

Physiological Conditioning of *Alibertia edulis* (Rich) Seeds

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Abstract

Physiological conditioning of seeds has been shown to increase the uniformity of seedlings; thus, it has been useful for propagating native tree species from the Brazilian Cerrado which, otherwise, are difficult to propagate successfully. The objective of this work was to evaluate the effect of physiological conditioning of *Alibertia edulis* seeds by soaking for 5 or 10 days in solutions of varying osmotic potential. After conditioning, seeds were dried down to original water content, sown on sheets of germitest paper inside gerbox plastic boxes, and incubated at 25°C. We evaluated the effect of conditioning by studying seed germination and vigor. Seed conditioning by osmotic pretreatment showed positive effects; however, germination and growth of seedlings from seeds conditioned at osmotic potentials of -0.3 to -0.7 MPa were reduced. Osmoconditioning for 10 days at -0.7 MPa resulted in increased percent germination, indicating that the longest imbibition period in the osmotic solution of the lowest osmotic potential (-1.3 MPa) favored the seed germination process. *A. edulis* seeds did not require conditioning to attain high germination rates; nonetheless, osmotic conditioning reduced average seed-germination time.

Keywords

Cerrado, Seeds Germination, Polyethylene Glycol, Osmotic Potential

1. Introduction

Rubiaceae ranks the fourth among angiosperms in species number and, in the Brazilian Cerrado, it is the fifth most representative family. Some Rubiaceae plants, such as those of the genus *Alibertia*, are of great importance, especially the *Alibertia edulis* (Rich) also known as quince and marmelo-do-cerrado, which is an arboreal species that may grow up to 8 m in height with a trunk diameter of 15 to 25 cm. The fruits are ovoid, approximately 2 to 4 cm in length by

2 to 4 cm in diameter, black in color when ripe, and widely appreciated by the population [1] [2].

Several studies have addressed the economic potential of Brazilian native species that are prominent for their nutritional or medicinal value. Some of these native fruits are an important support for wildlife and a complementary food supply for human rural populations. Additionally, they constitute an attractive and viable alternative source of income for small producers [2] [3]. However, the propagation of most native fruit-tree species is still a challenge, due to the difficulties encountered while handling their seeds.

Osmotic conditioning has been proven a useful technique to increase the efficiency of propagation of fruit species. This simple technique consists of controlling seed imbibition in an aqueous solution of polyethylene glycol (PEG) that allows controlled hydration of the seeds by limiting water absorption in a manner that the initial steps of germination are activated without reaching the visible germination phase during the procedure [4] [5] [6].

Osmotic conditioning is used to standardize and accelerate germination. It is accomplished by immersing the seeds in an osmotic solution for a previously determined period and at a certain temperature. Osmoconditioning provides higher germination uniformity, increases the emergence and seedling development index, and increases shoot growth rate even in soils with low water content. Thus, physiological conditioning of seeds allows faster germination, both at low and high temperatures, in addition to greater germination rate, resulting in overall more uniform stands [5] [7].

A few studies have been conducted to determine the best conditions for the physiological conditioning of seeds of different species. Successful results have been reported for seeds of *Guazuma ulmifolia* (Malvaceae) [8], *Tecoma stans* (Bignoniaceae), and *Cordia megalantha* (Boraginaceae) [9]. Nevertheless, results obtained from seed conditioning treatments are not always positive and there is still need to expand our knowledge about different aspects related with this technique [10], especially in the case of native fruit-tree species.

Thus, the objective of this work was to evaluate the effect of physiological osmoconditioning using PEG on the germination and vigor of seeds of *Alibertia edulis*.

2. Material and Methods

Ripe *A. edulis* fruits were collected directly from 12 wild trees located at the Santa Madalena farm in the city of Dourados State Mato Grosso of Sul, (22° 13' 18.54" S and 54° 48' 23.09" W, 437 m altitude), during the second half of November 2016. Fruits were taken to the Laboratory of Plant Nutrition and Metabolism of the Faculty of Agrarian Sciences, at the Federal University of Grande Dourados (UFGD) in Dourados-MS. In the laboratory, fruits were macerated in a sieve under running water, thus removing the pulp, until complete separation of the seeds from all fruit residues. All the seeds collected were then mixed to-

gether until fully homogenized, making up a single batch, and then placed in a single layer on plastic trays, until the water was completely evaporated from the surface of the seeds.

