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Tolerance to desiccation and production of encapsulated units of gabiroba (*Campomanesia adamantium*) seeds

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ABSTRACT

Campomanesia adamantium is a Brazilian Cerrado fruit crop species that presents a recalcitrant behavior, which compromises the viability of its seeds. Due to this seed feature, the objective of this study was to evaluate the preservation capacity of the species by the use of synthetic seeds. Two seed lots were maintained in a ventilation oven regulated at 30 °C for 30, 60, 120, 240 and 960 min, and then compared with the fresh seeds (control). After drying, the lots were evaluated according to the germination rate, emergence speed index (ESI), initial time, final time and mean time of germination, synchrony, relative frequency, germination speed and seed vigor. For the production of encapsulated units, 3 complexation times (15, 20 and 30 min) and 5 concentrations of sodium alginate (0, 1.5, 2, 2.5 and 3 g) were evaluated, with 3 replicates. For both experiments, Sisvar software was used, and the means were compared by Tukey test at 5%. Seed quality was compromised by desiccation, as the ESI decreases with increasing desiccation time. Regarding synchrony, there was no significant difference among the treatments, whereas for germination time, fresh seeds started and finished the process faster than the others, and the germination time increased as the drying treatments time increases. The emergence rate of fresh seeds was much higher than those exposed to desiccation. For synthetic seeds, the best complexation time was 20 min for 1.5, 2 and 3 g alginate, reaching 100% of the germinated encapsulated units.

Index terms: Cerrado fruit crop; recalcitrant seeds; seed germination; synthetic seeds.

INTRODUCTION

The economical and conservation potential of plant native species to Brazilian biomes has been studied in order to enable their domestication and sustainable commercial exploitation (Leite, 2012). Among these biomes, the Cerrado stands out as a savanna with the greatest plant biodiversity in the world, which has a large number of plant species that contribute to the production of food, fibers and other products that promote sustainable development (Reis; Schmiele, 2019).

Campomanesia adamantium (Myrtaceae), popularly known as gabiroba, is one of these Brazilian Cerrado fruit crops with potential for introduction into cultivation. The plant is a shrub with 0.40 to 1.00 m height, usually occurs in thickets, and its flowers are small with a whitish cream color. The fruits are rounded edible berries, with yellowish, juicy pulp, involving the seeds, are yellowish-green in color and ripen between September and November (Dousseau et al., 2011). Their sweet-tasting fruits can be consumed in natura or in the processed form such as pulp, juices, ice cream, sweets, liqueurs, or tanned in cachaça. Its cultivation grants small fruit growers an increase in income and an increase in family agribusiness, since its small size allows its association with other fruit species, enabling greater food production (Dousseau et al., 2011). However, there are still few studies on its cultivation and domestication (Vieira et al., 2010).

The propagation of gabiroba is carried out naturally through seeds. However, the fact that the seeds are recalcitrant, presenting a rapid loss

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of germination power, which need to be sown soon after their extraction from the fruits, not supporting storage, is a limiting factor for the sexual propagation of this species (Scalon et al., 2009; Dresch et al. 2013).

The application of tissue culture techniques in Cerrado fruit crops can solve or minimize multiplication problems, since many of them have difficulties with propagation such as heterogeneity in fruit maturation, dormancy, or the fact that their seeds are recalcitrant, compromising longevity and viability (Pinhal et al., 2011). Species with this particularity can be preserved alternatively by means of synthetic seeds. This technique consists of the encapsulation of different plant tissues that can be further used for in vitro or ex vitro culture regenerating plants (Rai et al., 2009; Nongdam, 2016). The main potential advantages of this method are related to the production of large amounts of propagules in a short period of time, maintenance of clonal identity, direct seeding in the field, eliminating the need for expensive acclimatization structures, such as seed trays and nurseries, the low cost per plant, and the possibility of long-term storage (Utomo et al., 2008). Various vegetable crops have been encapsulated to produce artificial seeds (for review see Abbas; Mahood; Alhasan, 2022).

