivity levels to desiccation. After 9 h exposed to silica gel (fast drying), and the moisture content reduced to 20% moisture, 50% of the seedlings

germinated normally. After drying at ambient temperature for 12.5 h (slow drying), to a 15% moisture level, 50% of the seedlings also germinated normally.

Slow drying to 15% of water content at ambient temperature minimized cellular damage caused by desiccation, facilitating normal restoration of water absorption by the seed after desiccation. This drying method may promote desiccation tolerance, because slow desiccation might allow the seed's defense mechanisms to act more efficiently (Silva et al., 2007). Fast dried seeds reached a critical germination potential earlier than slow-dried seeds, showing that drying speed is associated with the level of damage sustained by the seeds.

Damage because of fast removal of moisture may relate to desiccation sensitivity at the cellular level; thus,

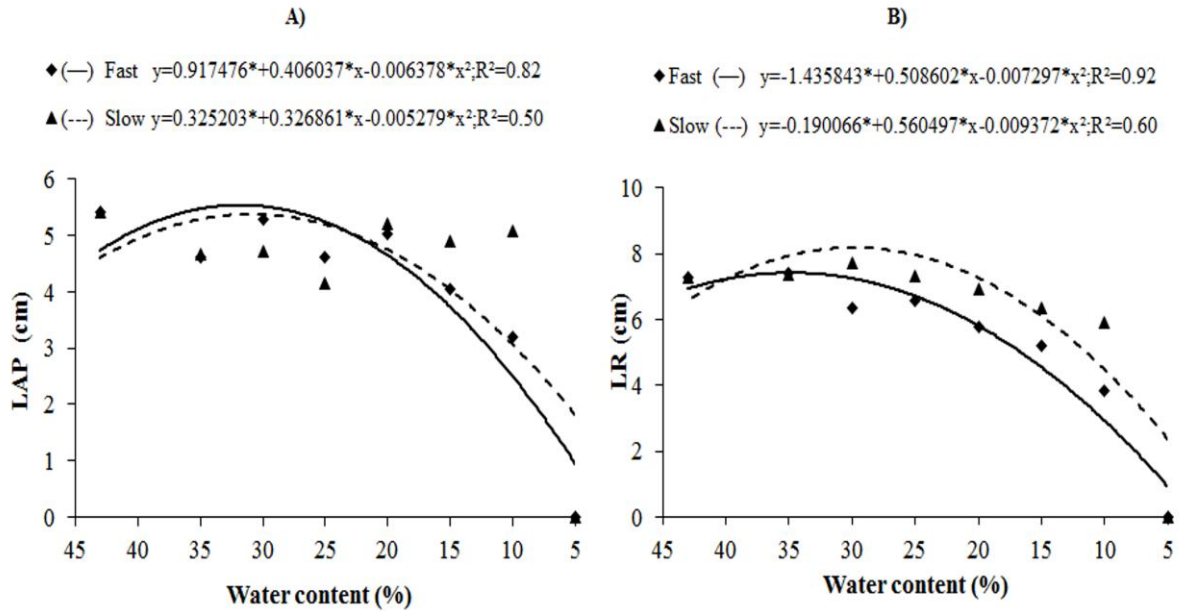


Figure 3. Length of aerial part (LAP) and length of root (CR) of *C. xanthocarpa* O. Berg. seedlings depending of fast and slow drying different water contents.