Seed water content was determined by oven drying at $105^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 24 hours, in four replications of 20 seeds each, according to the Rules for Seed Analysis [11]. After drying, seeds showed 14.5% water content (wet basis).

We used the equation cited by [12], for calculating the necessary quantities of PEG 6000 for the study of the effect of osmotic conditioning.

$$\Psi_{\text{os}} = -\left(1.18 \times 10^{-2}\right)C - \left(1.18 \times 10^{-4}\right)C^2 + \left(2.67 \times 10^{-4}\right)CT + \left(8.39 \times 10^{-7}\right)C^2T,$$

where Ψ_{os} = osmotic potential (MPa); C = concentration (grams of PEG 6000 Kg water⁻¹) and T = temperature ($^{\circ}\text{C}$), which was set at 25°C . Thus, to obtain an osmotic potential of -0.3 , -0.5 , -0.7 , and -1.3 MPa, aqueous solutions were prepared with 150, 200, 245 and 340 g of PEG 6000 per kilogram of distilled water, respectively. To prepare these solutions, the required amount of PEG 6000 was weighed and then mixed with distilled water until completely dissolved.

Seeds were arranged in a single layer on Petri dishes lined with “germitest” paper moistened with 12 mL of the various PEG 6000 solutions prepared as described. Osmo-treated seeds were soaked for either 5 or 10 days. These soaking periods were previously determined and similar to those used for conditioning of seeds of jenipapo (*Genipa americana* L.), which also belongs to the Rubiaceae family [13]. Subsequently, seeds were removed and washed under running water to remove excess soaking solution and the water content was determined after the conditioning periods. Seeds were dried down to original water content ($14.0\% \pm 2\%$) and submitted to the tests and determinations described below.

Germination Test

Seeds were initially washed in 1% sodium hypochlorite solution for 5 minutes, followed by rinsing under running water. Then, they were placed on two sheets of “germitest” paper previously moistened with distilled water to the equivalent to 2.5 times the mass of the dry paper in gerbox plastic boxes and maintained in germination chambers of the Biochemical Oxygen Demand (B.O.D.) type, at 25°C , under continuous white light. At the end of 30 days, the number of normal seedlings was recorded, *i.e.*, those that presented a primary root and a shoot longer than 1 cm. Data are expressed in percentage.

Average Germination Time

This was determined during the germination test by daily recording the number of germinated seeds in each replication. In order to estimate the average germination time, we used an equation of the index representing the average germination time, expressed in days [14].

Seedling Length

Twenty seeds were sown in the upper third of a paper roll previously moistened with water. After 30 days, the length of primary root, shoot, and total length of normal seedlings were recorded with the aid of a ruler with millimeter increments. Data are expressed in centimeters seedling⁻¹.

Fresh Mass Fresh mass was obtained by weighing the same normal seedlings used for length evaluation on an analytical precision balance (0.000 g), and data are expressed in grams seedling⁻¹.

Statistical Analysis

The experiment was laid in a completely randomized 5 × 2 factorial (osmotic concentrations and incubation times) design with four replications, each consisting of 50 seeds. Data were subjected to analysis of variance by the F test and when significant, means were submitted to regression analysis at 5% significance using the statistical software SISVAR [15].

3. Results and Discussion

Seeds of *A. edulis* showed 84% germination. During the conditioning periods, the seeds absorbed water slowly, as shown in **Table 1**. Although not statistically analyzed, the water content of osmoconditioned seeds differed little between the 5 and the 10 day soaking periods. Seeds soaked for 5 days showed higher water content at higher osmotic potential of the soaking solution. The same was not true for seeds soaked for 10 days in which case, water content varied between 43% and 48%, indicating a small variation in the pattern of water absorption by seeds (**Table 1**).

There was a significant interaction between time of imbibition and PEG concentration used for seed osmoconditioning (**Figure 1(a)**). Germination rate of seeds conditioned for five days was higher than that of seeds conditioned for 10 days, with minimum germination rates of 62.9% and 53.8%, respectively. These results may indicate that the longer conditioning period was likely detrimental to seed viability. Similar results were found by [16], who observed that soaking for 9 and 12 days reduced the efficiency of osmotic conditioning, whereas soaking for 3 and 6 days was more effective as osmotic conditioning with PEG (6000) and KNO₃ to enhance germination of lemon seeds (*Citrus limonia* Osbeck).