The objective of this study was to evaluate the tolerance of Campomanesia adamantium seeds to desiccation and their preservation capacity by the use of synthetic seeds. The complexation period and the sodium alginate concentration were also evaluated in order to establish an encapsulation protocol for this important Cerrado fruit species.

MATERIAL AND METHODS

Seed collection

Campomanesia adamantium seeds were collected at the Germplasm Bank of the Federal University of Jataí (-17.92341788591131, -51.71585666832501), and taken to the Plant Tissue Culture Laboratory of the same institution. The seeds were removed from the fruits and subjected to mechanical action (scrubbing) for mucilage removal. They were subsequently washed under running water for 10 min.

Evaluation of seed desiccation tolerance and physiological quality

Two seed lots were placed in a ventilated oven regulated at 30 °C, kept for 30, 60, 120, 240 and 960 min, and compared with seeds that were freshly harvested and superficially dried (control), totaling 6 treatments with 3 replicates each and 50 seeds per replicate (for each lot). The seeds were weighed before and after desiccation to obtain the lost water content, which was calculated by the formula described in the Rules for Seed Analysis (Brasil, 2009), which was:

100 x ((wet weight + tare) – (dry weight + tare)) / (wet weight + tare) – tare

After desiccation, in one of the lots, the physiological quality of the seeds was evaluated based on the loss of seed viability related to the gradual loss of membrane system integrity, evaluated by electrical conductivity according to Marcos Filho (2005).

EC (electrical conductivity) = reading / dry weight ($\mu S.\ cm^{-1}.\ g^{-1}$)

For this, the desiccated seeds were immersed in 75 mL of deionized water in a plastic container and kept in Bio-Oxygen Demand incubator (B.O.D) at 30 °C. After 24 h, electrical conductivity reading was performed. The other lot was taken to B.O.D. chamber regulated at 30 °C for germination evaluation.

Germination test

The seeds of the previous treatments underwent a germination test on the Germitex paper, moistened with distilled water with a volume (mL) equivalent to 2 times the mass of dry paper, in B.O.D chamber, regulated to maintain the constant temperature of 30 °C in the dark according to Dousseau et al. (2011).

The effect of the desiccation periods was evaluated by the germination test, and the evaluations were performed daily always at the same time, from the day the experiment was set up. Seedlings that reached 1 cm in length were considered germinated and normal. At the end of the test, normal seedlings were used for seedling production while non-germinated seeds were discarded.

The emergence speed index (ESI) was calculated using the formula described by Maguire (1962) and the initial time (ti), final time (tf), mean time (tm), synchrony (Z), relative frequency of germination (fr) and emergence rate (ER) were calculated using the formulas described by Santana and Ranal (2004). Initial date and final date of germination were considered to be the day of the first germinated seedling and the day from which germination stabilized, respectively.

Production of encapsulated units

For the production of encapsulated units, the seeds were placed in the sodium alginate solution and kept for 5 min, and then a pipette was used to collect each seed from the sodium alginate and carefully dripped into a solution of calcium chloride (50 mM) for capsule complexation. We have tested 5 concentrations (0, 1.5, 2.0, 2.5 and 3 g) of sodium alginate and 3 times of complexation (15, 20 and 30 min) in calcium chloride. Thus, there were 15 treatments (5x3), with 3 replicates each, and each replicate consisted of 50 seeds.

After the complexation period, the units formed were immersed in distilled water to remove excess calcium chloride and distributed in Gerbox boxes with Germitex paper (50 seeds per Gerbox box) and placed in B.O.D chamber regulated at 30 °C for germination control.

The germination rate (capsule rupture) was evaluated daily over a period of 15 days, and seeds whose seedlings (longer than 1 cm) were able to break the alginate capsule were considered germinated.