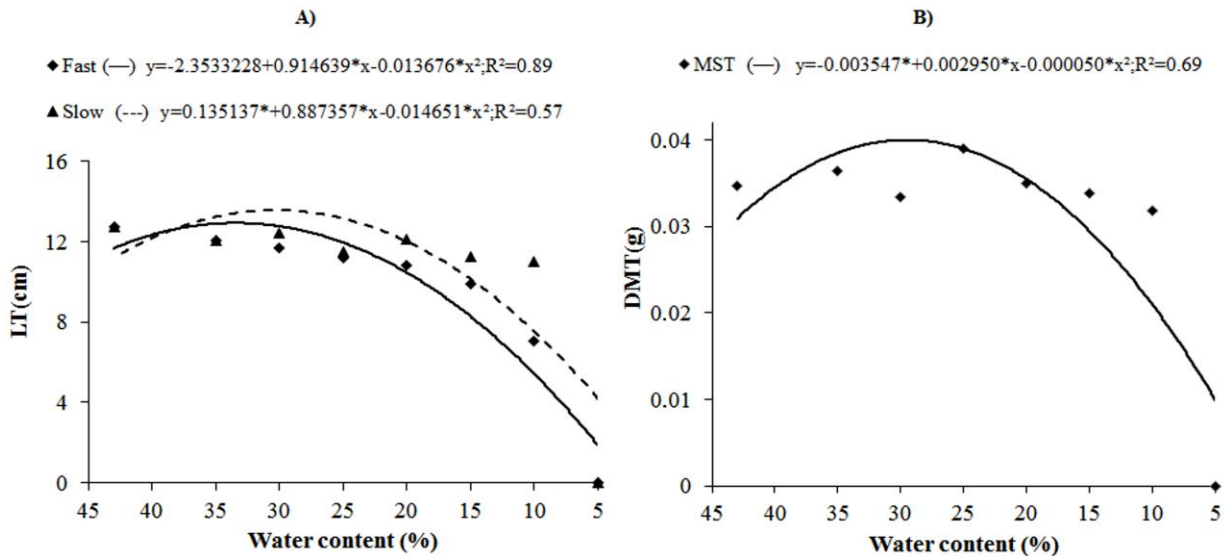


Figure 4. Length of total (LT) and dry mass total (DMT) of *C. xanthocarpa* O. Berg. seedlings depending of fast and slow drying different water contents.

after cells experience desiccation, they may present metabolic anomalies related to a higher intracellular concentration of solutes, altering the cell's ionic pressure and pH, and leading to protein denaturation, solute crystallization, and membrane damage (Nedeva and Nikolova, 1997; Black and Pritchard, 2002).

Some *Campomanesia* species have shown a response to desiccation similar to that by *C. pubescens*. In these

species, moisture reduction from 35 to 4% led to reductions in the germination potential and vigor, suggesting that these seeds are desiccation intolerant and may be classified as recalcitrant (Dousseau et al., 2011). For *C. adamantium* seeds, desiccation and moisture reduction below 21.1% by using silica gel (fast drying) and drying at ambient temperature to 17.2% water content (slow drying) damaged the seeds physiological

Table 1. Protrusion of the primary root (PPR) (%), percentage of normal seedlings (PNS) (%) and germination speed index (GSI) (c), length of aerial part (LAP), length of root (LR), length total (LT) e dry mass total (DMT) of *C. xanthocarpa* O. Berg. subjected to treatments of polyethylene glycol (PEG) associated or not with different concentrations of abscisic acid (ABA).

Treatment		Variables						
PEG (h)	ABA	PPR(%)	PNS (%)	GSI	LAP (cm)	LR (cm)	LT (cm)	DMT (g)
0.0	0.0	50.0 ^{a1}	43.3 ^a	0.682 ^a	4.65 ^a	5.90 ^a	9.88 ^a	0.0323 ^a
60	0.0	28.3 ^b	21.7 ^b	0.336 ^b	2.91 ^b	4.01 ^a	5.25 ^b	0.0338 ^a
60	10 ⁻³	15.0 ^c	13.3 ^c	0.183 ^b	3.69 ^b	4.52 ^a	8.21 ^a	0.0324 ^a
60	10 ⁻⁴	26.7 ^b	26.7 ^b	0.292 ^b	3.43 ^b	4.67 ^a	8.11 ^a	0.0405 ^a
60	10 ⁻⁵	23.3 ^b	23.3 ^b	0.300 ^b	4.45 ^a	5.54 ^a	10.00 ^a	0.0326 ^a
120	0.0	5.0 ^d	5.0 ^d	0.047 ^b	3.10 ^b	5.90 ^a	9.00 ^a	0.0349 ^a
120	10 ⁻³	0.0 ^d	0.0 ^d	0.000 ^c	0.00 ^c	0.00 ^b	0.00 ^c	0.0000 ^b
120	10 ⁻⁴	0.0 ^d	0.0 ^d	0.000 ^c	0.00 ^c	0.00 ^b	0.00 ^c	0.0000 ^b
120	10 ⁻⁵	16.7 ^c	15.0 ^c	0.264 ^b	3.99 ^a	5.56 ^a	9.56 ^a	0.0324 ^a
CV ²		20.1	20.7	27.9	17.7	32.5	18.7	24.9