Overall, *A. edulis* seeds soaked in solutions of low osmotic potential showed decreased germination, with only 55% of germinated seeds, previously osmoconditioned for 5 days at -0.5 MPa, and 51.8% of germinated seeds, previously osmoconditioned for 10 days at -0.3 MPa. However, osmoconditioning for 10 days at -0.7 MPa resulted in increased percent germination, indicating that the

Table 1. Water content of *Alibertia edulis* (Rich) seeds before and after osmotic conditioning with PEG (6000) solutions of known osmotic potential.

Freshly processed seeds	14%	
	Time of osmotic conditioning	
PEG (MPa)	5 days	10 days
-0.3	47%	45%
-0.5	43%	48%
-0.7	40%	45%
-1.3	41%	43%

longest imbibition period in the osmotic solution of lowest osmotic potential (−1.3 MPa) favored the seed germination process. Nevertheless, the extension of the conditioning period at a lower osmotic potential may indicate the loss of efficiency of the osmoconditioning technique for the seeds of *Alibertia edulis*.

Results from experiments with osmotic conditioning of seeds of several native forest species are controversial. In *Hancornia speciosa* (Gomes) seeds, also in the Brazilian Cerrado, seed germination and root length decreased after osmoconditioning of the seeds, indicating seed sensitivity to incubation in PEG [17]. Similar results were observed by [18] who reported no trend of improvement in the viability of wild peanut seeds (*Pterogyne nitens* Tul) with increased time of exposure of the seeds to osmoconditioning in a solution of PEG + KNO₃. Angico seeds (*Anadenanthera colubrina* (Vell) Brenan) soaked in a PEG 6000 solution of −0.8 MPa osmotic potential failed to absorb water, whereby seed germination was severely hampered relative to germination after osmoconditioning at an osmotic potential of −0.6 MPa [19].

On the contrary, we recorded a significant effect of osmotic potential on the mean seed germination time (Figure 1(b)), indicating the opportunity to optimize the duration of the germination test in combination with a reduction of the osmotic potential of the soaking solution used for seed osmoconditioning.

Although the reduction was subtle, in the absence of osmotic conditioning, the test had a maximum duration of 29 days, whereas after osmotic conditioning with −1.12 MPa, the maximum duration was 28 days, with a tendency to decrease (Figure 1(b)).

The beneficial effects of osmoconditioning in reducing germination time were also observed in seeds of papaya (*Carica papaya* L.) at osmotic potentials between −1.0 MPa and −1.5 MPa, with a positive effect on seed vigor, but without an influence from time of exposure to the conditioning solution [20]. Further, in seeds from *Dimorphandra mollis* Benth, another species native to the Cerrado, a positive effect was observed after osmotic conditioning with PEG of osmotic