Statistical analysis

For the desiccation, physiological quality and germination experiment, a completely randomized design was used with 6 treatments and 3 replicates, containing 50 seeds per replicate. Tukey test at 5% probability level was used to evaluate the means. For the encapsulated units, a completely randomized design was used with a 5 x 3 x 3 factorial scheme, corresponding to 5 concentrations of alginate, 3 times of complexation in calcium chloride and 3 replicates, with 50 seeds in each replicate. The means of capsule rupture were evaluated by Tukey test at 5% probability level. Both analyses were performed using Sisvar software (Ferreira, 2011).

RESULTS AND DISCUSSION

Desiccation tolerance, physiological quality and germination test

Regarding the tolerance to desiccation, the water content lost from gabiroba seeds was increasing as a function of drying time, reaching up to 36% of water lost in 960 min of drying (Figure 1a).

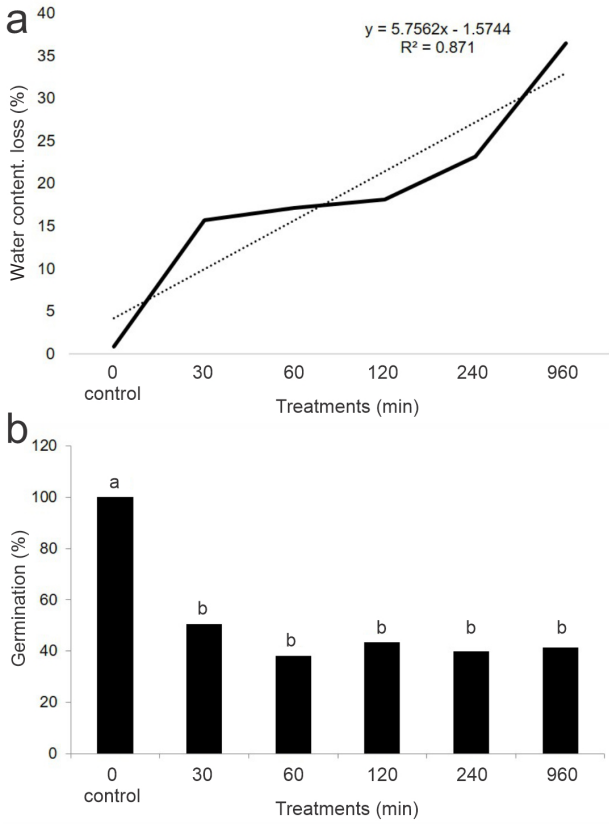

Figure 1: Percentage of water content loss (a) and germination (b) of *Campomanesia adamantium* seeds subjected to different desiccation period treatments. Means followed by the same letter do not differ from each other by Tukey test at 5% significance level.

The physiological quality of gabiroba seeds was affected by desiccation. In relation to germination percentage, the control treatment was the one with the highest germination rate, with no difference among the desiccation treatments (Figure 1b). However, the ESI reduced as the desiccation time increased (Table 1). These results corroborate those obtained by Dresch, Masettot

and Scalons (2015), who evaluated two methods to desiccate *Campomanesia adamantium* seeds and observed that the germination speed index decreases linearly with desiccation, regardless of the applied methods. These authors also claimed that *C. adamantium* seeds showed a recalcitrant behavior due to their sensitivity to desiccation. Melchior et al. (2006) also stated that the germination and ESI of *C. adamantium* seeds continuously decrease as the seed water percentage decreases, as observed here. This behavior seems to be conserved in the *Campomanesia* genus. Nunes et al. (2015) also reported that germination speed *C. xanthocarpa* seeds of is influenced by desiccation.

Seed germination in general is not perfectly synchronized, so it is possible to quantify this variation over time (Santana; Ranal, 2004). Here, the germination of *C. adamantium* fresh seeds was more synchronized than seeds subjected to desiccation (Table 1). However, no difference has been observed in the germination synchrony among the desiccation treatments (Table 1).