⁽¹⁾ Means followed by the same letter in the column do not differ significantly by Tukey test at 5% probability, ⁽²⁾ CV: Coefficients of Variation.

potential (Dresch, 2013). For *C. lineatifolia* seeds, the reduction of moisture to 16% caused a decrease in germination, suggesting recalcitrant behavior (Carvalho et al., 1997).

The length of aerial part, root and the whole plant growth (total) were affected negatively by the level of desiccation of the seeds, for both drying methods. These results suggest that seed desiccation hindered seedling growth, and may affect its ability to successfully establish in the soil, its ability to absorb water and nutrients and, therefore, it may ultimately limit plant survival. The results regarding total dry mass measurements showed that seed desiccation influenced biomass accumulation. Thus, seed moisture reduction might influence the seedling's ability to relocate resources. Similar results have been reported in *Cinnamomum zeylanicum* seeds after exposure to different desiccation levels; lower levels of moisture affected negatively plant length and seedling dry mass, for moisture levels lower or equal to 37.9% (Silva et al., 2012). Similar physiological consequences to desiccation, a reduction in plant length and dry mass, were observed in *Euterpe oleracea* seeds, for moisture levels of 30.3% and lower (Nascimento et al., 2007).

To reduce the desiccation sensitivity, treating the seeds exposed to osmotic pressures with different concentrations of ABA and later drying to 15% water content negatively influenced germination. Osmotic conditioning of the seeds by treating them with ABA might not activate the seed stress prevention mechanisms associated with moisture reduction. However, subjecting *C. adamantium* seeds to an osmotic potential of -1.48 MPa without adding ABA and drying to 15% moisture content reduced desiccation sensitivity, leading to a higher proportion of normally germinating seeds (84%) than that in the control (43%). Thus,

although these species belong to the same genus, they behave and respond differently to environmental factors and also to different treatments that may mitigate desiccation stress.

Desiccation tolerance can be promoted by osmotic conditioning of the seeds before desiccation (Maia et al., 2014). In addition, ABA offers protection to desiccation stress by activating genes that synthesize proteins, avoiding moisture loss, and regenerating damaged cells (Stacciarini-Seraphin, 2004). However, treating the seeds with ABA along with osmotic conditioning did not successfully reduce desiccation sensitivity in *C. xanthocarpa* seeds.

Slow-dried seeds exposed to ambient temperature of 25°C for 12.5 h maintained their viability even after lowering the water content to 15%. However, seeds, thus, treated should be sowed shortly after treatment to avoid further water loss. Future studies with seeds treated using PEG and ABA at different concentrations and duration are necessary to obtain better results for maintaining the viability of *C. xanthocarpa* seeds.

The seeds, however, may be desiccated under artificial conditions. The effectiveness of the desiccation would strongly depend on whether the seeds are intended to produce seedlings or be directly sown in a degraded area. *C. xanthocarpa* seeds are desiccation sensitive. In these seeds, reducing the water content below 20% by using silica gel or 15% by drying at ambient temperature reduces seed viability. Among the drying methods tested here, silica gel is proven to be the fastest; further, the drying time was shown to be directly associated with the seeds viability. Osmotic conditioning of the seeds, with or without ABA, did not reduce desiccation sensitivity. The use of this technique did not allow the conservation of seeds for the recovery of degraded areas.

Conflict of Interest

The authors declare they have no conflict of interest.

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