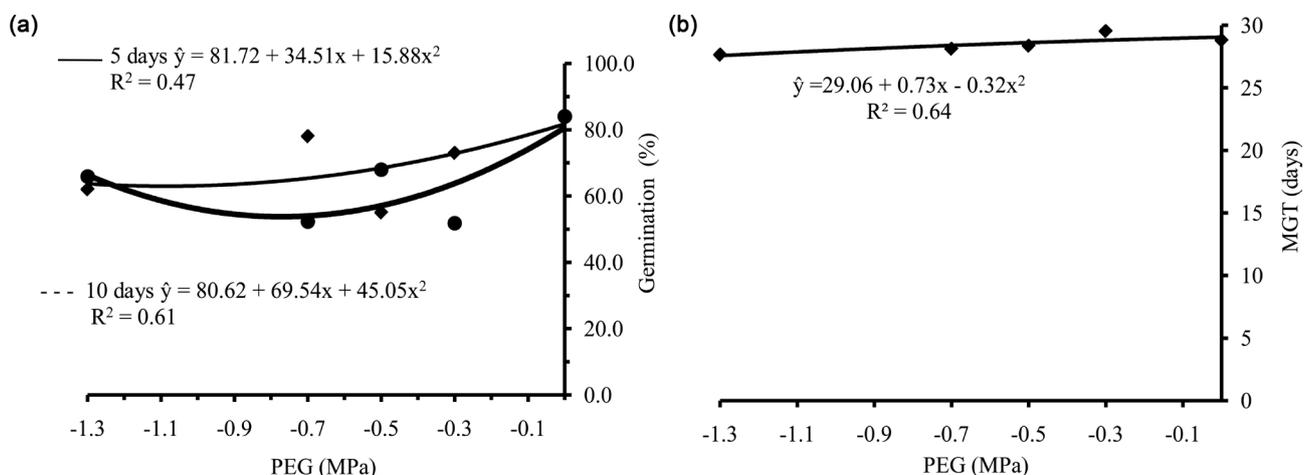


Figure 1. (a) Percent germination; (b) mean germination time (MGT) of *Alibertia edulis* (Rich) seeds submitted to different osmotic potentials by soaking in PEG 6000 solutions of known concentration for osmoconditioning.

potential of -0.7 MPa on the germination speed index (GSI) (7.4), whereas untreated seeds showed a GSI of 4.3 [21].

Emergence in seeds of *Parkia pendula* (Benth ex Walp) was more homogeneous after conditioning, whereas the average time for development of normal seedlings was significantly reduced by water conditioning in freshly harvested and stored seeds, indicating that hydro-conditioning was enough to increase the germination speed of the seeds and to eliminate differences among seed lots [22].

The increase in germination speed index favors the establishment of uniform field stands, thus providing positive results, as verified in seeds of maxixe cv. Nordestino, although conditioning in this case did not promote any change in seed germination [23]. In addition, there no increase observed in percent germination of melon seeds (*Cucumis melo*) osmotically conditioned at 25°C ; however, an increase in germination speed was verified. In this case, the authors proposed that some morphological, physiological, and biochemical changes occurring in seeds due to physiological conditioning are not yet fully elucidated [24]. Several events related to germination (genomic transcription and translation, respiration and energy metabolism, initial mobilization of reserves and DNA repair) also occur during physiological conditioning under the low water availability level to which seeds are exposed during osmoconditioning [25].

With respect to the effects on seedling growth in *A. edulis*, upon seed osmoconditioning, there was a significant isolated osmotic effect. Similar to the effect on percent germination, as osmotic potential of the soaking solution decreased between -0.3 and -0.7 MPa, minimum growth of root (2.15 cm), shoot (3.42 cm), total length (5.57 cm), and fresh mass of seedlings (0.027 g) was observed (Figures 2(a)-(d), respectively). These results may relate to the fact that physiological conditioning with osmotic solutions, such as PEG, involves seed exposure to low water potential, which allows only partial hydration of the seed material [26], thus causing osmotic stress, which probably reflects as oxidative damage to cellular components [27].

In general, conditioning of *A. edulis* seeds had positive effects associated with osmotic potential; in particular, there was a tendency for average time for seed germination to decrease. These results are in agreement with those found for seeds of watermelon (*Citrullus lanatus*) osmoconditioned with KNO_3 [28], seeds of *Psidium guineense* Swartz [29] and soybean (*Glycine max*) seeds of cv. M7211 [30] osmoconditioned with PEG. In all these cases, the beneficial effects of physiological conditioning were not evident as an increase in germination, but in the optimization of germination, together with a reduction of the time required for the establishment of the stand.

It is worth noting that *A. edulis* seeds are sensitive to the reduction of water content and prolonged storage, in which respect, they are classified as intermediate [3]. Thus, any osmotic stress caused by soaking in PEG solutions may have determined the limited effectiveness of the physiological conditioning technique