In relation to the initial time of germination, it is influenced by the drying treatments. Seeds that were not subjected to desiccation (control) and those that remained for up to 120 min started germination faster than those that remained 240 and 960 min in the drying process (Table 1). Fresh seeds (control) also finished their germination process faster than seeds subjected to desiccation treatments, and those that remained 960 min in drying took longer to finish germination, with no significant difference among the treatments. In general, the mean time of seed germination increased as the drying time increased (Table 1).

The emergence rate of seeds that were not desiccated was much higher than that of seeds subjected to desiccation, and those that remained exposed for longer periods had the worst results (Table 1). Oliveira, Santana and Santos (2011) also observed that the percentages of emergence of normal seedlings and emergence rate were reduced when *C. adamantium* seeds were dried in the shade for 24 h. Interesting, these same authors stated that drying treatments did not affect the germination synchrony and the initial and final times of *C. adamantium* seedlings emergence, which is not in accordance to the data reported here. Although, these authors also showed that the mean time of emergence of *C. adamantium* seedlings increased with the desiccation treatments, which corroborates the results presented here.

According to the relative frequency polygons (Figure 2), the germination of gabiroba seeds became slower as drying times increased. Fresh seeds that showed the highest germination percentage (Figure 1b) and lowest mean of germination time (Table 1), 40% of the seeds germinated in the period between the 2nd and 6th days after sowing (Figure 2a). In the treatment with 30 min of drying, 40% of the seeds germinated between the 3rd and 10th days (Figure 2b). In the treatments with longer periods of drying (240 and 960 min), the highest rates of daily germination occurred after the 8th day (Figure 2e, f). This behavior clearly confirms the recalcitrance of *C. adamantium* seeds, since the greater the reduction in seed water content, the greater the reduction in germination speed and seedling emergence, and the greater the initial, final and mean times of germination.

Table 1: Synchrony, initial, final, and mean time, emergence rate and emergence speed index (ESI) obtained in the germination test of *Campomanesia adamantium* (gabiroba) seeds, conducted with different desiccation treatments (0, 30, 60, 120, 240 and 960 min).

	Synchrony	Initial time	Final time	Mean time	Emergence rate	ESI
0	0.310 a	3.000 a	10.333 a	4.427 a	12.953 a	12.953 a
30 min	0.200 b	4.000 a	14.333 ab	7.123 b	3.817 b	3.890 b
60 min	0.110 b	4.000 a	14.667 ab	9.873 bc	2.397 bc	2.397 cd
120 min	0.190 b	4.000 a	12.667 ab	7.030 b	3.497 b	3.557 bc
240 min	0.187 b	9.000 b	14.333 ab	11.117 c	1.530 c	1.7633 d
960 min	0.127 b	10.000 b	16.333 b	12.180 c	1.583 c	1.807 d
CV	18.57%	7.20%	13.43%	15.66%	11.98%	10.67%

Means followed by the same letter do not differ from each other by Tukey test at 5% significance level.

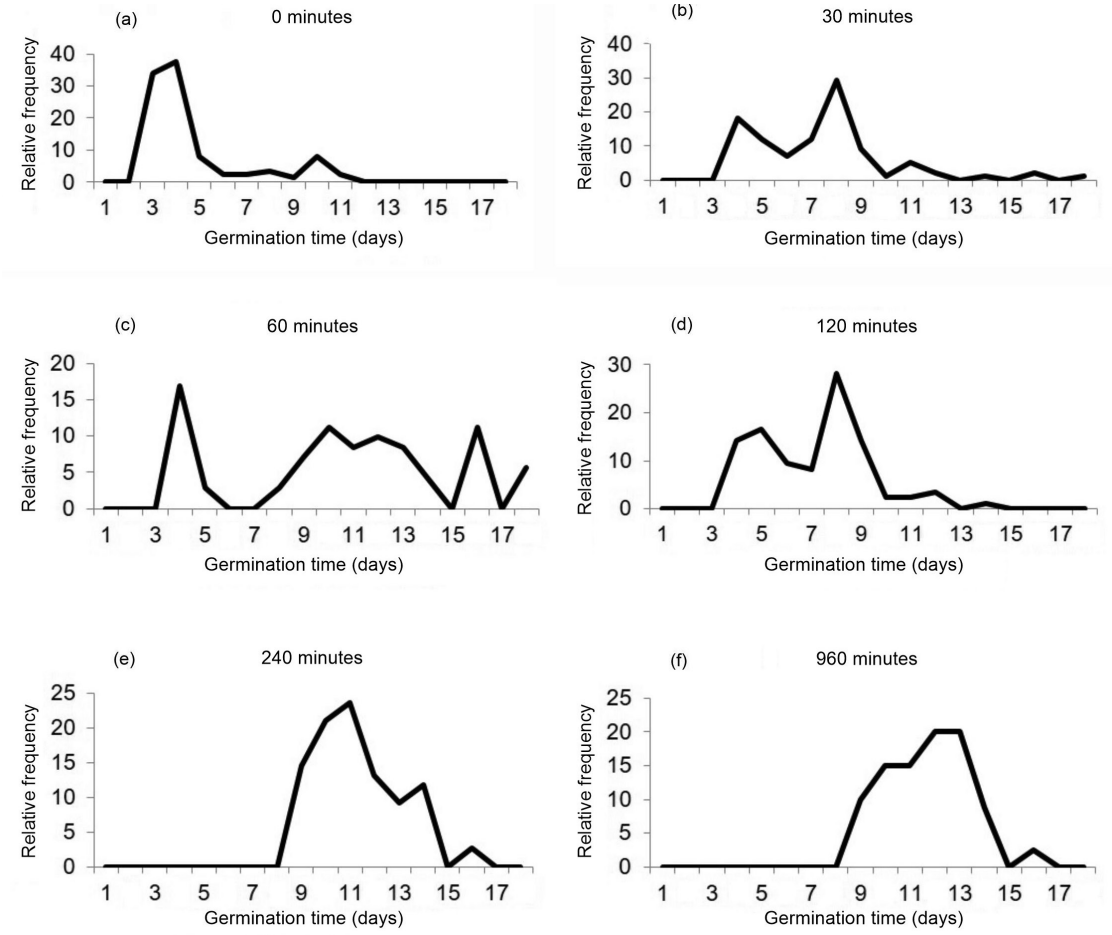

Figure 2: Distribution of the relative frequency of germination of *Campomanesia adamantium* (gabiroba) seeds subjected to different drying times.

The negative effect of desiccation on the physiological quality and vigor of the seeds was confirmed by the results of electrical conductivity, whose highest values were obtained as the water content of the seeds decreased (Figure 3). The value of electrical conductivity is measured as a function of the amount of leachates in the seed imbibition solution, which in turn is directly related to the integrity of cell membranes (Vieira; Krzyzanawski, 1999). Seeds that allow the exit of large amounts of electrolytes have greater conductivity, indicating greater permeability of membranes and, therefore, more advanced deterioration (lower vigor) (Popinigis, 1985).

These results confirm the damage caused by desiccation in the structure of cells as well as membranes. Membrane integrity is critically important for seed viability, since any improper rupture caused by desiccation can have immediate consequences for seeds during rehydration (Kermode; Finch-Savage 2002).

Production of encapsulated units

There was significant interaction between the factors studied in relation to the percentage of encapsulated units that managed to germinate and thus rupture the alginate capsule (Table 2). The significant effect of capsule concentration was verified only in the complexation time of 20 min, and the best results were obtained with concentrations of 1.5, 2 and 3 g of alginate, reaching 100% of the germinated encapsulated units.