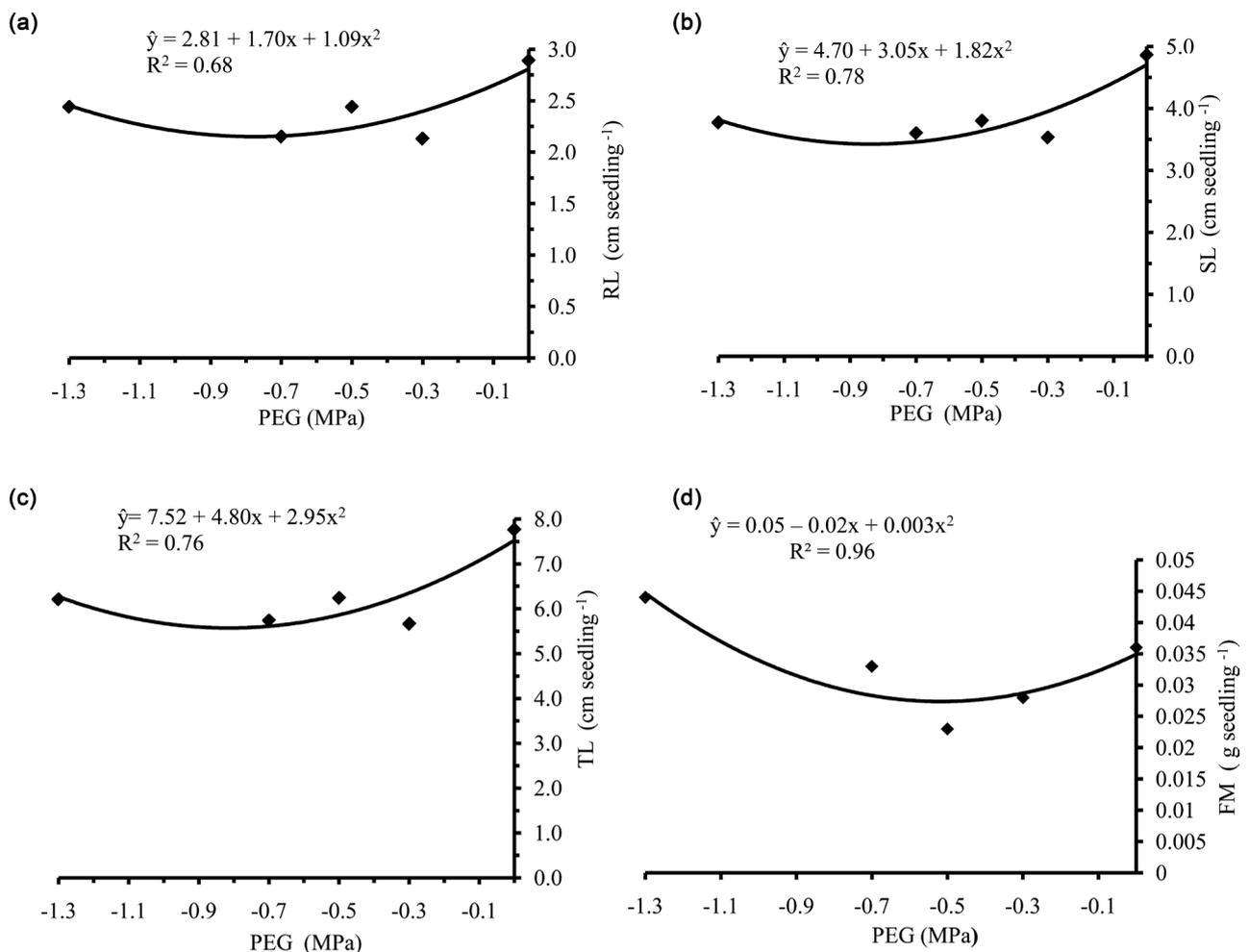


Figure 2. (a) Root length (RL); (b) Shoot length (SL); (c) Total length (TL); (d) Fresh mass of seedlings (FM) grown from *Alibertia edulis* (Rich) seeds subjected to different osmotic potentials (MPa) by soaking in PEG 6000 solutions for osmoconditioning.

for *A. edulis* seeds. Nonetheless, the use of technologies to maximize successful establishment of seedlings of any native forest species is foremost in importance, especially for those species that naturally produce few seeds of low longevity, such as *A. edulis*. Therefore, further studies should be carried out aimed to elucidate the behavior of *A. edulis* seeds when submitted to physiological conditioning and storage.

4. Conclusion

Physiological conditioning with PEG solutions of osmotic potentials between -0.3 and -0.7 MPa reduces germination and growth of *A. edulis* seedlings, but benefits the average time required for seed germination.

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