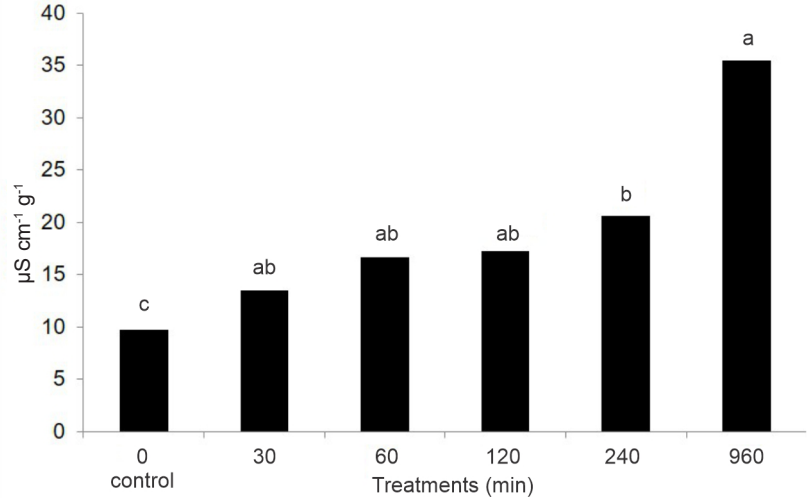

Figure 3: Effect of drying on the results of electrical conductivity of the imbibition solution of *Campomanesia adamantium* (gabiroba) seeds. Means followed by the same letter do not differ from each other by Tukey test at 5% significance level.

Table 2: Effect of capsule concentration and complexation time on germination percentage (capsule rupture) of encapsulated *Campomanesia adamantium* (gabiroba) seeds.

	Time (min)		
Concentration (g)	15	20	30
0	0 b	0 c	0 b
1.5	100 a	100 a	98.67 a
2	100 a	98 a	100 a
2.5	93.67 a	85.33 b	96.33 a
3	90.33 a	100 a	92.33 a

Means followed by the same letter do not differ statistically from each other by Tukey test at 5% significance level.

Campos (2014) evaluating the production of encapsulated units of *C. pubescens* obtained 100% capsule rupture using 20 min for complexation. Guedes, Costa and Pereira (2007) found no interaction between complexation times and concentration of alginate capsules for the production of synthetic long pepper seeds. However, the regeneration of encapsulated *Carica papaya* embryos was significantly affected by alginate concentration and complexation time, with the best results obtained at 2.5% alginate and 10 min of complexation (Castillo, 1998).

Sarmah, Borthakur and Borua (2010), working with encapsulated units of orchids, obtained the best results using 3% sodium alginate, while 2% of alginate produced malformed capsules and, at the highest concentration (4%), the capsules were more difficult to break. These authors also state that the frequency of conversion of explants into plants depends on the duration of exposure to calcium chloride, and 30 min was the ideal time. For *Flickingeria nodosa*, another epiphytic orchid, somatic embryos were encapsulated with sodium alginate (2%) dipped in calcium chloride (100 mM) solution and were incubated for 30 min to make firm, transparent and consistent synthetic seeds (Nagananda; Satishchandra 2023). Sodium alginate, in the presence of divalent and trivalent cations, is complexed and forms calcium alginate. The resistance and hardness of the capsule are important because, in some cases, excessive hardness of the capsule compromises or prevents the conversion of the explant into plants (Guerra; Torres; Teixeira, 1999).

CONCLUSIONS

Campomanesia adamantium seeds are intolerant to desiccation, as this condition reduces their physiological quality and vigor, proving their recalcitrant behavior. Sodium alginate concentration and the time of exposure to calcium chloride influence the frequency of conversion of encapsulated explants into seedlings, and satisfactory results are obtained with 1.5, 2, or 3 g of alginate in 20 min of complexation. These

data will contribute to the optimization of synthetic seed protocols for *Campomanesia* spp., in order to store these encapsulated units for a longer time, which may be useful in the conservation of gabiroba germplasm; and simplify the seed management of this important Cerrado fruit species.

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